# Seed priming with $H_2O_2$ confers better yield in mungbean by modulating the antioxidant enzymes activities and metabolic processes of roots and leaves under salt-alkali mixed stress condition

## Abstract

High salt concentrations and high pH occur simultaneously in nature, however, presently most of the studies have mainly focused on only salinity and salt-alkali combined stress research are comparatively very limited. Hydrogen peroxide is an important signaling molecule. However, the role of exogenously applied hydrogen peroxide ($H_2O_2$) under saline–alkaline stress is not known. The main objectives of present study was to assess role of exogenously applied $H_2O_2$ as seed priming in mitigating the harmful effect of saline–alkaline stress on differentially tolerant mungbean genotypes (TMB-37and MH-1314). Saline-alkaline stress significantly decreased the chlorophyll content, leaf relative water content (RWC) and yield while enhanced malondialdehyde (MDA), proline and antioxidant enzyme activity in root and leaf samples of both mungbean cultivars. Seeds priming were done with 0.01% $H_2O_2$ and distilled water. Seed priming with $H_2O_2$ significantly improved the yield and yield attributes along with increment in leaf chlorophyll content, RWC as well as accumulation of osmolytes. The activities of antioxidant enzymes, viz., SOD, CAT and POX were also significantly increased in both mungbean genotypes and especially the CAT activity both in root and leaf tissue. However, relatively higher improvement was observed in genotype TMB-37. In conclusion, exogenously applied 0.01% $H_2O_2$ improved the saline–alkaline tolerance, which was reflected in terms of enhanced photosynthetic pigments, RWC, proline accumulation, and antioxidant enzyme activity of root as well shoot tissues, especially the CAT activity. Moreover, 0.01% $H_2O_2$ appeared to be very effective in ameliorating the harmful effect of saline–alkaline stress on physiological traits, metabolic processes and yield.

**Key words:** Saline-Alkaline, seed priming, antioxidant enzyme, hydrogen peroxide, malondialdehyde, proline

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## 1. Introduction

The problem of soil saline-alkalization (SA) has grown more serious globally [1]. Currently, salt-alkali mixed stress is relatively less researched than salinity stress, which is the subject of the majority of research. High salt concentrations and high soil pH frequently occur together in nature, though, and their combined effects may be more detrimental to plant growth and development than the effects of either stress alone [2]. As a result, research on plant defense mechanisms against saline-alkaline mixed stress, selection of novel saline-alkali stress-tolerance genes and genotypes, and investigation of novel plant salt-alkali tolerance techniques are all of great practical significance for the long-term sustainability of agriculture. Saline-alkalization threatens more than 900 million hectares of land worldwide, according to data, and there are no practical ways to stop it from spreading [3]. The degree of salt-alkali condition is classed as mild (salt content less than 3 and pH 7.1-8.5), moderate (salt content 3 to 6 and pH 8.5-9.5), and severe (salt content more than 6 and pH greater than 9.5) based on salt content and pH value [4]. Majority of horticultural and leguminous crops are extremely vulnerable to saline stress [5]. Mungbean (*Vigna radiata* (L.) Wilczek) which is a crop of multiple use to people in the form of food, feed, fodder and green manure, is also highly sensitive to salt stress due to ion toxicity and oxidative stress, nutrient shortfall or imbalance, osmotic stress, water shortage, and reduced photosynthesis [6 & 7]. Due to the buildup of reactive oxygen species (ROS) in root cells, high soil pH significantly disrupts the pH stability of cells, degrades the integrity of cell membranes, damages root cells and reduces root vitality [8, 9]. The creation of resistant cultivars is a crucial strategy, but it is a time-consuming and expensive process [10]. Agronomic methods present a speedy answer to this issue in this situation [11, 12]. $H_2O_2$ is one such signalling molecule that serves multiple roles in plants at low levels and has been proven to be beneficial in reducing a variety of stresses (both abiotic and biotic) [13, 14 &15]. Higher amounts, however, encourage leaf senescence, which results in programmed cell death [16]. It was shown that treating seeds with low amounts of $H_2O_2$ has protective effects against various abiotic stressors in several plant species [17, 18, 19 & 20]. However, to the best of our knowledge, till now no reports are available regarding their role in alleviation of harmful effect of mixed saline-alkaline stress condition in differently tolerant mungbean genotypes.

The current study was undertaken with the notion that seed priming, an external application of signalling molecules, might significantly improve mungbean plant yields and reduce saline-alkaline stress. Hence, under mixed saline-alkaline stress conditions, it was intended to evaluate the impact of exogenously supplied $H_2O_2$ on root and leaf antioxidant enzyme activities, leaf water status and seed production in variously tolerant mungbean genotypes.

## 2. Materials and Methods

### 2.1.*Experimental site, meteorological condition, treatments and design*:

The present investigation was carried out in plantation pot (size 300 × 300 $mm^2$), in pot culture facility of Department of Botany, Plant Physiology & Biochemistry, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, situated at $25^0$ 97’ N latitude & $85^0$ 69’ E longitude at an altitude of 55 m above mean sea level. Biochemical parameters were analyzed in root and leaf tissues in the Abiotic Stress Physiology laboratory of the department. Sampling procedure was followed as per protocols and references mentioned in the subsequent sections. Experiments were conducted in four replications in factorial completely randomized design (CRD). The experiment was started from the third week of June in plantation pots and it lasted up to the end of September. During the course of experiment, the meteorological condition was average with maximum and minimum temperature of $33.9^oC$ and $26.0^oC$ respectively. The mean relative humidity was 77.46% and on an average 180 mm rainfall was received during the experimental period. Evaporation indicates water requirement for crop and its mean value during the experimental period was 4.72 mm.

### 2.2.*Soil type, plant materials, growth conditions and experimental detail*:

The soil used in control pot was clayey in texture having normal electrical conductivity and containing high amount of organic carbon, available phosphorus and potash. The saline-alkaline soil sample was sandy in texture, having high electrical conductivity, low organic carbon, low available phosphorus and high available potash. The soil composition and nutrients content are presented in table 1. The analysis of both types of soil was done in the Department of Soil Science, PGCA, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. The saline-alkali tolerant (TMB-37) and susceptible (MH-1314) mungbean genotypes used in the experiment were identified in preliminary studies. Seeds were surface sterilized with 0.01% $HgCl_2$ solution for 2 minutes, followed by thorough washing with

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DDW (double distilled water). The experiment was undertaken in a completely randomized design in the ambient condition. Thereafter, seeds of both the genotypes (TMB-37 and MH-1314) were soaked in distilled water ($T_0$, $H_2O$) and with 0.01% concentration of hydrogen peroxide ($T_1$, 0.01% $H_2O_2$) for 4 hours. Surface dried seeds were sown in plantation pots (300 × 300 $mm^2$) filled with normal and saline-alkaline soil separately. The standard agronomical practices were performed. Total three plants per pot were maintained till harvesting. After 40 days of seed germination, data was collected for various growth, physiological and biochemical parameters, whereas the yield attributes were recorded at maturity stage.

**Table 1**. Physio-chemical properties of control and saline-alkaline soil

| **Particular** | **Control** | **Saline-alkaline** |
|---|---|---|
| Soil depth | 0-15 cm soil depth | 0-15 cm soil depth |
| **Mechanical determination** | | |
| Sand (%) | 18.09 | 48.09 |
| Silt (%) | 41.71 | 32.71 |
| Clay (%) | 38.53 | 19.20 |
| Texture | Clayey | Sandy |
| **Chemical determination** | | |
| Soil pH | 8.27 | 8.87 |
| Electrical conductivity (ds $m^{-1}$ at 25°C) | 0.25 | 4.30 |
| Electrical conductivity of saturation extract (ds $m^{-1}$) | 1.3 | 7.4 |
| Organic carbon (%) | 1.18 | 0.25 |
| Available nitrogen (kg $ha^{-1}$) | 225 | 95 |
| Available $P_2O_5$ (kg $ha^{-1}$) | 63.98 | 31.58 |
| Available $K_2O$ (kg $ha^{-1}$) | 226 | 305 |
| Zn (ppm) | 0.98 | 0.60 |
| Cu (ppm) | 0.80 | 0.81 |
| Fe (ppm) | 2.72 | 4.49 |
| Mn (ppm) | 3.58 | 2.77 |
| B (ppm) | 0.31 | 0.31 |

### 2.3. ***Determination of leaf chlorophyll content*:**

According to Arnon [21], the amount of photosynthetic pigments (chlorophyll a and chlorophyll b) was measured. After being thoroughly homogenised in 5 ml of an 80% (v/v) acetone solution, fresh leaf tissue weighing 0.1 g was centrifuged at 3000 g for 15 minutes after being maintained at 4°C overnight. For the purpose of estimating Chl. a and b, the optical density of the supernatant was measured at 663, 645, and 480 nm. Equations - and - were used to compute the chlorophyll contents.

$$\mathbf{Chl}\boldsymbol{a} = [(12.25 \times OD_{663}) - (2.79 \times OD_{647})]$$

$$\mathbf{Chl}\boldsymbol{b} = [(21.50 \times OD_{647}) - (5.10 \times OD_{663})]$$

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### 2.4.***Determination of relative water content (RWC)*:**

RWC was calculated using Turner's approach [22]. First, the leaf's fresh weight was determined. The leaf was then left in a dish with distilled water overnight. After recording the turgid weight of the rehydrated leaves, the leaf tissue was dried for three days at 70 °C in a hot air oven. The following equation was used to determine the RWC:

**RWC = (fresh weight - dry weight) / (turgid weight –dry weight) × 100**

### 2.5.***Lipid peroxidation***

According to Behera et al. [23], MDA (malondialdehyde) content measurement created by thiobarbituric acid reacting components was used to measure lipid peroxidation. 100 mg of a frozen leaf sample were homogenised in 5 ml of Tri Chloro acetic acid that contained 0.1%. Centrifuging the homogenate at 10,000 g for 5 minutes at $4^0$C. 1.2 ml of 0.5% thio barbituric acid produced in 20% tri chloro acetic acid was combined with an aliquot of 0.3 ml supernatant, and the mixture was incubated at $95^0$C for 30 minutes. Samples were centrifuged at 10,000 g for 10 minutes at $25^0$ C after the reaction was stopped for 5 minutes in an ice bath. Using a Hitachi U-2000 double-beam UV/Vis spectrophotometer, absorbance was measured at 532 nm (Hitachi, Lake Sherwood, MO, USA). Malondialdehyde (MDA) concentration was calculated by subtracting the nonspecific absorbance at 600 nm. Malondialdehyde (MDA) concentration was determined using an extinction coefficient of 155 mmol$^{-1}$ cm$^{-1}$. Lipid peroxidation was expressed as MDA content in nmol g$^{-1}$ of fresh weight.

### 2.6.***Antioxidant enzyme activity and proline content*:**

For superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.3.6) and peroxidase (POX, EC 1.11.1.7) Frozen tissue was homogenized in 0.1 M tris HCl buffer at pH 7.8 with 5 ml of 4% polyvinyl pyrrolidone per gram of fresh weight, 1 mM dithiothreitol, and 1 mM ethylene diamine tetraacetic acid. Centrifuging the homogenate at 20,000 x g at 40°C. Supernatant was used to measure the activity of the enzymes. Nitro blue tetrazolium's photoreduction at 560 nm was used to measure SOD [24]. In terms of enzyme units per milligramme of protein, one unit of SOD activity is equal to the quantity needed to block the photo reduction of nitro blue tetrazolium by 50%. According to Samantary et al. [25] A reaction mixture including an enzyme aliquot, 50 mM phosphate buffer (pH 7.0), and 10 mM $H_2O_2$ was used to measure the CAT activity. At 240 nm, the breakdown of $H_2O_2$ was examined. By spectrophotometrically evaluating the rate of color development at 436 nm due to guaiacol oxidation, POX activity was determined as the rate of breakdown of $H_2O_2$ by POX, with guaiacol serving as the hydrogen donor according to Lin et al. [26]. The Bradford method was used to calculate how much protein was present in the enzyme extract [27]. The procedure recommended by Bates et al. [28] was used to determine the proline content in mungbean leaf and root samples. L-proline was used as the standard, and the proline content was determined from a standard curve.

### 2.7. ***Estimation of yield*:**

Different yield parameters including pod length, number of pods per cluster, number of pods per plant, number of seeds per pod and yield per plant were measured at the maturity stage.

### 2.8.***Statistical analysis*:**

Statistical Analysis Package, OPSTAT, CCSHAU, Hisar, Haryana, India was used for analysis of variance (ANOVA). For mean separation for significant differences across treatments at $P< 0.05$ significance level, Duncan's multiple range test (DMRT) was used. The results were provided as means with standard errors from three replicates (n=3).

## 3. Results

### 3.1.Chlorophyll content

It was observed saline-alkaline stress significantly declined the chlorophyll *a* and chlorophyll *b* contents in both tolerant as well as susceptible mungbean genotypes with respect to the control condition (Table 2). The hydrogen peroxide treated ($T_1$, 0.01% $H_2O_2$) plants maintained higher chlorophyll *a* as well as chlorophyll *b* contents in both tolerant (TMB-37) and susceptible (MH-1314) mungbean genotypes under normal and salinity stress conditions. The chlorophyll *a* content was 14.22% and 13.24% higher in tolerant and susceptible genotypes respectively under saline-alkaline stress condition, when compared with untreated stress condition. Also, chlorophyll *b* content was 13.24% and 12.26% higher in tolerant and susceptible genotypes respectively under saline-alkaline stress condition, when compared with untreated stress condition.

### 3.2. Relative water content

It was observed that saline-alkaline stress significantly decreased the relative water content of leaf in both tolerant as well as susceptible mungbean genotypes with respect to the control (Table 2). The per cent increase observed in relative water content of tolerant genotype (TMB-37) in normal and saline condition under treatment, T1 (0.01% $H_2O_2$) was 5.38% and 12.52% respectively, whereas in susceptible genotype (MH-1314) the values were 2.62 % and 8.60% respectively, when compared with untreated normal and saline conditions.

### 3.3. Lipid peroxidation

The lipid peroxidation was measured in terms of MDA (malondialdehyde) content (Table 2). Saline-alkaline stress significantly ($P<0.05$) increased the lipid peroxidation in leaf and root in both mungbean genotypes. Greater lipid peroxidation was observed in root as compared to leaf. The percent increase in MDA contents of leaf and root was 18.13% and 22.61% respectively in tolerant genotype, and 38.60% and 47.52% respectively in leaf and root of susceptible genotype under saline stress over the control condition. The treatment with hydrogen peroxide (0.01%) significantly decreased the lipid peroxidation in leaf and root of both tolerant genotypes under control and saline-alkaline stress conditions. Significant decrease was observed in MDA content in hydrogen peroxide treated plant under salinity stress condition in leaf (13.23%) and root (17.21%) of tolerant genotype as well as leaf (11.81%) and root (14.29%) of susceptible genotype as compared with untreated stress.

### 3.4. Antioxidant enzyme activity and proline content

Plants have evolved a well-developed network of antioxidant systems to scavenge reactive oxygen species (ROS) generated during stress conditions. The results of the present study indicate that the activity of antioxidant enzymes such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) increased significantly ($P < 0.05$) in plants grown from $H_2O_2$ primed seed (Table 2). The treatment with hydrogen peroxide (0.01%) significantly increased the superoxide dismutase activity in leaf and root of both mungbean genotypes under control and saline-alkaline stress conditions. The

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percent increase in SOD activity in leaf and root of tolerant genotype in salinity stress condition under treatment $T_1$ was observed to be 30.89% and 89.02% respectively, whereas in leaf and root of susceptible genotype, it was 23.27% and 82.95% respectively as compared with untreated stress condition. The seed priming with signaling molecule ($H_2O_2$) further significantly raised SOD activity and higher increase was observed in root tissues than that of leaf in both the genotypes. Significant increase in the catalase activity in leaf and root of both genotypes under control and saline-alkaline stress conditions was also observed. The significant enhancement in catalase activity in leaf and root of tolerant genotype in saline-alkaline stress condition under treatment $T_1$ was 87.58% and 90.77% respectively, whereas in leaf and root of susceptible genotype, increase was 72.23% and 74.02% respectively as compared to untreated stress condition. The treatment with hydrogen peroxide (0.01%) also significantly increased the peroxidase activity in leaf and root of both genotypes under control and saline-alkaline stress conditions. The increase in peroxidase activity in leaf and root of tolerant genotype in saline-alkaline stress condition under treatment $T_1$ was 33.95% and 32.73% respectively, whereas in leaf and root of susceptible genotype, it was observed as 18.04% and 17.44% respectively when compared with untreated stress condition. Among enzymatic antioxidants activity, the per cent increase (90.77%) in catalase activity was higher in hydrogen peroxide treated root tissues of mungbean plant.

### 3.5. *Proline content*

Saline-alkaline stress significantly ($p < 0.05$) increased the proline content in leaf and root in both tolerant and susceptible mungbean genotypes with respect to the control condition (Table 2). The seed priming with hydrogen peroxide (0.01%) further significantly enhanced the proline content in leaf and root of both mungbean genotypes. The per cent increase in proline content in leaf and root of tolerant genotype in saline-alkaline stress condition under treatment $T_1$ to the tune of 43.02% and 73.96% respectively, whereas in leaf and root of susceptible genotype, it was 31.85% and 64.14% respectively when compared with untreated saline-alkaline stress condition. However, percent increase was higher in root tissues compared to leaf tissues of both genotypes.

**Table 2** Effect of seed priming with $H_2O_2$ on SOD, CAT, POX, proline and lipid peroxidation in leaf and root, RWC, and chlorophyll contents of mungbean (*Vigna radiata* L.) seeding under normal and saline-alkaline stress conditions ($T_0 = H_2O$, $T_1 = 0.01\ \%\ H_2O_2$).

| Genotypes | Soil | Treatments | SOD (Units $mg^{-1}$ protein $min^{-1}$) | | CAT (µmol $H_2O_2$ $mg^{-1}$ protein $min^{-1}$) | | POX (µmol TG $mg^{-1}$ protein $min^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | | | Leaf | Root | Leaf | Root | Leaf | Root |
| TMB – 37 | Control | $T_0$ | $16.7 \pm 0.02^{e}$ | $34.4 \pm 0.02^{g}$ | $12.3 \pm 0.01^{f}$ | $21.9 \pm 0.06^{f}$ | $2.4 \pm 0.02^{e}$ | $2.2 \pm 0.15^{bc}$ |
| | | $T_1$ | $18.0 \pm 0.02^{d}$ | $38.3 \pm 0.02^{e}$ | $13.0 \pm 0.05^{e}$ | $25.0 \pm 0.02^{e}$ | $2.5 \pm 0.01^{d}$ | $2.2 \pm 0.02^{bc}$ |
| | Saline | $T_0$ | $19.2 \pm 0.02^{c}$ | $60.9 \pm 0.02^{c}$ | $19.9 \pm 0.03^{c}$ | $36.1 \pm 0.03^{c}$ | $3.02 \pm 0.01^{b}$ | $2.8 \pm 0.13^{b}$ |
| | | $T_1$ | $25.0 \pm 0.48^{a}$ | $115.2 \pm 0.29^{a}$ | $37.3 \pm 0.04^{a}$ | $68.9 \pm 0.09^{a}$ | $4.0 \pm 0.03^{a}$ | $3.7 \pm 0.18^{a}$ |
| MH -1314 | Control | $T_0$ | $15.4 \pm 0.02^{h}$ | $33.6 \pm 0.01^{h}$ | $10.7 \pm 0.13^{g}$ | $20.3 \pm 0.72^{g}$ | $2.3 \pm 0.72^{e}$ | $2.0 \pm 0.17^{c}$ |
| | | $T_1$ | $15.9 \pm 0.02^{g}$ | $36.0 \pm 0.08^{f}$ | $11.0 \pm 0.02^{g}$ | $22.5 \pm 0.04^{f}$ | $2.3 \pm 0.06^{e}$ | $2.0 \pm 0.22^{c}$ |
| | Saline | $T_0$ | $16.5 \pm 0.02^{f}$ | $57.3 \pm 0.01^{d}$ | $16.2 \pm 0.01^{d}$ | $31.0 \pm 0.01^{d}$ | $2.6 \pm 0.02^{c}$ | $2.3 \pm 0.35^{bc}$ |
| | | $T_1$ | $20.4 \pm 0.18^{b}$ | $104.8 \pm 0.24^{b}$ | $27.9 \pm 0.03^{b}$ | $54.0 \pm 0.06^{b}$ | $3.0 \pm 0.16^{b}$ | $2.7 \pm 0.25^{b}$ |

| Proline (µg $g^{-1}$FW) | | Lipid Peroxidation (nmol TBARS $g^{-1}$FW) | | RWC (%) | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) |
|---|---|---|---|---|---|---|
| Leaf | Root | Leaf | Root | | | |

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|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| 62.6 ± 0.13$^{g}$ | 71.9 ± 0.03$^{g}$ | 34.4 ± 1.72$^{de}$ | 42.8 ± 1.09$^{de}$ | 77.9 ± 0.243$^{cde}$ | 1.03 ± 0.003$^{d}$ | 0.70 ± 0.003$^{abc}$ |
| 69.2 ± 0.03$^{e}$ | 80.9 ± 0.09$^{e}$ | 31.9 ± 1.49$^{e}$ | 39.1 ± 0.22$^{e}$ | 81.4 ± 0.48$^{a}$ | 1.12 ± 0.003$^{a}$ | 0.75 ± 0.12$^{a}$ |
| 80.9 ± 0.07$^{c}$ | 100.5 ± 0.03$^{c}$ | 40.7± 0.78$^{bc}$ | 52.5 ± 1.87$^{c}$ | 68.5 ± 0.09$^{gh}$ | 0.84 ± 0.009$^{i}$ | 0.64±0.01$^{abcde}$ |
| 115.8 ± 0.13$^{a}$ | 174.7 ± 0.21$^{a}$ | 35.3 ± 1.55$^{de}$ | 43.4 ± 1.73$^{de}$ | 77.1 ± 0.05$^{cde}$ | 0.96 ± 0.003$^{f}$ | 0.73 ± 0.02$^{ab}$ |
| 60.8 ± 0.13$^{h}$ | 70.0 ± 0.06$^{h}$ | 36.8 ± 1.41$^{cd}$ | 52.0 ± 1.91$^{c}$ | 75.9 ± 0.77$^{de}$ | 0.98 ± 0.003$^{ef}$ | 52.0 ± 1.91$^{c}$ |
| 64.6 ± 0.13$^{f}$ | 74.9 ± 0.13$^{f}$ | 34.4 ± 0.99$^{de}$ | 48.4 ± 4.76$^{cd}$ | 77.9 ± 0.17$^{c}$ | 1.07 ± 0.003$^{c}$ | 0.65 ± 0.03$^{abcde}$ |
| 76.3 ± 0.17$^{d}$ | 95.7 ± 0.06$^{d}$ | 50.8 ± 0.94$^{a}$ | 76.8 ± 0.65$^{a}$ | 64.5 ± 0.14$^{i}$ | 0.71 ± 0.002$^{m}$ | 0.52 ± 0.03$^{e}$ |
| 100.7 ± 0.26$^{b}$ | 157.2 ± 0.26$^{b}$ | 44.9 ± 1.62$^{b}$ | 65.8 ± 1.71$^{b}$ | 70.0 ± 0.06$^{g}$ | 0.81 ± 0.004$^{j}$ | 0.58 ± 0.02$^{cde}$ |

### 3.6.Yield and yield attributing components

The yield and yield attributing components in both mungbean genotypes were studied (Table 3 & figure 1). The pod length, number of pods per cluster, seeds per pod and seed yield of hydrogen peroxide ($T_1$, 0.01% $H_2O_2$) treated plant were significantly higher in both mungbean genotypes in control as well as saline-alkaline stress condition. The increase in pod length was 10.98% and 9.46% in tolerant and susceptible genotypes respectively in hydrogen peroxide treated plant under saline-alkaline stress condition. The increase in number of pods per cluster was 21.11% and 15.0% in tolerant and susceptible genotypes respectively under saline-alkaline stress condition. The increase in total number of pods per plant was 22.34% and 17.50% tolerant and susceptible genotypes respectively under saline-alkaline stress condition. The increase in total number of seeds per pod was 23.08% and 16.67% in tolerant and susceptible genotypes respectively under saline-alkaline stress condition. The seed priming with hydrogen peroxide ($T_1$, 0.01% $H_2O_2$) enhanced the seed yield in both tolerant and susceptible genotype in control and saline-alkaline stress condition. The percent increase in seed yield was 18.40% and 13.16% in tolerant and susceptible genotypes respectively under saline-alkaline stress condition as compared with untreated stress condition.

**Table 3**: Effect of pre-seed soaking with $H_2O_2$ on pod length, number of pods cluster$^{-1}$, total number of pods plant$^{-1}$ and number of seeds pod$^{-1}$ of mungbean (*Vigna radiata* L.) genotypes under control and salinity stress conditions.

| Genotypes | Soil | Treatment | Pod Length (cm) | Number of pods cluster$^{-1}$ | Total number of pods plant$^{-1}$ | Number of seeds pod$^{-1}$ |
|---|---|---|---|---|---|---|
| TMB – 37 | Control | $T_0$ | 6.5 ± 0.29$^{ab}$ | 6.7 ± 0.33$^{c}$ | 17.0 ± 0.58$^{c}$ | 10.7 ± 0.67$^{ab}$ |
|  |  | $T_1$ | 7.1 ± 0.06$^{a}$ | 7.9 ± 0.15$^{a}$ | 20.7 ± 0.33$^{a}$ | 11.7 ± 0.33$^{a}$ |
|  | Saline | $T_0$ | 5.8 ± 0.37b$^{cd}$ | 6.0 ± 0.33$^{de}$ | 15.7 ± 0.33$^{c}$ | 8.7 ± 0.67$^{cd}$ |
|  |  | $T_1$ | 6.4 ± 0.21$^{ab}$ | 7.3 ± 0.09$^{b}$ | 19.1 ± 0.22$^{b}$ | 10.7 ± 0.33$^{ab}$ |
| MH -1314 | Control | $T_0$ | 5.8 ± 0.44b$^{cd}$ | 5.6 ± 0.03$^{ef}$ | 13.7 ± 0.33$^{d}$ | 9.3 ± 0.67$^{bc}$ |
|  |  | $T_1$ | 6.3 ± 0.33$^{abc}$ | 6.4 ± 0.07$^{cd}$ | 16.0 ± 0.58$^{c}$ | 10.00±0.58$^{abc}$ |
|  | Saline | $T_0$ | 4.9 ± 0.23$^{d}$ | 4.7 ± 0.33$^{g}$ | 12.0 ± 0.58$^{e}$ | 7.0 ± 0.58$^{d}$ |
|  |  | $T_1$ | 5.4 ± 0.21$^{cd}$ | 5.4 ± 0.09$^{f}$ | 14.1 ± 0.67$^{d}$ | 8.2 ± 0.60$^{cd}$ |

Figure 1: Effect of pre-seed soaking with $H_2O_2$ on yield plant$^{-1}$ mungbean (*Vigna radiata* L.) genotypes under control and salinity stress conditions.

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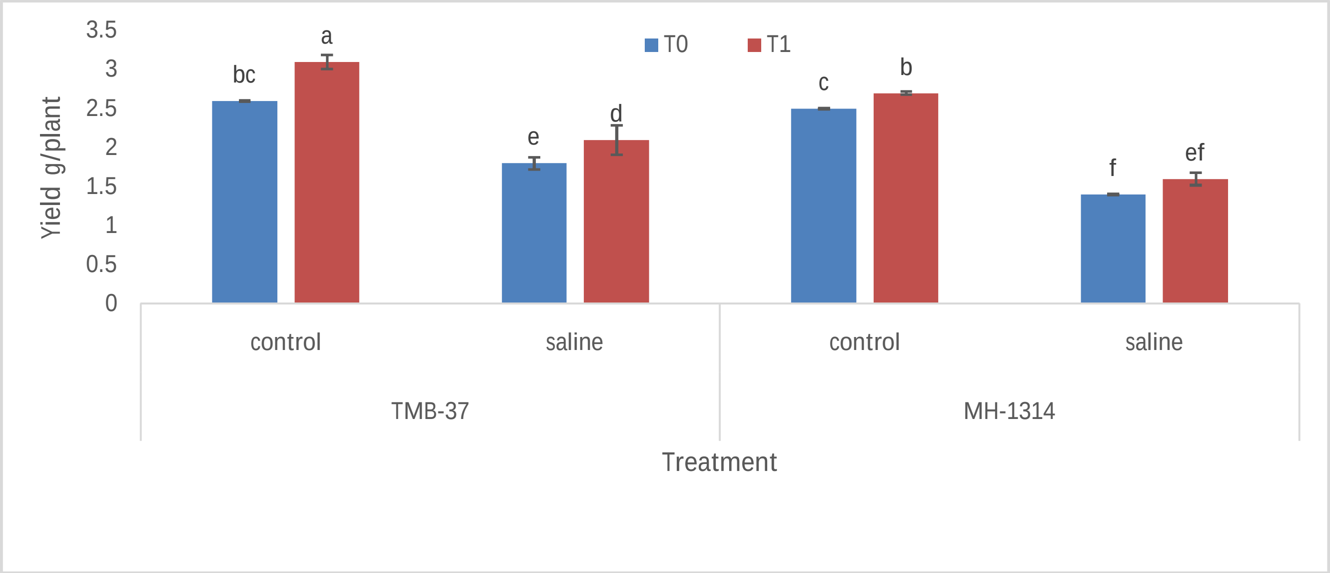

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## 4. Discussion

Saline-alkaline soil has a detrimental impact on plant growth and development because it raises pH levels, causes ion toxicity, oxidative stress, and osmotic stress. [3 & 29]. In addition to ion-toxicity and osmotic stress, the generation of reactive oxygen species (ROS) under alkaline stress in root cells causes high pH to greatly disturb cell pH stability, cell membrane integrity, severely injure root cells, and impair root vitality [30 & 9]. Under stress conditions, the plant cell membrane structure and function are first disrupted [31]. In the present study, membrane lipid peroxidation was estimated in root and leaf in terms of MDA produced, in both mungbean genotypes under saline–alkaline stress condition. Compared to leaf tissues, higher MDA was produced in root tissues of susceptible genotypes (Table 2). It is documented that under alkaline stress condition accumulation of reactive oxygen species in root cells are responsible for severe damage of root cells and decrease root vitality [30]. Present investigations depict that exogenously applied $H_2O_2$ decreased the MDA contents in saline-alkaline stressed plants of both mungbean genotypes and greater percent decrease in MDA content was observed in root compared to leaf tissue of both genotypes. In present study, activity of antioxidant enzyme was also increased significantly which we have discussed in latter section, might be responsible for lower MDA content in treated plant as compared to untreated plant under stress condition. It is also reported by others that the priming with low concentration $H_2O_2$ has the potential to stimulate antioxidative defense mechanism under salinity stress condition [32, 33 & 34]. However, the present result of priming with low concentration of signaling molecule ($H_2O_2$) which was very effective in protecting the root tissue under saline-alkaline stress condition by preventing the root cell damage and membrane lipid peroxidation, has not been reported earlier regarding effectiveness of $H_2O_2$, especially for saline-alkaline stress condition. It functions as a signalling molecule in regulating plant response at low concentrations [35]. A prior study revealed that the putative signal transduction mechanism involved in the exogenous administration of the signalling molecule ($H_2O_2$) efficiently increased plant tolerance and caused physiological changes in stressed plants [36, 34]. Both the tolerant and susceptible genotypes in the current study experienced lower physiological characteristics like RWC during saline-alkaline stress, and exogenous $H_2O_2$ therapy reversed this trend in comparison to saline-alkaline treatment alone (Table 2).These findings supported earlier research that demonstrated the benefits of $H_2O_2$ mediated signals for root development, water absorption capacity, leaf water potential, rate of metabolism, and growth [20 & 38] which demonstrated that exogenous application of $H_2O_2$ increased the leaf RWC in mungbean plant under ideal conditions, provided additional support for our findings. One significant photosynthetic pigment, chlorophyll, is what powers photosynthesis by collecting light energy and transforming it into chemical energy. Plant growth and development are impacted by the depletion of chlorophyll concentration, which lowers the photosynthetic ability [39]. The green pigment contents of mungbean genotypes also decreased significantly, however, $H_2O_2$ treated plant maintained higher chlorophyll contents in both genotypes under saline–alkaline stress. The content of chlorophyll *a* was particularly important in this process because its change was the most significant (Table 2). The chlorophyll degradation in susceptible mungbean was more significant, under the same condition of saline–alkaline stress and the alleviation effect of exogenous $H_2O_2$ on chlorophyll was evident (Table 2). Previous research demonstrated that oxidative stress is one of the stresses brought on by saline-alkaline stress in tomato leaves [40]. Excessive ROS buildup causes oxidative cellular damage, which results in cell death [41]. Under stressful circumstances, low levels of $H_2O_2$ appear to be crucial for the activation of antioxidant enzymes. $H_2O_2$ boosts the activities of SOD, CAT, and POX, as well as the scavenging of ROS and the plant's ability to produce antioxidants [38 & 34]. Exogenous $H_2O_2$ treatment raised SOD, CAT, and POX activities in both root and leaf tissues in the current investigation, with a bigger % increase seen in the root tissues of tolerant genotypes under control and saline-alkaline stress conditions (Table 2). However, till now no studies has been done for saline –alkaline stress condition. This might be one of the reasons of less damage caused to root tissues, which was reflected in terms of increased yield in treated plant compared to untreated stress condition. Seed treatment with signaling molecule ($H_2O_2$) has

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already been reported to increase activities of antioxidants in stressed leaves in wheat crop under saline condition alone however, no study reported the response of plant under saline-alkaline stress condition. In present investigation, saline-alkaline stress significantly reduced the pod size, number of pods per cluster, total number of pods, and number of seeds per pod and grain yield of both mungbean genotypes (Table 3 & figure 1). However, exogenously applied low dose $H_2O_2$ (0.01%) as seed priming was found effective to improve yield as well as yield attributes under control and saline-alkaline stress conditions of both mungbean genotypes. It is also reported in salt-stressed wheat plants that $H_2O_2$ given exogenously as seed priming proved effective in reducing the detrimental effects of salinity on plant biomass output [42]. The exogenously given $H_2O_2$ and SNP were shown to be helpful in boosting the development and yield of salt-stressed wheat genotypes whether treated separately or in combination as seed priming [34]. Salinity hinders a variety of physiological and biochemical processes, such as photosynthesis, antioxidant status, water relations, and ionic equilibrium, which all affect plant development [43]. It was said that seed priming with modest concentrations of $H_2O_2$ might lessen the negative effects of high levels of NaCl salinity on plants growth by lowering salt-mediated membrane damage, which has been connected to $H_2O_2$'s ability to trigger oxidative defence mechanisms [32 & 33]. Yet our study was also the first to reveal an increase in mungbean production and yield characteristics under saline-alkaline stress conditions.

## 5. Conclusion

In conclusion, exogenously applied $H_2O_2$ significantly increased the enzymatic activities of antioxidant enzymes (CAT, POX and SOD) in root as well as leaf tissues of both genotypes of mungbean under saline-alkaline stress condition. However, maximum increase was observed in root tissues. The 0.01% $H_2O_2$ also significantly improved the chlorophyll contents and maintained better cellular water relations through cellular osmotic adjustment by accumulation of proline. The low concentration of 0.01% $H_2O_2$ was very effective in alleviation of harmful effect of saline-alkaline stress which was reflected in terms of higher yield in treated plant, especially in saline-alkaline stress condition.

**Declarations:**

Ethics approval: No ethical issues were violated in this study.

Consent to participate: All authors agree to participate.

Consent for publication: All authors agree for publication.

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