



Magnetopriming regulates antioxidant defense system in soybean against salt stress

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ABSTRACT

The field experiment was conducted to study the influence of magnetopriming with static magnetic field (SMF) of 200 mT for 1 h) on growth, nitrogen fixation, photosynthesis, antioxidative system and yield of soybean under salt stress. The results revealed the adverse effect of salinity on growth, photosynthesis, nitrogenase activity and yield. Salt stress significantly elevated the level of hydrogen peroxide (H₂O₂) and ascorbic acid (ASA) and the activities of superoxide dismutase (SOD), ascorbic acid peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (POD) in leaves of soybean seedlings emerged from unprimed seeds. However, α-tocopherol content was reduced with the increase concentration of NaCl but it was enhanced in leaves emerged from magnetoprimed seeds. On the other hand leaf area, specific leaf weight, photosynthesis, nitrogenase activity and ASA/DHA ratio were significantly increased whereas H₂O₂, ASA and antioxidant enzymes were reduced significantly by SMF pre-treatment; which ultimately improved the biomass accumulation, yield and harvest index of soybean under both the saline and non saline conditions. These results indicated that SMF pre-treatment compensated for the negative effects of salinity stress, consequently soybean plants do not have to deflect their metabolic energy in detoxification of ROS produced under salt stress. Thus application of magneto-priming could scavenge or alleviates the harmful effects of salinity stress at the field performance of soybean plants and it can be used in agriculture to better growth and increased yield under adverse abiotic stress conditions.

1. Introduction

Salinity is one of the major factors limiting the agricultural crop production. High salt imposes negative impacts on growth, nodulation, agronomy traits, seed quality and quantity, and thus reduce the yield of soybean (Kataria and Verma, 2018). Soybean (*Glycine max* L. Merr.) is the most important legume crop in the world (Ferguson and Gresshoff, 2009), having high-quality protein (about 40% of seed) and oil (about 20% of seed). Salt-induced osmotic stress as well as sodium toxicity trigger the formation of ROS such as superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂), and hydroxyl radical (•OH), which can damage mitochondria and chloroplasts by disturbing cellular constitution (Mittler, 2002; Banu et al., 2009, 2010). ROS can act as signaling molecules, intervene many key physiological processes. Excess production of ROS is toxic to plants and causes oxidative damage to cellular constituents, leading to cell death (Noctor and Foyer, 1998; Banu et al., 2009, 2010). Plants have enzymatic and non-enzymatic antioxidant defense systems to defend cells against the harmful effects of ROS. The major ROS-

scavenging antioxidant enzymes are SOD, APX, GR and POD. Several effects have been studied on the components of antioxidant defense systems in plants induced by salt stress (Mittova et al., 2003; Demiral and Türkan, 2005; El-Shabrawi et al., 2010; Hasanuzzaman et al., 2014).

It is well-known that salt inhibits soybean seed germination and post-germinative growth, which eventually leads to decrease in yield (Hamayun et al., 2015; Baghel et al., 2016; Kataria et al., 2017a). It has also been reported that legumes, especially their symbiotic performance are inhibited by abiotic stresses such as UV-B, drought and salinity (Kataria et al., 2017b). The salt stress inhibits the shoot and root biomass, nodule formation, number of pods, and yield of soybean (Hamayun et al., 2010). Navarro et al. (2011) found that the biological fixation of atmospheric N₂ by the symbiotic association between the plant and soil bacteria belongs usually to the genus *Bradyrhizobium* and *Sinorhizobium* that are jointly called “soybean rhizobia”. Rhizobia contaminate the legumes roots and stimulate the formation of nodules, where nitrogen fixation takes place (Beattie and Handelsman, 1989).

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Soybean production has global importance; however its development is highly suffered from the problem of salinity stress. So it is necessary to identify the mitigation strategies to protect the soybean plants from the adverse effects of salinity. Among the modern agronomic practices, magneto-priming is one of the important eco-friendly techniques to improve the seed vigour, growth, and yield of plants under abiotic stresses like salinity, drought and UV-B (Kataria et al., 2017a,b; Baghel et al., 2018; Hozayn et al., 2018). Previously, we have reported that SMF pre-treatment (200 mT for 1 h) to the soybean seeds ameliorate the adverse effect of salt stress and it enhanced the percentage germination, biomass accumulation and photosynthetic performance of soybean plants (Baghel et al., 2016; Kataria et al., 2017a,b). However, the mechanism behind this amelioration of salt stress by magnetopriming has not been identified yet. The stimulation in percentage germination and seedling growth due to magnetopriming by SMF has been found to be mediated through the increase in water uptake and production of ROS in the germinating soybean and maize seeds under salt stress (Kataria et al., 2017a,b). There are reports on positive effect of magnetopriming on growth, efficiency of PS II, photosynthesis and yield on the crops like wheat, barley, maize, soybean, and mungbean under abiotic stresses like salt, drought, cadmium and UV-B stress (Anand et al., 2012; Chen et al., 2011; Kataria et al., 2017a,b; Baghel et al., 2016, 2018; Hozayn et al., 2018). To best of our knowledge this is the first report showing the effect of static magnetic field (200mT for 1 h) on role of ROS and antioxidant defense system in magnetoprimed induced growth, photosynthesis, nitrogenase activity, pod yield and harvest index of soybean plants under salt stress. The aim of the present study is to identify the role of ROS and the activation or deactivation of antioxidant defense systems that might be involved in ameliorating the effects of magnetopriming of soybean seeds grown under salinity stress in field conditions. A dose–response study on soybean seedling showed that SMF of 200 mT for 1 h was proven best dose for improvement in early seedling characteristics and growth in soybean (Shine et al., 2011; Fatima et al., 2017). Hence in the present study SMF of 200 mT for 1 h pre-treatment chosen as suitable SMF strength for mitigation of adverse effect of salt stress in soybean.

2. Materials and methods

2.1. Plant material

Seeds of soybean (*Glycine max* L. Merrill) var. JS-335 were collected from Directorate of Soybean Research, Indore (M.P.), India.

2.2. Magnetic field generation and magnetic field treatment

For treating seeds with SMF, an electromagnetic field generator "Testron EM-20" (Testron Instrument, Delhi, India) with variable magnetic field strength (50–500 mT) with a gap of 5 cm between pole pieces was used. The pole pieces of the electromagnet were cylindrical in shape with 9 cm diameter and 16 cm length. The total number of turns of copper coil per pole piece was 3000 and resistance of the coil was 16 Ohm. For the electromagnet a DC power supply (0–85 V/10 A) with constantly variable output current was used.

Previously, it has been reported that pre-treatment of SMF (200 mT for 1 h) to seeds stimulated the seed germination, seedling vigor, growth and photosynthesis of soybean as compared unprimed ones (Shine et al., 2011; Fatima et al., 2017); therefore in the present study we exposed soybean seeds to SMF of 200 mT (1 h). Soybean seeds were exposed to SMF of 200 mT in a cylindrical-shaped sample holder of holder of 42 cm³ capacity, made from a non-magnetic thin transparent plastic sheet. One hundred visibly sound and healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field and treated for 1 h and the room temperature was maintained at 25 °C during the treatment. By regulating the current in the coils of the electromagnet, the required strength of

the magnetic field was obtained. Between the poles the strength of the magnetic field was measured by a gauss meter. The local geomagnetic field was < 10 mT. SMF treatments in the experiments were run simultaneously along with unprimed controls.

2.3. Experimental details

The experiments were conducted on the terrace of Department of Biochemistry, Devi Ahilya University, Indore (Latitude- 22°43'N) India during October 2017 to January 2018. The SMF treated (magneto-primed) and untreated (unprimed) seeds of soybean var. JS-335 were treated with fungicides viz Bevistin and Diathane M at 2 gm/kg seeds and then before sowing, these seeds were inoculated with powder of Rhizobium culture (National Fertilizer limited, New-Delhi, India) 3 g/kg seeds. The seeds of identical size and shape were sown in plastic nursery bags (34 × 34 cm) filled with a combination of thoroughly sifted soil, sand and cow-dung manure in the proportion of 2:2:1 by volume. These plastic nursery bags were treated with saline solutions with varying electrical conductivity that was 0, 4, 6,8,10 dSm⁻¹ (equivalent to 0, 25, 50, 75 and 100 mM NaCl). In order to attain the required salinity level in the soil of nursery bags was added with these salt solutions on three consecutive days before sowing. Each bag received three doses of 500 ml of different concentrations of 0, 25, 50, 75 and 100 mM NaCl solution. The controls of magnetoprimed and unprimed (0 mM NaCl) were irrigated with tap water only. Three plants of uniform size were maintained in each bag. Samples were arranged in completely randomized designs with three replications. All the data are presented in triplicates (3n); five plants from each replica were taken for the recording of all the parameters studied. We have used 2 bags for per replica and total of 6 bags were kept for each treatment for analysis. Plants were sampled and analyzed for the growth indices such as leaf area and specific leaf weight (SLW) at 45 days after emergence of seedlings (DAE). Area of third trifoliolate leaves was measured using portable laser leaf area meter CID-202 scanning planimeter (CID Inc., USA). SLW was calculated from leaf biomass data using formula given by Hunt (1982). For leaf dry weight, third trifoliolate leaves and for total biomass accumulation, whole plant parts were oven-dried at 105 °C for 24 h to obtain constant weight on an analytical balance. The rate of photosynthesis (*P_n*) were recorded in third trifoliolate leaves of soybean at 45 DAE by using a portable Infra Red Gas Analyser (LI-6200, LICOR Inc., Lincoln, USA). The measurements were taken on sunny days at 11:00 to 12:00 a.m. at ambient temperature 30 to 35 °C temperature, 1200–1600 mM m⁻² s⁻¹ PAR at the leaf surface at the time of measurement. The number of pods per plant, total biomass accumulation, seed yield and total biological yield were measured at harvested maturity on 120 DAE. Similarly, 6 bags with 3 plants each were separately kept for harvest yield data. For seed yield the weight of seeds per plants and for biological yield the weight of the above ground parts per plant were measured. Harvest index was calculated by the following formula: HI = Seed yield/Biological yield (Kataria and Guruprasad, 2012).

2.4. Antioxidant defense system

Non-enzymatic-antioxidants and antioxidative enzymes were measured in third trifoliolate leaves at 45 DAE. All the enzymes were extracted from fresh leaves and assays performed at 4 °C.

2.5. Antioxidants

2.5.1. Estimation of ascorbic acid

Ascorbate (reduced form of ascorbate) and dehydroascorbate (oxidized form of ascorbate) were estimated based on the reduction of ferric to ferrous ions with ascorbic acid in acid solution followed by the formation of a red-chelate between ferrous ion and 2,2'-bipyridyl (Arakawa et al., 1981). 200 mg of leaf tissue was homogenized with 2 ml ice cold 5% TCA containing 4% PVP-40 (w/v). The homogenate

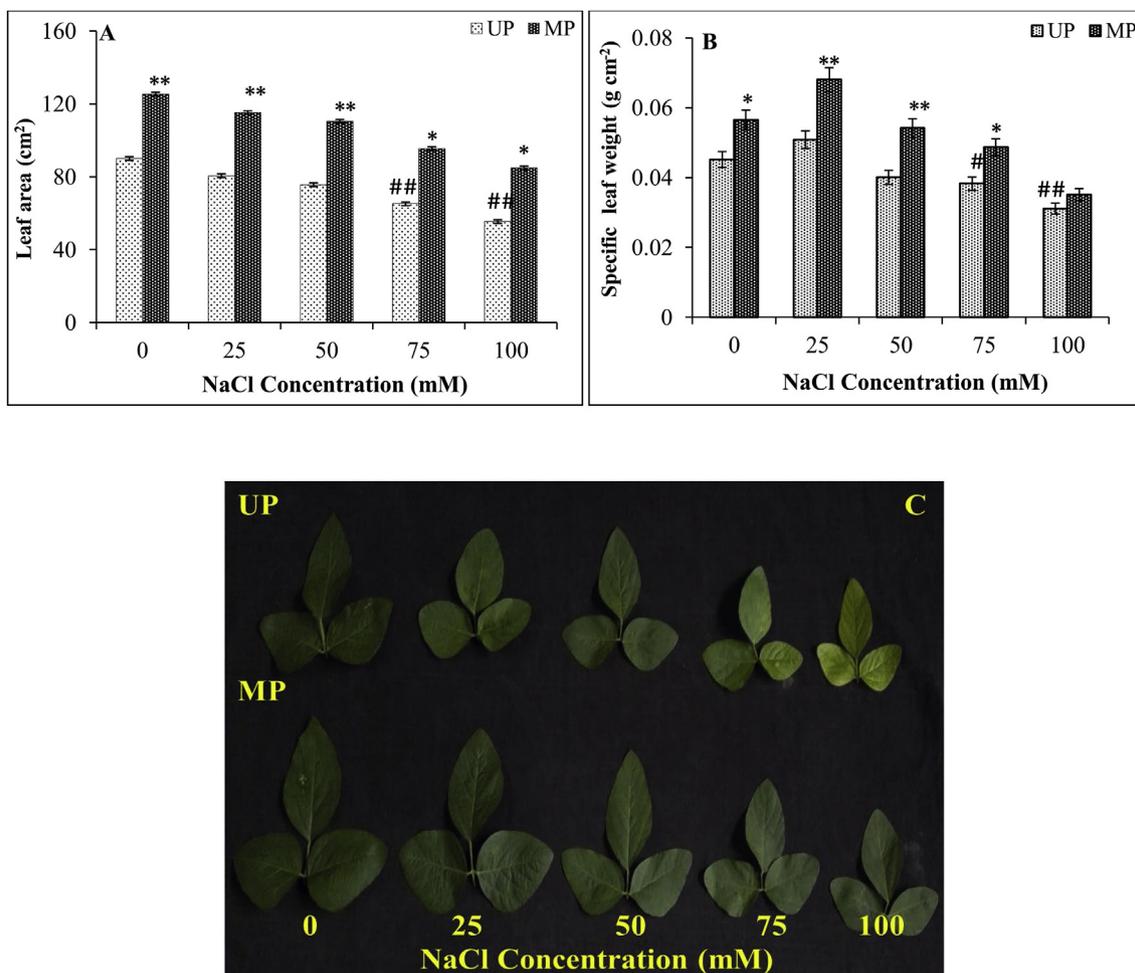


Fig. 1. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on (A) leaf area and (B) specific leaf weight of third trifoliolate leaves and (C) photograph showing leaf area of third trifoliolate leaves with different concentrations of NaCl at 45DAE. The vertical bar indicates \pm SE for mean. ## $p < 0.01$; # $p < 0.05$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds; ** $p < 0.01$; * $p < 0.05$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

was filtered through four layers of muslin followed by centrifugation at $14,000 \times g$ for 10 min at 4 °C. The supernatant was used for total ASA assays. Total ASA was determined as the reduction of dehydroascorbic acid (DHA) to ASA by dithiothreitol.

2.5.2. Estimation of α -Tocopherol

500 mg of leaf tissue was homogenized in 25 ml of absolute alcohol, 0.5 ml 10% alcoholic pyrogallol and 2–3 boiling chips. Solution was transferred to a conical flask, refluxed for 5 min, 2.5 ml saturated aq-KOH was added through the condenser. The solution was again refluxed for another 5 min. The sample was cooled in an ice bath and 25 ml of cold water added along with 25 ml of petroleum ether. Solution was then transferred to 250 ml separating funnel. The lower aqueous phase was decanted for re-extraction with 25 ml of petroleum ether. The ether fraction was collected. Solution was washed 3–4 times with distilled water containing alcoholic pyrogallol. Petroleum ether was evaporated and the remaining matter was redissolved in a little benzene (0.2 ml) volume was made up to 10 ml with absolute alcohol (Walker and Slinger, 1975).

To 1 ml of the above-mentioned solution, 0.2% alcoholic FeCl₃ and 1 ml 0.5% alcoholic α, α -dipyridyl test solution were added. Volume was made up to 5 ml with absolute alcohol. After 10 min the absorbance was read at 520 nm. The amount of tocopherol present was calculated from the standard curve prepared with 0.12–1.25 mg tocopherol/ml (Pearson et al., 1970).

2.6. Antioxidative enzymes

Superoxide dismutase (SOD) activity was measured according to the method of Beauchamp and Fridovich (1971) by measuring ability of the enzyme extract to inhibit the photochemical reduction of NBT. One unit of SOD was defined as the enzyme activity (per mg protein), which inhibited the photo reduction of NBT to blue formazan, by 50%. Ascorbate peroxidase (APX) activity was measured by the method of Nakano and Asada (1981). The activity was calculated as μM ascorbic acid oxidized/min/mg protein. Glutathione reductase (GR) activity was determined at 25 °C by following the method of Rao et al. (1996). Enzyme activity was calculated using extinction coefficient ($6.2 \text{ mM}^{-1} \text{ cm}^{-1}$). The GR activity was expressed as μM NADPH oxidized/min/mg protein. Peroxidase (POD) was assayed by the method of Maehly (1955). Activity was calculated as change in OD/min/mg protein.

2.7. Nodules fresh weight and nitrogenase activity in root nodules

Nodules were taken out from the harvested soybean plants at 45 DAE. Then roots were washed and dried on filter paper and weight was recorded in gm/plant for all the treatments. Nitrogenase (EC 1.18.6.1) activity was determined in roots of soybean with nodules by the acetylene reduction assay (ARA) (Hardy et al., 1973). Three roots with intact nodules were excised and incubated in a 25-ml incubation vessel sealed with a flanged rubber septum. 5 ml of the air was withdrawn

from the incubation vessel (via the rubber septum in the cap with a syringe) and replaced with an equal volume of acetylene (C_2H_2). After 60 min incubation at 200 °C, 2 ml of gas sample was withdrawn and analyzed by gas chromatography (DANI make, DPC-100, Italy) for ethylene formation. The incubation vessel was filled with water to measure the volume occupied by the root (air space). Nodulated roots were removed from the incubation vessel, fresh weight of root with the nodules were taken after blotting. Standard curve for ethylene was developed for analysis. Nitrogenase activity was calculated for the linear phase of reaction and expressed as Nitrogenase activity = n mole C_2H_4 produced/g fresh weight of root nodule/h.

2.8. Statistical analysis

All the data are presented in triplicates ($n = 3$); five plants from each replica were taken for the recording of all the parameters studied. The data are expressed as means \pm SE and analyzed by the Student *t*-test using Microsoft excel tool. $###p < 0.001$; $##p < 0.01$; $#p < 0.05$ indicate the significant difference between non-saline and saline condition in soybean plants emerged from unprimed seeds; $***p < 0.001$; $**p < 0.01$; $*p < 0.05$ indicate significant difference between SMF primed (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions.

3. Results

The area and specific leaf weight of third trifoliolate of soybean were significantly reduced with increasing level of salinity from 0 to 100 mM NaCl in soybean plants emerged from unprimed seeds at 45 DAE (Fig. 1 A, B, C). Fig. 1 A shows the dramatic change in leaf size of soybean plants raised from magnetoprimed and unprimed seeds under saline and non-saline conditions. High salinity levels induced by the supply of 50, 75 and 100 mM NaCl resulted in significant reduction of leaf area by 16, 28 and 38% and a reduction of specific leaf weight by 12, 15 and 31%, respectively in unprimed soybean seedlings (Fig. 1A and B). On the other hand, SMF pre-treatment of soybean seeds significantly enhanced both the leaf area and specific leaf weight as compared to their unprimed ones in saline as well as non-saline conditions. Magnetoprimed seedling showed maximum promotion of 53% at 100 mM NaCl in leaf area and 35% at 50 mM NaCl in specific leaf weight of third trifoliolate leaves of soybean as compared to their unprimed seedlings (Fig. 1A and B).

In the present study, salt stress has been found to cause oxidative stress by the production of ROS in the leaves of soybean plants from unprimed seeds. Supply of increasing concentration of NaCl increased the level of H_2O_2 in the leaves of soybean plants emerged from unprimed seeds (Fig. 2). However, in the plants that emerged from magnetoprimed seeds, H_2O_2 content was significantly decreased by 46%, 39%, 44%, 48% and 40% at 0, 25, 50, 75 and 100 mM salinity respectively as compared to their unprimed seeds (Fig. 2).

Antioxidants such as ASA and α -Tocopherol content were determined to examine the effect of magnetopriming on soybean under salinity. As the concentration of NaCl increased, the level of total ASA were increased but the level of DHA decreased in leaves of unprimed plants (Fig. 3A and B). However, SMF treatment reduced the level of total ASA and DHA as compared to their unprimed ones in saline as well as non saline conditions. Therefore, the ratio of ASA/DHA increased after pre-treatment with SMF in both saline and non saline conditions as compared to their unprimed ones (Fig. 3A,B,C).

The α -tocopherol content reduced in leaves of plants from unprimed seeds with increasing concentrations of NaCl but it was significantly enhanced by SMF treatment under both saline and non-saline conditions. The increase in the level of α -tocopherol was 36%, 20%, 74%, 72% and 38% observed at 0, 25, 50, 75 and 100 mM NaCl respectively in the plants which were emerged from magnetoprimed seeds as compared to the plants emerged from unprimed ones (Fig. 4).

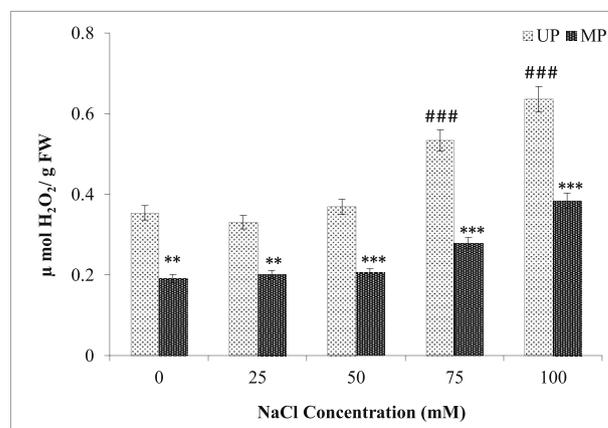


Fig. 2. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on H_2O_2 content with different concentrations of NaCl at 45DAE. The vertical bar indicates \pm SE for mean. $###p < 0.001$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds; $***p < 0.001$; $**p < 0.01$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

To investigate whether magnetopriming alleviates the adverse effect of soil salinity in soybean seedlings, the role of antioxidant defence system in soybean was analyzed. The activities of major ROS-scavenging antioxidant enzymes, including SOD, APX, GR, and POD were determined. The activity of antioxidant enzymes i.e. SOD, APX, GR, and POD were found to increase with increase in NaCl concentrations from 0 to 100 mM in the leaves of plants emerged from unprimed seeds (Fig. 5A and B,C,D). On the other hand activity of all of these antioxidant enzymes were decreased in the plants that emerged from magnetoprimed seeds under saline as well as non-saline conditions as compared to their unprimed seeds (Fig. 5A and B,C,D).

Number of root nodules (data not given) and fresh weight of root nodules were also increased in plant that emerged from SMF treated seeds under saline and non saline condition (Fig. 6A). To indicate the potency of root nodules nitrogenase activity was measured in the present study in seedling emerged from unprimed and magnetoprimed seeds under salt stress. Our results showed that the nitrogenase activity decreased as the salinity increases in root nodules of seedling emerged from unprimed seeds (Fig. 6B). SMF treatment enormously enhanced the activity of nitrogenase enzymes in root nodules of soybean by 161%, 157%, 108%, 92% and 85% at 0, 25, 50, 75 and 100 mM salinity as compared to their unprimed seeds (Fig. 6B).

The results revealed that as the concentration of NaCl increases the total biomass accumulation, rate of photosynthesis, crop yield in terms of number of pods/plant and harvest index were decreased in plants emerged from unprimed seeds (Fig. 7A and B,C,D). The maximum reduction of 54% was noted in rate of photosynthesis at 100 mM NaCl in the plants emerged from untreated seeds as compared to plants grown in non-saline conditions (0 mM NaCl). The plants emerged from SMF primed seeds showed increase in total biomass accumulation and *Pn*; they have large number of pods per plant and higher harvest index as compared to unprimed seeds under both saline and non-saline conditions (Fig. 7A,B,C,D). An increase of 22.5% in *Pn* was recorded in the plants that emerged from SMF treated seeds as compared to unprimed ones in non saline conditions (0mM NaCl) and SMF treatment caused 46%, 76%, 95% and 133% increment in *Pn* at 25, 50, 75 and 100 mM NaCl (Fig. 7B).

The harvest index was increased by 15%, 29%, 43%, 53% and 34% respectively at 0, 25, 50, 75 and 100 mM salinity via SMF treatment as compared to their unprimed ones (Fig. 7 D).

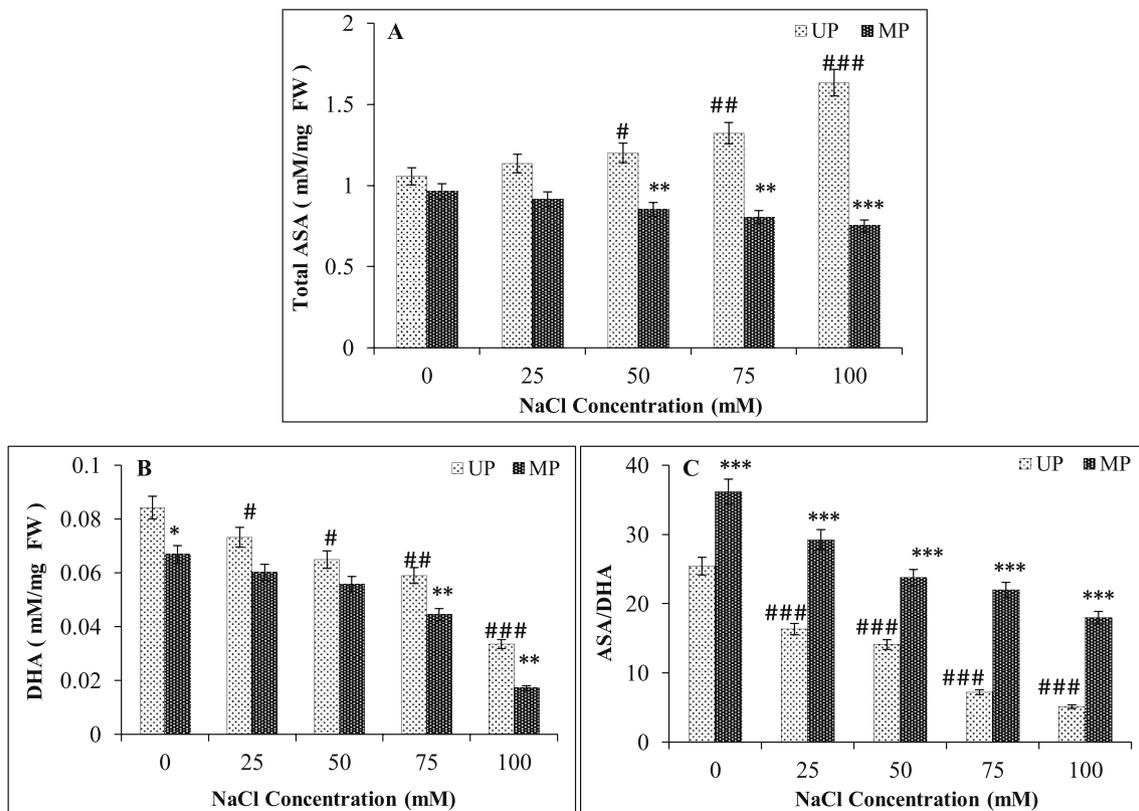


Fig. 3. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on (A) Total ASA, (B) DHA and (C) ASA/DHA with different concentrations of NaCl at 45 DAE. The vertical bar indicates \pm SE for mean. ### $p < 0.001$; ## $p < 0.01$; # $p < 0.05$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds; **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.05$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

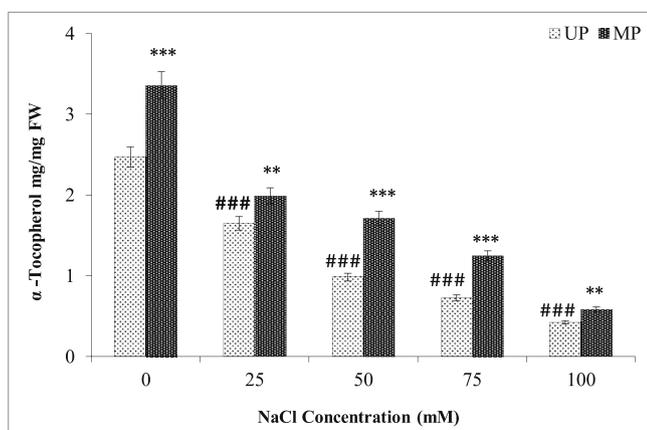


Fig. 4. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on α -tocopherol with different concentrations of NaCl at 45 DAE. The vertical bar indicates \pm SE for mean. ### $p < 0.001$ indicate significant differences between non-saline and saline condition in plants emerged from unprimed seeds; **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.05$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

4. Discussion

Salt stress is an important abiotic stress that crucially affects the growth, nitrogen fixation and productivity of soybean (Amirjani, 2010; Kataria and Verma, 2018). Leaf area signifies the measure of plant growth, which can be severely affected by salt stress. The results of present study revealed that salt stress reduced the leaf area, specific leaf weight, nitrogenase activity and seed yield of soybean plants emerged

from unprimed seeds. Previously, it has been found that all the developmental stages of soybean are adversely affected by salinity stress and it is considered as a salt-sensitive glycophyte (Phang et al., 2008). Wahid et al. (2014) found that the leaf dry weight was affected more than leaf area, resulting in reduced SLW in sugarcane by salt stress. Salt stress significantly reduces seed germination (Kataria et al., 2017a), plant height and leaf area (Baghel et al., 2016), decreases the number of pods and seeds (Phang et al., 2008; Baghel et al., 2016) during soybean development. However, in the present study magnetopriming has been found to improve the leaf growth, specific leaf weight, nitrogenase activity, and seed yield under salt stress. These results are in agreement with earlier report of Baghel et al. (2016). These authors measured only growth, photosynthetic efficiency, photosynthesis and yield, however in the present study we have evaluated the effect of SMF pre-treatment on oxidative stress caused by salt stress along with growth, antioxidant defense mechanism, nitrogen fixation and harvest yield in the soybean plants under salt stress.

Salinity is generally account to enhance production of ROS particularly H_2O_2 (Ozgun et al., 2013), which can harm major cell components such as proteins, lipids and nucleic acids (Apel and Hirt, 2004; Gill and Tuteja, 2010). In response to increasing salinity, we have also observed an increase in H_2O_2 content (a common ROS) in the leaves of soybean plants emerged from unprimed seedlings, similar report has been reported in several plant species (Jithesh et al., 2006; Ozgun et al., 2013). To prevent oxidative damage caused by ROS under salt stress plants employ several enzymatic and non-enzymatic antioxidants (Jithesh et al., 2006; Ozgun et al., 2013). The low-molecular-weight antioxidant substance such as ASA is involved in ROS detoxification in plants (Hameed and Khan, 2011). In the present study, total ASA contents increased under saline conditions in soybean leaves of plants from magnetoprimed seeds; which may be due to its increased biosynthesis.

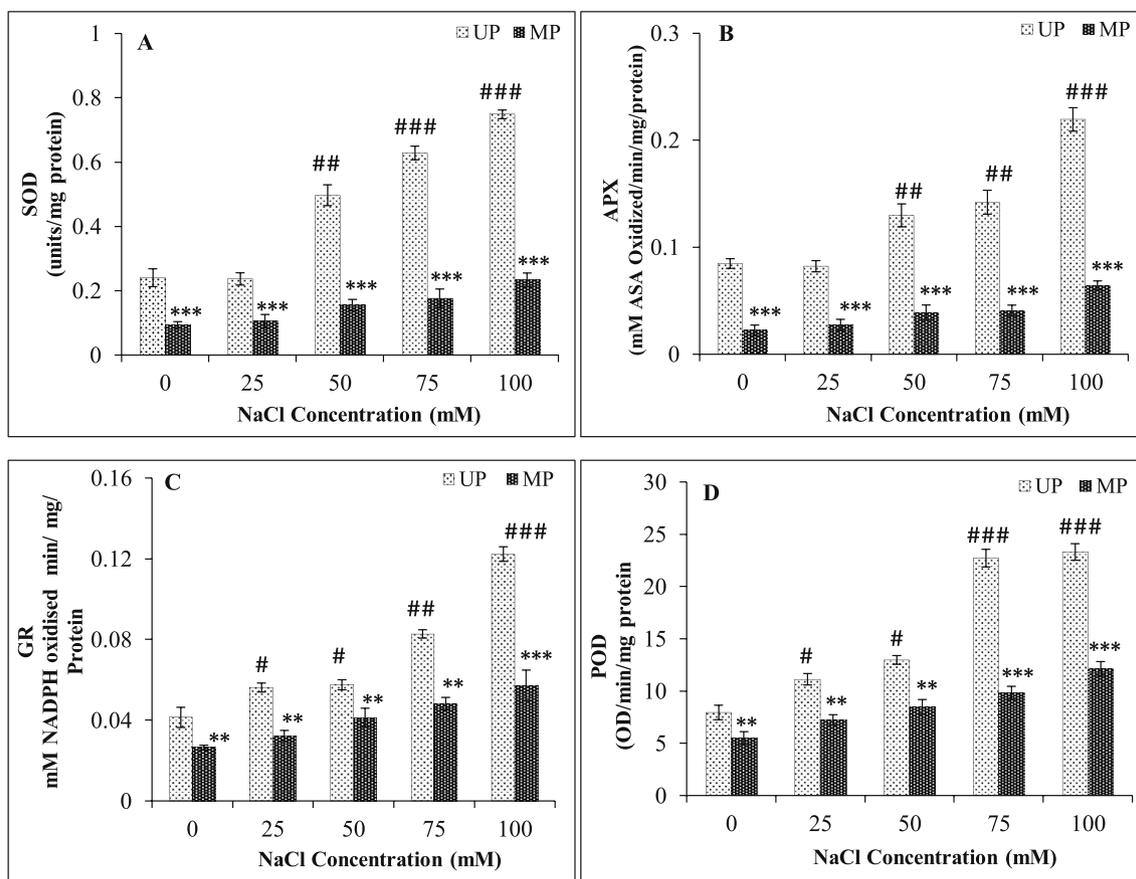


Fig. 5. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on (A) SOD, (B) APX, (C) GR and (D) POD with different concentrations of NaCl at 45 DAE. The vertical bar indicates \pm SE for mean. $^{\#}$ $p < 0.01$; $^{\#}$ $p < 0.05$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds and *** $p < 0.001$; ** $p < 0.01$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student t-test.

ASA plays a major role in salt tolerance in many plants species (Hameed et al., 2012; Ozgur et al., 2013). It decreases the ROS directly as well as by the Asada–Halliwell–Foyer pathway (Noctor and Foyer, 1998; Gest et al., 2012). It also recovers the lipid soluble antioxidant α -tocopherol (Lushchak and Semchuk, 2012). The results of our study showed that 100 mM NaCl severely affects the total ASA level, increasing the oxidation of ASA to DHA and this resulted in decreased ASA/DHA ratio in leaves of plants emerged from unprimed seeds (Fig. 3 C). Magnetopriming increased the ratio in the presence and absence of NaCl and indicated that SMF treatment effectively alleviated the effect of NaCl by increasing the ASA/DHA ratios (Fig. 3 C).

Our data also showed a significant decrease in the antioxidant (ASA) and antioxidant enzymes (SOD, APX, GR and POD) activities while increase in ASA/DHA ratio and α -tocopherol content in the leaves of the plants that were emerged from magnetoprimed seeds in saline as well as non-saline conditions. It is generally assumed that increase in α -tocopherol contribute in the plant stress tolerance, while decreased levels favour oxidative damage (Munne-Bosch, 2005). Similarly, in our study leaves of magnetoprimed seedlings show higher amount of α -tocopherol in saline conditions; which may be due to its role in plant stress tolerance.

In the present study, in addition to decrease amount of α -tocopherol, increased contents of ASA and increased SOD, APX, GR and POD activity in the leaves of plants emerged from unprimed seeds under salinity; played the major roles in detoxification process of ROS (Fig. 5). Increasing activities of antioxidant enzymes and improving antioxidant metabolism in plants was one of the most important ways to enhance salt tolerance of plants (Mao et al., 2004). The similar increase in the activity of antioxidant enzymes in the present study was

also observed and indicating their role in ROS detoxification under salinity stress. Under abiotic stress, SOD is the first line of defence against ROS (Alscher et al., 2002). Under saline conditions increased activity of SOD has been shown in plants (Parida et al., 2004). Several studies showed that maintaining a high level of antioxidant enzymes will help a plant to protect itself against oxidative damage caused by rapidly scavenging toxic levels of ROS in its cells and restoring the redox homeostasis. Salt stress preferentially enhanced the content of H_2O_2 and the activities of SOD, APX, and GPX in leaves of rice plant (Lee et al., 2001).

Root growth has also been positively affected by SMF pre-sowing treatment. Root length, biomass and root surface area has been demonstrated to be significantly increased in seedlings exposed to SMF strength from 0 to 250 mT (Vashisth and Nagarajan, 2010; Shine et al., 2011). Nitrogen (N) metabolism is also affected by salinity, possibly by decreasing the activity of enzymes involved in N metabolism (Mansour et al., 2002; Santos et al., 2002). Salt-tolerant genotypes of chickpea have better nodulation and symbiotic N_2 fixation capacity as compared to the sensitive genotypes (Rao et al., 2002). These studies suggested that salt-induced decline in growth and yield of legume crops like soybean is to some extent because of decrease in activity of N_2 fixation (Van Hoorn et al., 2001). Under saline conditions, addition of genistein (a nod gene inducer) enhanced the soybean nodulation and growth (Miransari and Smith, 2007, 2009). Similarly, biochemical analysis of nodules in the present study has revealed plants from magnetoprimed seeds showed increased nitrogen fixation by the enhancement in the nitrogenase activity in the root nodule of soybean in non-saline and saline conditions. The decrease in nitrogenase activity was observed due to increasing salinity in the root nodules of seedling from unprimed

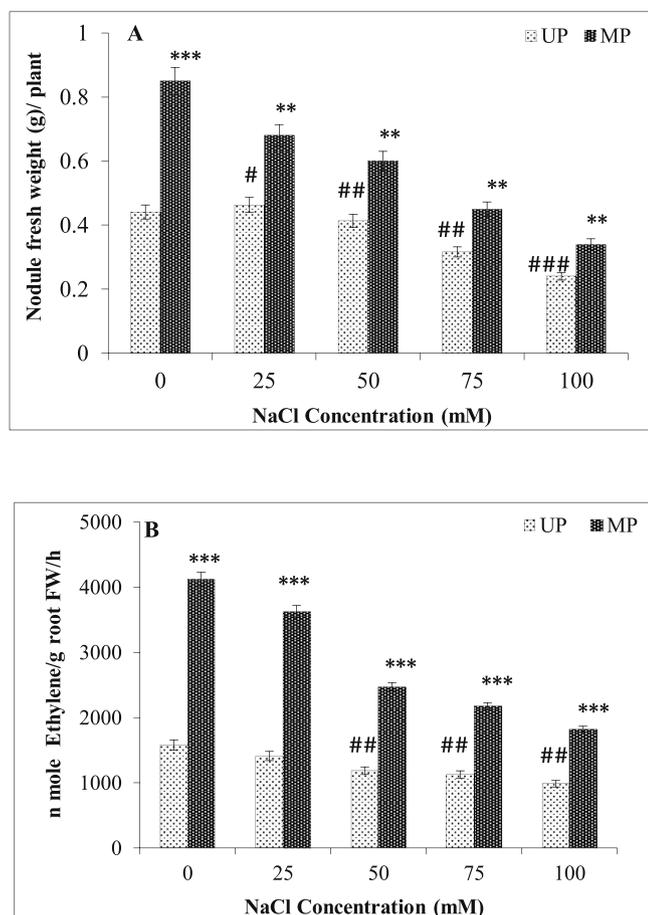


Fig. 6. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on (A) fresh weight of nodules per plant and (B) nitrogenase activity in root nodules at different concentrations of NaCl at 45 DAE. The vertical bar indicates \pm SE for mean. ## $p < 0.01$; # $p < 0.05$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds; *** $p < 0.001$; ** $p < 0.01$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

seeds. This decrease in nitrogenase activity in root nodules due to reduction in nodule weight, plant growth as well as increased ROS level in the soybean seedlings emerged from unprimed seeds under salinity. Nitrogenase activity is extremely vulnerable to ROS and ROS can robustly hamper its activity (Alquères Sylvia et al., 2010). Likewise, Amirjani (2010) found that at 200 mM salt concentration, the nitrogenase activity showed decrease of 60%. Application of compost has been found to improve nitrogen fixation significantly by increase in nitrogenase activity under salt stress conditions in soybean (EL-Sabagh et al., 2016). In the same way, our results also showed that magnetopriming improved the plant growth through improved nodulation and nitrogen fixation in both normal and salt stress conditions.

However, stresses and oxidative damage are interlinked and are accountable for the photosynthesis and yield losses (Wahid et al., 2014; Negrao et al., 2017). At moderate to low salinities the changes in growth rate or yield are often the only visual responses to salt tolerance (Shannon, 1985; Negrao et al., 2017). Salinity can reduce the crop yield with a significant metabolic attempt afford to plant adaptation, growth maintenance and stress responses with a consequent decrease in rate of photosynthesis and yield (Munns and Gilliam, 2015; Negrao et al., 2017; Kataria and Verma, 2018). In the present study, we have also found decrease in rate of photosynthesis, biomass accumulation and harvest yield of soybean in terms of number of pods per plant and harvest index; our data showed 20% reduction in harvest index of

soybean var. JS 335 at 75 mM NaCl as compared to seed yield at 0 mM NaCl in the plants that emerged from unprimed seeds. Similarly, EL-Sabagh et al. (2015) found that salt stress (10 mM) caused decrease in seed yield of 65% and 68% in soybean cultivars (Giza-35 and Giza-82) respectively. While plants emerged from magnetoprimed seeds showed 53% promotion in the harvest index as compared to their unprimed ones at 75 mM NaCl. Munns (2002) suggested that increase of the harmful level of Na^+ in leaves under salinity consequences the necrosis and early leaf senescence. The less supply of photosynthates (Grodzinski et al., 1998; Komor, 2000) due to leaf senescence or defoliation caused eventually decreased the yield under salinity. Under saline conditions due to decrease in osmotic potential of soil, the water as well as K^+ and Ca^{2+} uptake by the plant decreased (Munns et al., 2006; Khan et al., 2016). On the other hand magnetoprimed seeds showed higher water uptake in soybean and maize and lower Na^+/K^+ ratio in wheat even under salinity (Rathod and Anand, 2016; Kataria et al., 2017a,b). Rathod and Anand (2016) found that the sodium exclusion in magnetoprimed wheat seeds maybe beneficial in enhancing the salt tolerance to wheat genotypes.

Beneficial effects of SMF towards abiotic stresses have been reported previously in number of studies, including under cadmium stress in mungbean (Chen et al., 2011), under salt stress in soybean (Baghel et al., 2016) and salt and drought stresses in explants of wheat mature embryo (Sen and Alikamanoglu, 2014). Chen et al. (2011) reported that SMF alleviate the toxic effects of cadmium salts through increasing the photosynthetic rate and reducing the lipid peroxidation in mungbean seedlings. PMF pre-treatment helped in the regeneration of soybean under salt stressed condition (Radhakrishnan et al., 2012; Radhakrishnan and Kumari, 2013). SMF treatment of maize and soybean seeds enhanced the growth, photosynthesis and yield under soil water and salt stress (Anand et al., 2012; Baghel et al., 2016, 2018). PMF pre-treatment of soybean seeds has the potential to counteract the adverse effects of salt stress on calli growth by improving primary and secondary metabolites (Radhakrishnan et al., 2012). In the present study, we have measured the oxidative stress and identified the role of ROS and antioxidant defense system in ameliorating the harmful effect of salt stress. This is the first report to reveal that increase in the leaf growth, nitrogen fixation, photosynthesis, pod yield and harvest index of soybean under salt stress may be due to decrease in content of ROS and ASA and decrease in the activities of antioxidant enzymes and higher amount of α -tocopherol in the leaves of the soybean plants emerged from magnetoprimed seeds. Magnetopriming also increase the cell membrane permeability and ion transport in the ion channels which consequently affects some metabolic pathways activities (Galland and Pazur, 2005); which ultimately improved growth, photosynthesis and yield of crop plants.

5. Conclusions

The results of present study revealed that in addition to increase in H_2O_2 content, the antioxidant level like total ASA and activities of antioxidant enzymes like SOD, APX, GR and POD were also higher under salinity stress in plants from unprimed seeds. ROS (H_2O_2) content and antioxidants and antioxidant enzymes activities were lower and ASA/DHA ratio and α -tocopherol were higher in magnetoprimed plants under saline as well as under non-saline conditions. These results indicate that in the presence of salt stress, higher H_2O_2 content activates the antioxidant defense system in leaves of unprimed plants and (ii) SMF pre-treatment to the seeds eliminates the need for the defense against harmful salinity stress and leads to enhancement of primary metabolism, photosynthesis, nitrogen fixation and improved the yield of soybean. This increased growth by SMF pre-treatment appears to be due to the lower level of free radicals and scavenging enzymes in plants that emerged after magnetoprimed seeds. This suggested that SMF ameliorated the adverse effect of stress by restricting the production of free radicals. The metabolic energy that would have been utilized for

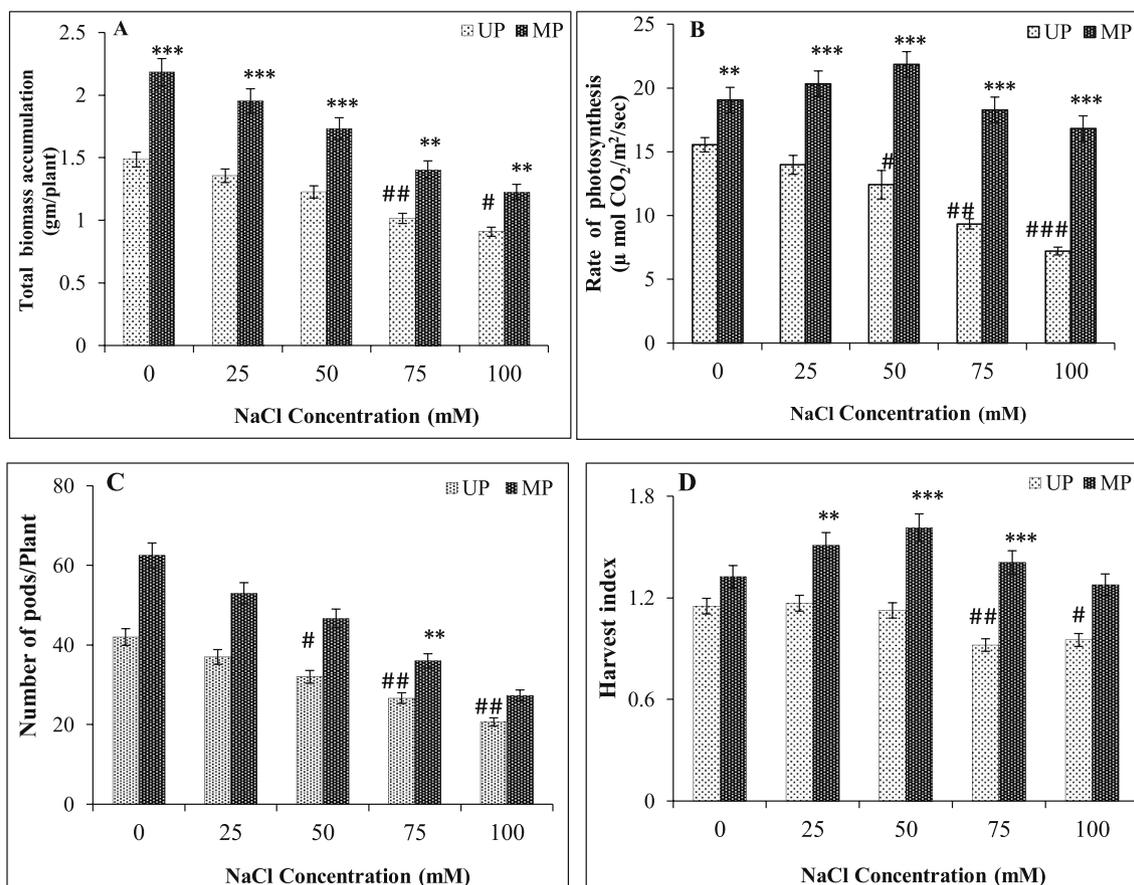


Fig. 7. Impact of SMF (200 mT for 1 h) pre-treatment of soybean seeds on (A) total biomass, (B) rate of photosynthesis, (C) number of pods per plant and (D) harvest index of soybean plants with different concentrations of NaCl. The vertical bar indicates \pm SE for mean. ## $p < 0.01$; # $p < 0.05$ indicate significant difference between non-saline and saline condition in plants emerged from unprimed seeds; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ indicate significant difference between SMF (MP) and unprimed (UP) seedlings grown in saline as well as non-saline conditions according to Student *t*-test.

scavenging these free radicals was now efficiently utilized towards maintaining growth of the plant in magnetoprimed seeds under salt stress conditions. The combinations of all these measurements suggest that the influence of magnetic field can lead to a better establishment of soybean seedlings, plant development and production under salt stress. Pre-seed SMF treatments could be used to enhance the growth and yield production by minimizing the salt-induced adverse effects on different crop plants.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbab.2019.101090>.

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