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SK conceived, designed,
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LB helped in experiments; SK
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ORIGINAL RESEARCH PAPER

Mitigation of adverse effects of salt stress on germination, growth, photosynthetic efficiency and yield in maize (*Zea mays* L.) through magnetopriming

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The efficiency of magnetopriming was evaluated for mitigation of the detrimental effects of salt stress on maize germination, growth, photosynthesis, and yield of maize plants. Maize seeds were pretreated with 200 mT of static magnetic field (SMF) for 1 h to assess the impact of SMF on the germination, seedling vigor, growth of plant, photosynthetic performance, ROS content, and yield under salt stress. The seedling characteristics of maize were negatively influenced by salt stress. However, SMF-pretreated maize seeds showed relatively higher germination percentage and germination stress tolerance index as compared to untreated seeds in saline and nonsaline conditions. The detrimental effect of NaCl induced salt stress was also observed on growth, yield, and different physiological characteristic of maize plants. The results showed that SMF-pretreated seeds enhanced seedling vigor, growth parameters such as plant height, leaf area, and biomass accumulation at different concentrations of NaCl (0, 25, 50, 75, and 100 mM) as compared to untreated seeds. Photosynthetic pigments, quantum yield of PSII photochemistry (Fv/Fm), phenomenological fluxes such as electron transport per leaf CS (ETo/CSm) and density of reaction centers (RC/CSm), the performance index (PI) were high in the leaves of plants that emerged from SMF-pretreated seeds as compared to untreated seeds. This stimulatory effect of SMF treatment of seeds was also revealed in the rate of photosynthesis and stomatal conductance, which results in improved yield of maize plants under saline conditions. The leaves from plants of SMF-treated seeds showed decreased hydrogen peroxide (H₂O₂) when compared with untreated seeds in both conditions. SMF ameliorates the adverse effect of salt stress in maize plants, by reducing H₂O₂ and increasing growth, photosynthetic performance, and yield under salt stress. For improvement of salt tolerance, magnetopriming with SMF of 200 mT for 1 h to dry seeds of maize can be efficiently used as a presowing treatment.

Keywords

electron transport; performance index; PSII; static magnetic field; tolerance index

Introduction

Plants are exposed to diverse abiotic environmental stress factors that influence their growth and development throughout their life cycle [1]. Agricultural yield is reduced through a variety of these factors like drought, high temperature, soil erosion, salt, and UV-B stress [2]. Amongst these, soil salinity is the major agricultural trouble in arid and semiarid regions that causes fields infertile and diminishes the yield of crop plants [2]. The new inclination of the current agriculture to more sustainable ways of agriculture has shown various new eco-friendly methods that must have a low ecological impact and also contribute to increasing crop yields. The physical seed pretreatment

via magnetopriming is one of the significant methods worth attention because it induces germination, growth, and development of plants [3–5]. Magnetic fields (MF) are extensively used by several researchers, as they complement the needs of organic agriculture. Various types of magnetic fields have been used in plants with their remarkable results [6,7]. Magnetopriming with static magnetic fields (SMF) augmented the germination percentage in many seeds, and the SMF-treated plants grow earlier than untreated ones [4,5,8,9]. Further, SMF have been found to provide protection against heat stress [10], salt stress [4,11,12], drought [13,14], UV-B stress [5], and cadmium stress [15] without adversely affecting the environment.

Maize (*Zea mays* L.) is an important cereal crop and it is moderately sensitive to salt stress. It is the basic food for human consumption besides its uses as a feed for livestock throughout the world [16]. It has been found that carbon fixation is very sensitive to salt stress in maize [17] that severely reduces the production of maize worldwide. In spite of the positive effects of SMF on plants under abiotic stresses, to date very few reports are available on investigation of the interaction of SMF and salt stress with the parameters related to chlorophyll *a* (Chl *a*) fluorescence, photosynthesis, and yield, especially in maize plants. Thus, there is a need to clarify the consequences of SMF pretreatment of maize seeds under salinity. Previously, it has been reported that SMF pretreatment caused enhancement in the percentage germination and vigor of maize seedlings under salt stress [4]. The present study was carried out in order to understand the biochemical and physiological changes related with SMF biostimulation of maize seeds. In this study, we have demonstrated the effect of SMF pretreatment on the vigor of seedling, plant growth, Chl *a* fluorescence, photosynthesis, and yield of maize plants under salt stress. Substantial enhancement in germination and growth as well as higher photosynthetic efficiency induced by SMF pretreatment consequently improved the yield of maize plants under saline and nonsaline conditions. SMF strength of 200 mT (1 h) has been proved to be best for improving germination and growth parameters of maize [4,9,18] in controlled lab and field conditions, and it has also been reported that maize var. JM 216 is sensitive to salt stress [4].

Material and methods

The breeder seeds of maize (*Zea mays* L.) var. JM 216 were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara (M. P.), India.

Magnetic field generation and treatment

In the present study, an electromagnetic field generator Testron EM-20 (Testron India, Delhi, India) was used for seed priming with variable horizontal magnetic field strength (50–500 mT) and a gap of 5 cm between pole pieces [19]. The pole pieces were in cylindrical shape, with a diameter of 9 cm and 16 cm in length. The number of turns per coil was 3,000 and the resistance of the coil was 16 ohms. A DC power supply (80 V / 10 A) with continuously variable output current was used for the electromagnet. The digital Gauss meter (model DGM-30-Testron, India) measured the magnetic field strength produced between the poles. The probe is made up of indium arsenide crystal and encapsulated to a nonmagnetic sheet of 5 × 4 × 1 mm; it could measure 0–2 T with the full scale range in increments of 5 mT. The variation in temperature during the course of seed exposure was 25 ± 1°C. The maize seeds were pretreated with SMF of 200 mT for 1 h in a cylindrical shaped sample holder of 42 cm³ capacity, made from a nonmagnetic thin transparent plastic sheet. Under a uniform magnetic field, hundred seeds kept in the plastic container were placed between both poles of the electromagnet.

Seed germination

Seed germination was measured according to the method of ISTA [20]. Four replications, each with 25 seeds, were placed between two layers of moist germination paper

by distilled water in control (with no salt solution, 0 mM NaCl) and respective saline solution (25, 50, 75, 100 mM NaCl) for salt stress treatment. These germination papers were rolled carefully and were wrapped in a sheet of wax paper to reduce surface evaporation. They were then placed in a seed germinator (Remi Instruments Ltd., India) at 25°C in an upright position. The germination percentage was calculated after 8 days. Five such seedlings from each replicate were randomly taken for measuring seedling length. Further, they were dried at 60°C for 72 h and dry weight was measured. Seedling vigor was calculated according to Abdul-Baki and Anderson [21]:

$$\text{Vigor index I} = \text{Germination \%} \times \text{Seedling length}_{(\text{Root}+\text{Shoot})}$$

$$\text{Vigor index II} = \text{Germination \%} \times \text{Seedling dry weight}_{(\text{Root}+\text{Shoot})}$$

Germination stress tolerance index (GSTI)

The germination stress tolerance index (GSTI) was estimated using the following formula [22]: $PI = n_{d1} (1.00) + n_{d2} (0.75) + n_{d3} (0.50) + n_{d4} (0.25)$, where, *PI* is promptness index, n_d is the number of seeds germinated on the day of observation [23], n_{d1} , n_{d2} , n_{d3} , and n_{d4} represent number of seeds germinated on the first, second, third, and fourth day, respectively.

A GSTI for magnetoprimed and unprimed seeds was calculated in terms of percentage as follows: $GSTI = (PI \text{ of salt stressed seeds} / PI \text{ of control seeds}) \times 100$.

Germination related enzymes

α -Amylase activity. After 72 h of seed imbibition, the germinating seedlings of maize (100 mg) were homogenized in 5 mL of chilled 80% acetone and centrifuged at 15,000 rpm for 10 min at 4°C. Then, the supernatant was discarded and the pellet was dissolved in 10 mL 0.02 M phosphate buffer (pH 6.4) and again centrifuged at 15,000 rpm for 20 min at 4°C; the resultant supernatant served as α -amylase enzyme extract. The α -amylase activity was determined by the procedure of Sawhney et al. [24]. The enzyme activity was expressed as mg starch hydrolyzed mg protein⁻¹ h⁻¹.

Protease activity. After 72 h of seed imbibition, the germinating seeds (1 g) of maize were homogenized in 5 mL of phosphate buffer (0.2 M, pH 7.6), then the homogenate was filtered with Whatman No. 1 filter paper and centrifuged at 12,000 rpm for 30 min at 4°C. The resultant supernatant was used for the enzyme assay following the procedure given by Kunitz [25]. The enzyme activity was expressed as mg protein hydrolyzed g⁻¹ fresh weight.

Reactive oxygen species (ROS) involved in germination

After 72 h of seed imbibitions, the ROS were quantified in the seedlings emerged from SMF treated and untreated seeds of maize under saline and nonsaline conditions.

Superoxide radical was estimated by the reduction of XTT (sodium, 3'-[1-(phenylamino-carbonyl)]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate) [26]. Twenty germinating seeds were incubated in 15 mL of potassium phosphate buffer (50 mM, pH 7.0) containing 500 μ mol XTT overnight on a shaker in darkness at 25°C. Changes in the absorbance were observed at 470 nm in a Shimadzu (Japan) spectrophotometer (UV 1601).

H₂O₂ was estimated by formation of titanium–hydroperoxide complex [27]. As per requirement, seedlings or third leaf (0.5 g) of maize were homogenized in 5 mL of cold acetone. The homogenate was filtered via Whatman No. 1 filter paper followed by the addition of 2 mL of titanium reagent and 2.5 mL of ammonium hydroxide solution to precipitate the titanium–hydroperoxide complex. The reaction mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The precipitate was dissolved in 2 mL of 2 M concentrated sulfuric acid and recentrifuged. The supernatant was read at 415 nm in a Shimadzu spectrophotometer (UV 1601) against blank and expressed as μ mol H₂O₂ g⁻¹ of seedlings or leaves.

Field experiments

The field experiments were performed on the terrace of the School of Biochemistry, Devi Ahilya University, Indore, India (latitude 22.48° N), during the period from October 2017 to January 2018 under ambient environmental conditions. The uniform in size and shape maize seeds were treated with fungicides like Bevestin and Thiram at 2 g/kg seeds before sowing. The maize seeds were sown in plastic nursery bags (34 cm height × 34 cm breadth; filled with mixture of sand, soil, and manure – 1:2:1 by volume). These plastic nursery bags were supplemented with saline solutions prepared from NaCl of different electrical conductivity that was 0, 4, 6, 8, 10 dSm¹ (equivalent to 0, 25, 50, 75, and 100 mM NaCl). The required salinity level was obtained in the soil by adding the salt solutions on 3 subsequent days before sowing. Three doses of 500 mL of different concentrations of 0, 25, 50, 75, and 100 mM NaCl solution were poured in each nursery bag of particular treatments. A conductivity meter (Analab Scientific Instruments, India) was used to measure the electrical conductivity of different salinity levels. For controls (0 mM NaCl), tap water was poured into the bags. In each nursery bag, three plants of uniform size were maintained.

On forty-fifth day after emergence of seedlings (DAE), all the growth attributes were measured in maize plants obtained from SMF (200 mT for 1 h) pretreated and untreated seeds under nonsaline and saline conditions (0 to 100 mM NaCl). The aboveground growth parameters of maize like plant height, leaf area, and plant dry weight were measured. The plants were removed from the plastic nursery bags and were gently washed to remove the adhering soil particles. Plant height was measured from the soil level to shoot tip. The area of the third leaf of maize was measured using a portable laser leaf area meter CID-202 scanning planimeter (CID Bio-Science, USA) and total biomass accumulation was measured after oven drying of whole plant parts at 60°C for 72 h.

Photosynthetic pigments

Photosynthetic pigments were extracted by dimethyl sulfoxide (DMSO) (Merck, India) [28] from the third leaves of 45-day-old maize plants. For the extraction of Chl, fresh leaf discs (25 mg) were transferred into a test tube containing 10 mL of DMSO. The contents were incubated at 65°C for 3 h and volume was made up to 10 mL with DMSO. The content was allowed to settle down and the absorbance was recorded at 470, 649, and 665 nm with a Shimadzu UV/VIS 1601 Spectrophotometer (Kyoto, Japan). The Chl *a*, Chl *b*, and total Chl content were calculated by the equations of Wellburn and Lichtenthaler [29] and the content was expressed as mg g⁻¹ leaf fresh weight.

PSII efficiency

Chl *a* fluorescence was measured at 45 DAE in third leaves of maize plants that emerged from SMF (200 mT for 1 h) pretreated and untreated seeds under nonsaline and saline conditions, using a plant efficiency analyzer (Handy PEA; Hansatech Instruments, UK). Whole plants were adapted to darkness for 30 min, transients were induced by red light (peak at 650 nm) of 600 Wm² (3,200 μE m⁻² s⁻¹) provided by an array of six light emitting diodes, focused on the leaf surface in the clips on a spot of 4 mm diameter to provide homogenous illumination over the exposed area of the sample. Data were recorded for 1 s with 12 bit resolution; the data acquisition was every 10 μs for the first 2 ms and every 1 ms thereafter [30]. All the measurements were taken at 25 ± 1°C. The following parameters were calculated from the OJIP transients: (i) maximum quantum yield of primary photochemistry (Fv/Fm); (ii) a value that is comparative to the activity of the water-splitting complex on the donor side of the PSII (Fv/Fo); (iii) phenomenological energy fluxes per excited cross section (CSm; m refers to maximum fluorescence, Fm): absorption (ABS/CSm), electron transport (ETo/CSm), trapping (TRo/CSm), dissipation (DIO/CSm), and concentration of reaction centers (RC/CSm); (iv) performance index (PI) that gives important information about the vitality and the state of plants [31]. All these parameters and the energy pipeline leaf model were calculated using BioLyzer HP

3 software (the chlorophyll fluorescence analyzing program by Bioenergetics Laboratory, University of Geneva, Switzerland).

Photosynthetic gas exchange

The rate of photosynthesis (Pn) and stomatal conductance (gs) were recorded at mid-day in fully expanded third leaves of maize plants at 45 DAE in each treatment on a sunny day via a portable infra red gas analyzer (LI-6200, LICOR, USA). At the time of measurement, the atmospheric conditions were as follows: the photosynthetic photon flux density (PPFD) was 1,300–1,600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO₂ concentration (350–380 ppm), air flow (500 $\mu\text{mol s}^{-1}$), air temperature (22°C), and 70% relative humidity.

Crop yield

At harvest maturity (120 DAE), the yield parameters were measured in maize plants emerged from SMF (200 mT for 1 h) treated and untreated seeds under nonsaline and saline conditions (0 to 100 mM NaCl). Yield characteristics like weight of cobs/plant and size and number of seeds in cobs were measured in three replicates of five plants each.

Statistical analysis

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. The data are expressed as means +SE and analyzed by the Student *t* test using Microsoft Excel tool. ^{***} $p < 0.001$; ^{**} $p < 0.01$; ^{*} $p < 0.05$ denote significant differences between nonsaline and saline conditions in maize plants that emerged from unprimed seeds, and ^{***} $p < 0.001$; ^{**} $p < 0.01$; ^{*} $p < 0.05$ denote statistically significant differences between seedlings of magnetoprimed with SMF pretreatment (MT) and untreated (UT) seeds grown in saline and nonsaline conditions according to Student *t* test.

Results

Effect of SMF on the early seedling characteristics of maize under salt stress

The data showed that the percentage germination decreased in SMF treated and untreated maize seeds with the increasing concentration of NaCl from 0 to 100 mM (Fig. 1A). However, the seedlings emerged from SMF treatment showed higher percentage germination at each concentration of NaCl used in the present study as compared to untreated ones. The maximum increase of 16% in percentage germination was found at 100 mM NaCl concentration in magnetoprimed seeds as compared to untreated ones (Fig. 1A).

The tendency of enhancement in vigor index I by magnetopriming was similar to the seedling length, and vigor index II was parallel to the dry weight of seedlings. Increasing concentrations of NaCl decreased the vigor index I and II in maize seedlings emerged from untreated seeds, and a maximum decrease of 20% in vigor index I and 28% in vigor index II was found at 100 mM NaCl as compared to untreated control (0 mM NaCl) (Fig. 1B,C). While on the other hand, at 100 mM NaCl the magnetoprimed seeds showed maximum of 30% enhancement in vigor index I and 70% in vigor index II as compared to untreated ones (Fig. 1B,C).

GSTI was reduced with the increasing concentration of salt stress in both untreated and SMF pretreated seeds (Fig. 1D). The lowest GSTI of 76 was observed at 100 mM salinity level in untreated seeds. On the other hand, the magnetoprimed seeds were successful in increasing relatively the highest GSTI of 117 at 25 mM NaCl, while at

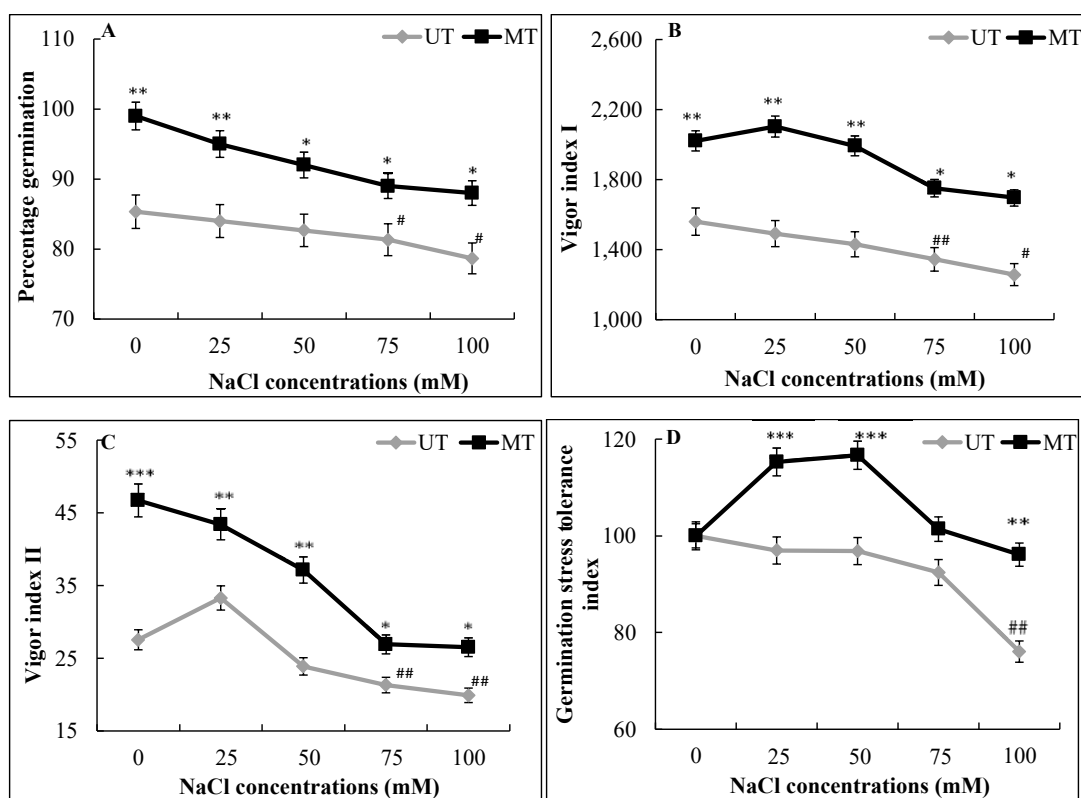


Fig. 1 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on percentage germination (A), vigor index I (B), vigor index II (C), and germination salt tolerance index (D) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

100 mM the GSTI was lower (96), but a relative increase was noticed as compared to untreated seeds.

Hydrolytic enzyme activities, like α -amylase and protease activity, were estimated after 72 h imbibitions in SMF-treated and untreated maize seeds under saline and non-saline conditions (Fig. 2A,B). At 100 mM NaCl, the α -amylase and protease activities decreased by 23% and 14% as compared to control (0 mM NaCl) seedlings emerged from untreated seeds. However, magnetoprimed seeds had higher activities of both the enzymes in saline and nonsaline conditions. At 100 mM NaCl, α -amylase activity was significantly increased (76%) by magnetopriming (200 mT for 1 h) as compared to untreated ones (Fig. 2A).

The maize seedlings that emerged from SMF-pretreated seeds showed enhanced production of $\cdot\text{O}_2^-$ and H_2O_2 as compared to seedlings emerged from untreated seeds under nonsaline and saline conditions (Fig. 2C,D). H_2O_2 was also increased in magnetoprimed seedlings of maize at all concentrations of NaCl used; at 100 mM NaCl concentration, there was 26% higher production of H_2O_2 observed as compared to untreated ones (Fig. 2C). SMF pretreatment promoted the rate of $\cdot\text{O}_2^-$ production to increase by 52%, 33%, 24%, 26.4%, and 26% at 0, 25, 50, 75, and 100 mM NaCl, respectively, in comparison to untreated seeds (Fig. 2D).

Effect of SMF on growth of maize plants under salt stress in field conditions

SMF (200 mT for 1 h) pretreatment of seeds was efficient in improving all the measured aboveground growth parameters of maize under salt stress and nonstress conditions. With increasing salinity, the plant height, leaf area, and plant dry weight were reduced as compared to the seedlings that emerged from untreated seeds under nonsaline conditions. The maximum 35% decrease was found in plant height, 50% in leaf area, and 71% in plant biomass on 45 DAE (Fig. 3A–C) at 100 mM salinity (NaCl) compared to control (0 mM NaCl) grown in nonsaline conditions. While SMF-pretreated seeds showed increase in all these parameters as compared with untreated seeds under saline and nonsaline

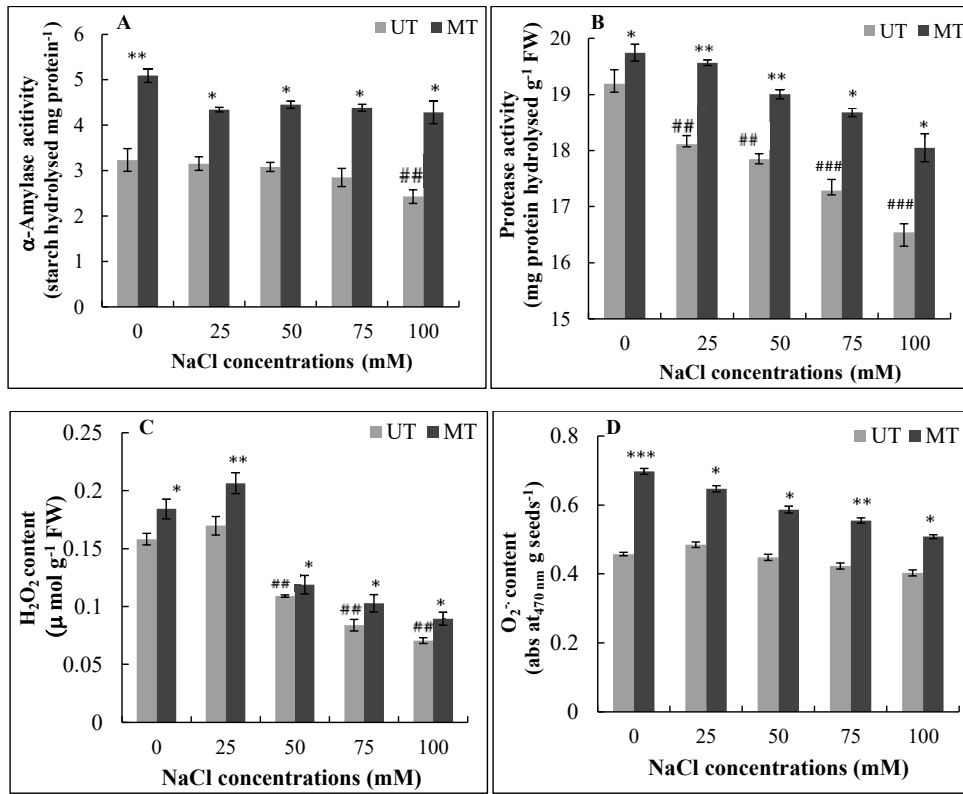


Fig. 2 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on activity of α -amylase (A), protease (B), H₂O₂ content (C), and O₂⁻ content (D) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

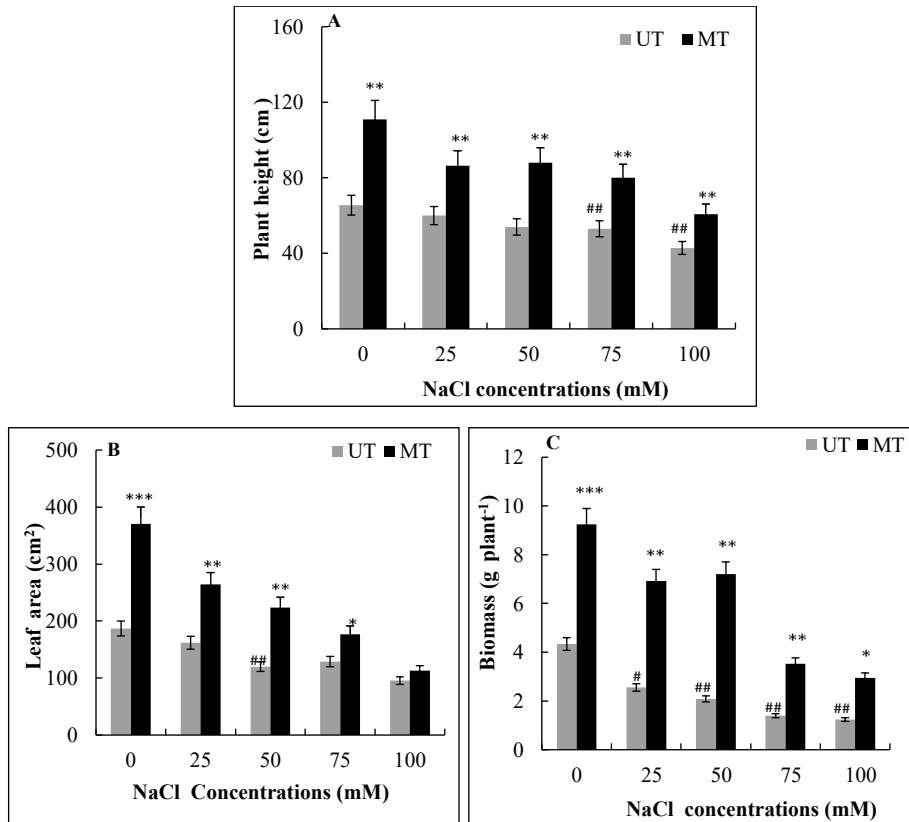


Fig. 3 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on plant height (A), leaf area (B), and plant dry weight (C) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

conditions. Plant height was enhanced by 69% at 0 mM and 41% at 100 mM salinity by SMF treatment (Fig. 3A). Leaf area was enhanced by 98% at 0 and 18% at 100 mM salinity (Fig. 3B). Total plant biomass of maize also increased by 113%, 170%, 245% 152%, and 137% in the seedlings that emerged from SMF-treated seeds in comparison to untreated seeds at 0, 25, 50, 75, and 100 mM salinity, respectively (Fig. 3C).

Photosynthetic pigments under salt stress

Chl content was enhanced in plants that emerged from SMF-treated seeds as compared to untreated ones. Salt stress caused reduction in both chlorophyll *a* and *b* in the third leaves of maize, thereby total Chl declined (Fig. 4A–C). SMF treatment (200 mT for 1 h) enhanced the total Chl content by 26%, 38%, 58%, 38%, and 46% at 0, 25, 50, 75, and 100 mM salinity, respectively, as compared to untreated seeds (Fig. 4C). This increase in the content of total Chl was because of greater enhancement in chlorophyll *b* (83%, 72%, 58%, 42%, and 39%, respectively, at 0, 25, 50, 75, and 100 mM salinity) than in chlorophyll *a* (12%, 29%, 58%, 46%, and 40%, respectively, at 0, 25, 50, 75, and 100 mM salinity) (Fig. 4B,C). Due to this, the chlorophyll *a/b* ratio decreased after SMF treatment; a 40% reduction was found at 0 mM and a 25% reduction was found at 25 mM NaCl (Fig. 4D).

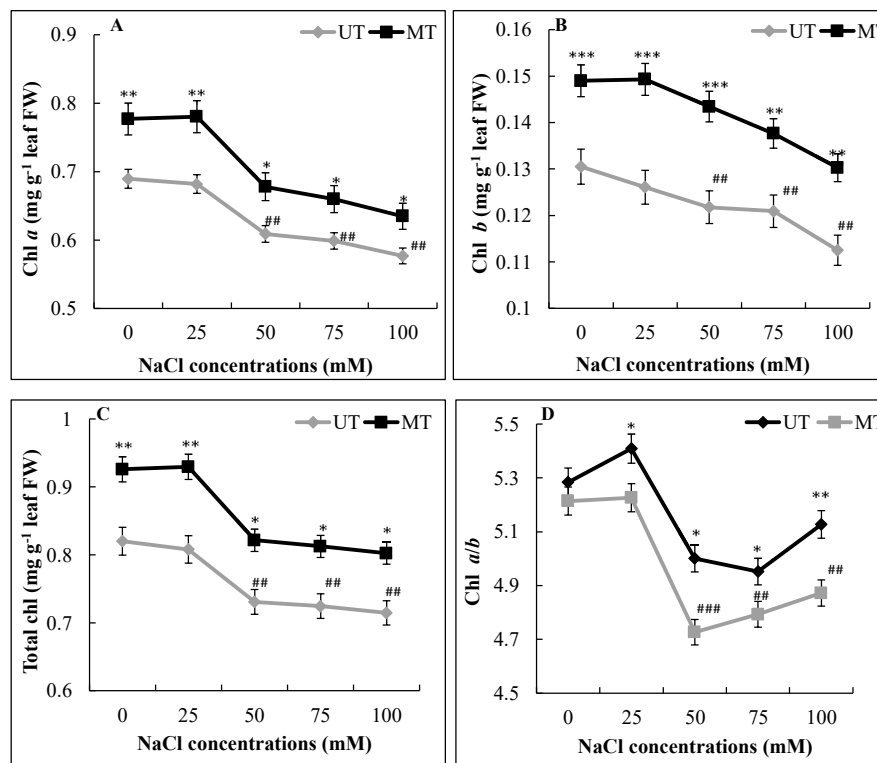


Fig. 4 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on Chl *a* (A), chl *b* (B), total chl (C), and chl *a/b* ratio (D) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

Effect of SMF on PSII efficiency under salt stress

The maximum quantum efficiency of PSII (calculated from F_v/F_m), F_v/F_o , and RC/CS_m marginally decreased with the increased concentrations of NaCl used (Fig. 5A,B,D). The values of F_v/F_m and F_v/F_o were higher in SMF treatment, but we did not find any significant difference between SMF-treated plants and untreated plants under salt stress and nonstress conditions (Fig. 5A,B). The RC/CS_m remarkably improved by 120% and 122% at 0 and 100 mM NaCl, respectively, in leaves of SMF-treated plants in comparison to untreated plants (Fig. 5C).

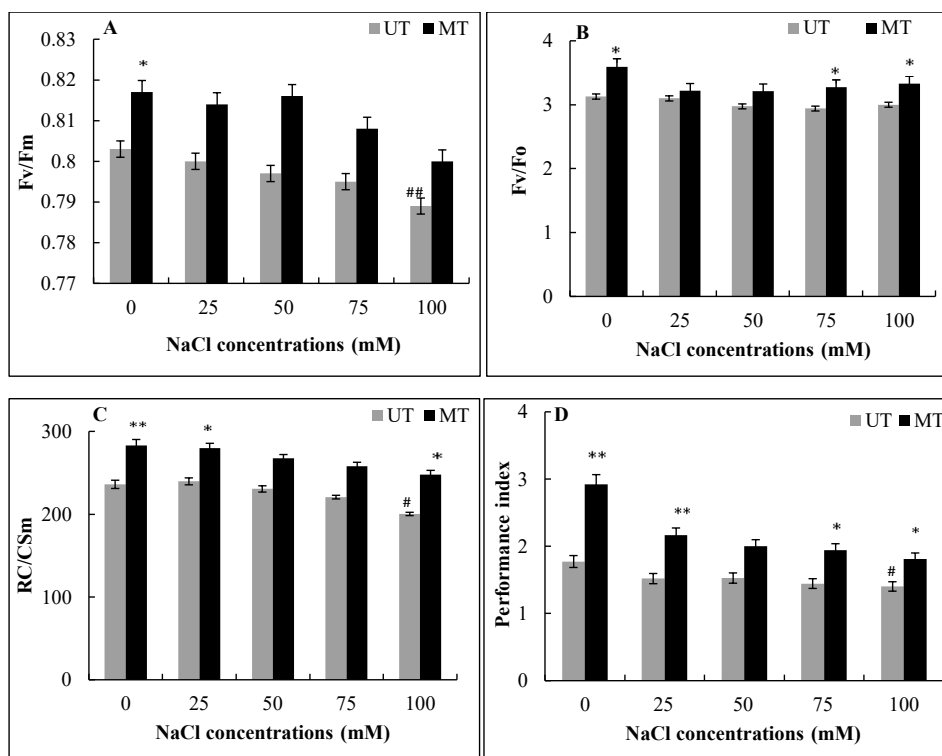


Fig. 5 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on Fv/Fm (A), Fv/Fo (B), RC/CSm (C), and PI (D) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

PI is an indicator of sample vitality and it is the most sensitive parameter calculated by the equation of the JIP test (the JIP test is a tool to analyze polyphasic rise of Chl *a* fluorescence transient – OJIP labeled phases). At 100 mM salt stress, PI decreased by 21% in leaves of plants that emerged from untreated seeds. However, a significant increase of 65%, 42%, 31%, 34%, and 29% at 0, 25, 50, 75, and 100 mM NaCl, respectively, was observed in the leaves emerged from SMF-treated seeds as compared to untreated seeds (Fig. 5D).

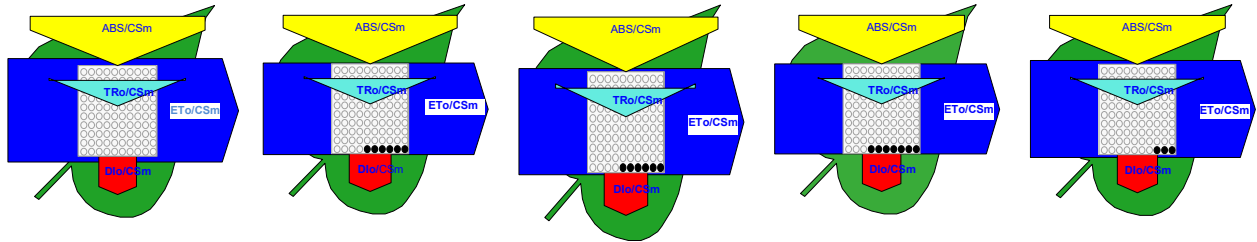
A phenomenological leaf model of the maize plants emerged from SMF-pretreated seeds produced by Biolyzer HP 3 software represents a higher number of active reaction centers per unit area in comparison to untreated ones (Fig. 6A,B). In this representation, open circles stand for the active reaction center, and plants emerged from SMF-pretreated seeds had more active reaction centers along with higher efficiency of electron transport, which is indicated by the greater thickness of the arrow in the phenomenological leaf model as compared to untreated ones at all concentrations of NaCl used (Fig. 6A,B).

Effect of SMF on photosynthesis under salt stress

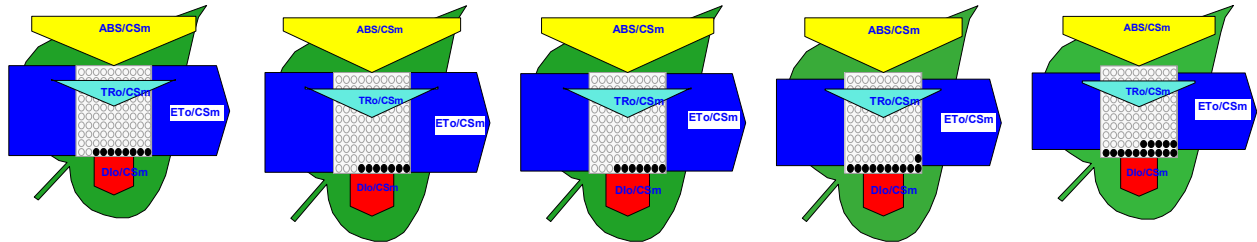
The rate of photosynthesis (*P_n*) along with stomatal conductance decreased with the increased salinity level from 0 to 100 mM NaCl. However, a significant increase in *P_n* and stomatal conductance was observed after SMF treatment (200 mT for 1 h) in saline and nonsaline conditions (Fig. 7A,B).

There was a 21% reduction in *P_n* in the leaves of plants emerged from untreated seeds at 100 mM NaCl. However, a 60% increase was recorded in *P_n* of leaves of plants that emerged from SMF-treated seeds as compared to the plants emerged from untreated seeds in nonsaline conditions (0 mM NaCl), and SMF pretreatment caused 67%, 54%, 29%, and 28% increase in *P_n* at 25, 50, 75, and 100 mM NaCl, respectively (Fig. 7A).

(A) 200 mT for 1 hour (MT)



(B) Untreated (UT)



0

25

50

75

100

NaCl concentrations (mM)

Fig. 6 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on leaf model showing phenomenological energy fluxes per excited cross section (CSm; m refers to maximum fluorescence, Fm) of maize leaves; MT (A), UT (B). Each relative value is represented by the size of the proper parameters (arrow), empty circles represent reducing quinone reaction centers (active), full black circles represent nonreducing quinone reaction centers (inactive or silent).

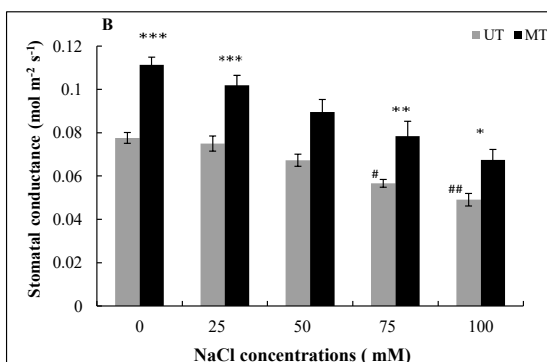
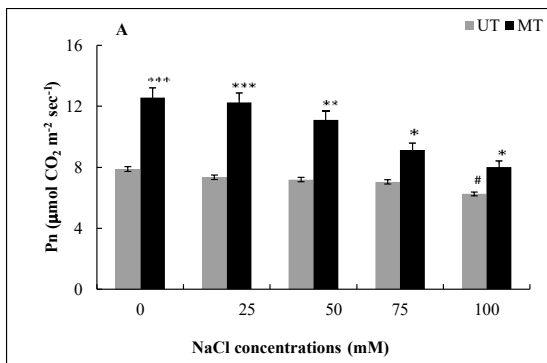


Fig. 7 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on rate of photosynthesis (A) and stomatal conductance (B) under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

Effect of SMF on ROS content under salt stress

It is well known that salt stress causes oxidative stress by the production of H₂O₂ in plants. The results showed that as the concentration of NaCl increased, the level of H₂O₂ also increased in the leaves of plants emerged from untreated seeds. H₂O₂ significantly decreased by 30%, 31%, 36%, 32%, and 32%, respectively, at 0, 25, 50, 75, and 100 mM NaCl in comparison to untreated seeds (Fig. 8).

Effect of SMF on maize yield under salinity stress

The yield parameters like number of seeds, arrangement of seeds per cob, and cob size per plant were reduced with the increasing concentrations of NaCl in maize plants emerged from untreated seeds, as shown in Fig. 9A. However, plants emerged from SMF-pretreated seeds showed remarkable enhancement in yield parameters as compared to untreated ones in saline and nonsaline conditions (Fig. 9A,B). The improvement in the weight of cobs due to SMF pretreatment was 27%, 22%, 27%, 29%, and 14% as compared to their untreated controls, respectively, at 0, 25, 50, 75, and 100 mM salinity (Fig. 9B).

Discussion

In the present study, salt stress drastically affected the early seedling characteristics and growth parameters of maize plants

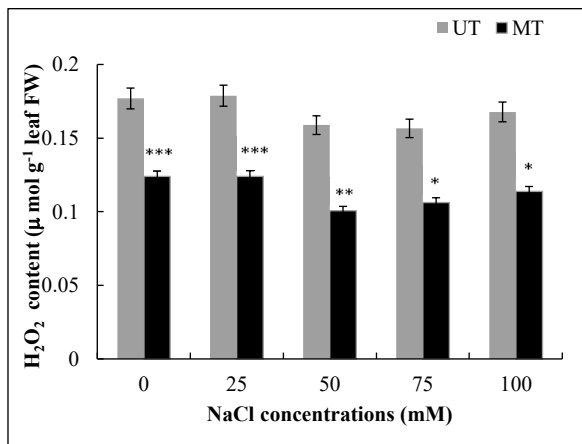


Fig. 8 Effect of SMF (200 mT for 1 h) pretreatment of maize seeds on H₂O₂ content in leaves under salt stress. The data are expressed as means \pm SE and analyzed by the Student *t* test.

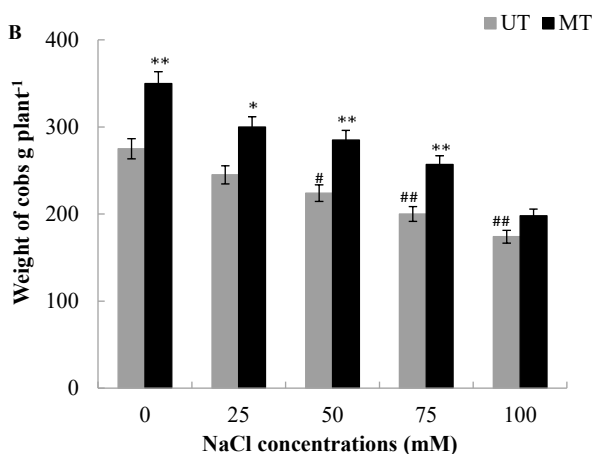
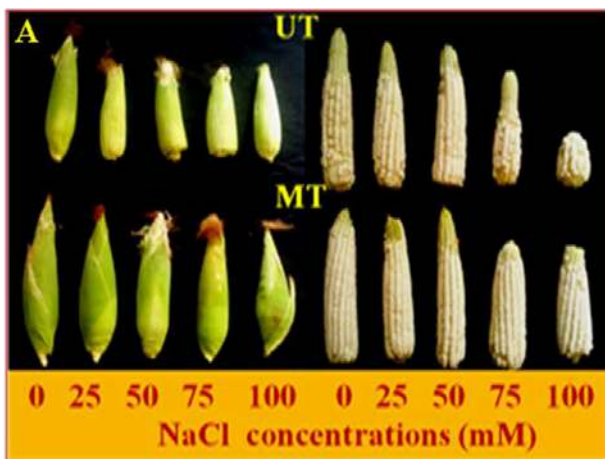


Fig. 9 Effect of SMF (200 mT for 1 h) pretreatment on maize yield under salt stress; photograph showing the size of cobs and arrangement of seeds in maize cobs (A) and weight of cobs/plant in maize plants (B). The data are expressed as means \pm SE and analyzed by the Student *t* test.

emerged from untreated seeds. The percentage germination and GSTI decreased considerably with increased level of salinity in both untreated and SMF treated seeds. On the other hand, higher values of percentage germination and GSTI were observed in SMF-treated seeds in both saline and nonsaline conditions. The GSTI was used to understand variation in the rate of germination due to osmotic stress [32]. The reason for relative increase in GSTI of magnetoprimed seeds might be due to the acceleration in osmotic potential which induces the water uptake necessary for mobilization of nutrients essential for the germination. As found earlier, seedlings emerged after SMF pretreatment have higher water uptake in both saline and nonsaline conditions [4].

Alpha- and beta-amylase enzymes catalyze the breakdown of stored carbohydrate reserves of the seed into monosaccharides which are then consumed by the growing seedlings afterwards during germination. SMF-treated seeds of maize showed higher α -amylase and protease activities as compared to untreated seeds under nonsaline and saline conditions in the present study. Germination and ROS accumulation are closely associated, and seed germination performance may be directly linked to the internal content of ROS [33]. It is well known that ROS such as O₂⁻ and H₂O₂ act as major signals and secondary messengers in regulating plant acclimation responses to environmental stresses [34]. In our study, we also found a higher level of O₂⁻ and H₂O₂ in seedlings emerged from SMF-pretreated seeds in both salt stress and nonstress conditions in comparison with untreated seeds. Thus, it seems that the increase in ROS level during germination due to salinity stress may be contributing to elevating the rate of germination. Similarly, the increased rate of germination was linked with a higher amount of O₂⁻, H₂O₂ and higher α -amylase and protease activities in SMF-pretreated seeds of chickpea, soybean, and maize under salt stress [4,12].

Salt stress reduces the plant growth severely and limits the crop production [2]. In the present study, plant height, leaf area, and biomass accumulation were significantly reduced in maize plants grown under different levels of salinity (0 to 100 mM NaCl) at 45 DAE. On the other hand, SMF pretreatment alleviates the growth inhibition in maize plants caused by salt stress. Similarly to the growth of plants, photosynthesis is one of the primary processes, drastically affected by salinity [2,35]. In the present study, we observed that the photosynthetic pigments, PSII efficiency, and photosynthesis in maize leaves were reduced under salt stress. However, SMF pretreatment significantly increased all of these parameters in maize plants grown under saline and nonsaline conditions. SMF-treated plants showed a higher amount of Chl *a*, *b* and total Chl than in the plants emerged from untreated seeds under both conditions. SMF has been shown to increase the chlorophyll content in sugar beet [36] and in soybean [8]. Similar effects of magnetopriming on chlorophyll content have been found in soybean under salt, UV-B and water stress [5,11,13]. In

the present study, the extent of promotion by SMF pretreatment was more in Chl *b* than in Chl *a* as compared to untreated ones. In the same way, seed pretreatment with SMF and electromagnetic field increased the Chl contents (*a* and *b*) in nonstress and drought stress conditions in soybean and maize [13,37]. The days to emergence, flowering, and

maturity were shortened, chlorophyll content was high, and proline contents were lower after SMF pretreatment to cumin seeds [38]. The lower level of Chl under salt stress may be due to higher Na^+ accumulation in leaves, which degrades the chlorophyll or reduces the magnesium (Mg^{2+}) [39]. The enhancement in the chlorophyll content by electromagnetic treatment might be because of the paramagnetic properties of chloroplast, which can enhance the rate of seed metabolism [36].

To monitor the alteration in the photosynthetic apparatus and its functional competence, chlorophyll *a* fluorescence is widely used as a detecting tool for the effect of salt stress on plants [40]. Our results also showed that the OJIP parameters of maize leaves, like maximal quantum efficiency of PSII (calculated from F_v/F_m) and the efficiency of the water-splitting complex on the donor side of PSII (as inferred from F_v/F_o), were reduced by salt stress and both F_v/F_m and F_v/F_o were enhanced in the leaves of SMF-treated seeds but not significantly in both saline and nonsaline conditions. Pereira et al. [41] found a similar decrease in (F_v/F_o) ratio, which consequently caused the destruction in electron transport of the photosynthesis. Similarly to our results, pre-exposure of seeds to electromagnetic and SMF treatment does not cause any significant changes in F_v/F_m and F_v/F_o under water deficit in corn and saline conditions in soybean [11,37]. Salt stress caused injuries to the oxygen-evolving complex and reduced the quantum yield of PSII electron transport and efficiency of PSII in maize [40]. The RC/CS_m , which stands for the active reaction centres at F_m , was also a good indicator for assessment of the effects of salt stress on plants. Our data showed that the active reaction centres decreased at higher level of salinity, which indicates that salt stress decreases the efficiency of PSII. RC/CS_m was decreased by 15% at 100 mM NaCl, and it was significantly increased via SMF pretreatment in comparison to untreated controls in saline conditions. It appears that the plants under salinity (100 mM NaCl) had much less chlorophyll per cross section and fewer active units (open circles) than control (0 mM NaCl) plants that emerged from untreated seeds.

PI is a collective measure of three partial performances, the amount of photosynthetic reaction centers (RC/ABS), the maximal energy flux which reaches the PSII reaction center (TR_o), and the electron transport at the onset of illumination (ET_o) [42]. The results of our experiments demonstrated the largest decrease (21%) in PI at 100 mM NaCl stress; SMF pretreatment resulted in a 65% increase in PI in comparison to untreated control (0 mM NaCl) plants grown in nonsaline conditions and an increase of 34% and 29% was observed at 75 and 100 mM NaCl by SMF pretreatment. The increase in PI_{ABS} was also found in maize plants after SMF pretreatment under nonstress conditions [18].

PI and RC/CS_m , derived from JIP test, demonstrate a greater sensitivity than F_v/F_m , which is in agreement with numerous findings [43,44]. These parameters provide information on the heterogeneity of PSII reactive centers, whereas F_v/F_m reflects the effectiveness of all PSII units along with both active reactive centers and inactive reactive centers [45]. The phenomological leaf model, presented in Fig. 6B, revealed that the leaf model generated from leaves of plants emerged from untreated seeds had a lower level of calculated electron transport (ET_o/CS_m) and a higher number of inactive reaction centers. But on the other hand, ET_o/CS_m and less inactive reaction centers were observed in SMF-treated plants (Fig. 6A).

This is the first report on the changes in OJIP parameters along with the rate of photosynthesis and yield after SMF pretreatment in maize plants under salt stress. Our results showed that a lower chlorophyll content under saline conditions negatively affects the fluorescence parameters of maize plants, which eventually may cause the reduction in the photosynthesis and yield of maize. Previously, it has been observed that chlorophyll content, photosynthesis, and stomatal conductance increased in maize plants emerged from different electromagnetic and SMFs pretreatments under irrigated and water stress conditions [14,37].

Under salt stress, lower photosynthetic pigments, reduced stomatal conductance, and damage to the photosynthetic apparatus are the important reasons for decrease in the fixation of carbon in maize plants [17]. Our results also showed that under salinity, the decrease in rate of photosynthesis was robustly associated with reduced growth, photosynthetic pigments, and stomatal conductance in maize. The promotive effect of the application of SMF on the growth and photosynthesis data under salt stress reported in this paper are in agreement with the earlier reports on soybean and wheat [11,46].

ROS production was increased by the salt stress and resulted in oxidative stress in plants. Our data showed that SMF treatment caused a decrease in H₂O₂ production in saline and nonsaline conditions at 45 DAE in field conditions. It indicates that SMF ameliorated the salt stress; thus, plants need not require any defense mechanism to degrade the ROS that are usually produced under salt stress. Hence, in magnetoprimed seeds the metabolic energy was efficiently employed to sustain the growth and development of the maize plants. This increased growth and Pn by SMF pretreatment might be because of fewer ROS in plants that emerged from SMF-pretreated seeds.

The weight of cobs/plant was reduced with the increased NaCl concentrations in plants that emerged from untreated seeds. However, significant enhancement in the weight of cobs/plant was found in plants emerged from SMF-pretreated seeds (Fig. 9B) in comparison to their untreated controls in both saline and nonsaline conditions. Previously, a decrease in grain number and weight and a consequential decrease in maize grain yield have been reported under salt stress [47,48].

In conclusion, most of the damages due to salt stress are mediated through ROS. Resistance to any kind of stress is closely linked to detoxifying ability of plants to counteract the ROS. There is a close link between ROS and SMF treatment. Our results indicate that SMF has a dual beneficial impact on ROS; SMF enhanced the ROS at the germination stage facilitating seedling vigor and higher percentage rate of germination and later reduced the ROS in mature leaves to facilitate higher electron transport, efficiency of PSII, PI, and photosynthesis, which ultimately results in higher yield of maize under salt stress. Thus, the present study provides the evidence that SMF pretreatment of seeds can enhance the plant salt tolerance. Plant salt tolerance also depends on the maintenance of K⁺ and Na⁺ homeostasis. Sodium is the most important toxic ion that hinders the uptake and transport of K⁺, which disturbed the stomatal modulation and caused water loss and lowered the growth, photosynthesis, and dry matter accumulation in maize [49]. On the other hand, SMF treatment mitigates the harmful effect of salt stress to some extent, and in the present study it might have been due to the maintenance of plant water status by osmotic alteration compared to untreated controls, as it has been earlier reported in maize [4]. SMF treatment may also cause increases in the K⁺ content as reported by Rathod and Anand [46] in wheat seedlings under salt stress. A lower Na⁺/K⁺ ratio and exclusion of sodium in magnetoprimed seeds may also be helpful in imparting salt tolerance to maize. Our results indicate that magnetopriming of dry seeds of maize can be successfully used as a presowing treatment for alleviating salinity stress. Thus, SMF seed pretreatment can be used in agriculture to obtain better growth and increased yield under adverse abiotic stress conditions. Pre-exposure of seeds to SMF is one of the feasible safe and cost-effective physical presowing treatments to enhance postgermination growth and development of plants and the crop stand. Pre-exposure of seeds to SMF has potential to increase crop production per unit area of land without having any detrimental effects toward any environmental component. In future, it may be a good tool for improving agricultural production in several economically important crops since it mitigates the adverse effects of abiotic stresses.

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Łagodzenie niekorzystnego wpływu stresu solnego na kiełkowanie, wzrost, wydajność fotosyntetyczną oraz plon kukurydzy (*Zea mays* L.) poprzez magnetyczną stymulację nasion

Streszczenie

W doświadczeniu oceniono efektywność magnetycznej stymulacji nasion w celu łagodzenia szkodliwego wpływu zasolenia na kiełkowanie, wzrost, fotosyntezę i plonowanie kukurydzy (*Zea mays* L.). Nasiona kukurydzy wstępnie traktowano 200 mT statycznym polem magnetycznym (SMF) przez 1 godzinę w celu oceny jego wpływu na kiełkowanie, żywotność siewek, wzrost roślin, wydajność fotosyntetyczną, zawartość ROS i plonowanie w warunkach zasolenia. Zaobserwowano negatywny wpływ stresu solnego na badane cechy siewek kukurydzy. Nasiona wstępnie traktowane SMF charakteryzował stosunkowo wyższy procent kiełkowania i wskaźnik tolerancji na stres podczas kiełkowania w porównaniu z nasionami nietraktowanymi SMF, zarówno w warunkach zasolenia jak i jego braku. Szkodliwy wpływ indukowanego NaCl stresu solnego stwierdzono również w odniesieniu do wzrostu, plonowania oraz różnych parametrów fizjologicznych roślin kukurydzy. Wyniki wskazują, że traktowanie nasion SMF wpływało na zwiększoną żywotność siewek oraz parametrów wzrostu roślin, takich jak wysokość, powierzchnia liści i biomasa w warunkach zróżnicowanego zasolenia (0, 25, 50, 75 i 100 mM NaCl) w porównaniu z roślinami uzyskanymi z nasion nietraktowanych SMF. Zawartość barwników fotosyntetycznych, maksymalna wydajność kwantowa fotosystemu PSII (Fv/Fm), fenomenologiczne przepływy energii, takie jak transport elektronów we wzbudzonej powierzchni fotosyntetycznej liścia CS (ETo/CSm) oraz gęstość centrów reakcji (RC/CSm); wskaźnik vitalności (PI) był wyższy w roślinach uzyskanych z nasion wstępnie traktowanych SMF w porównaniu z nasionami niepoddanymi jego działaniu. Zastosowanie SMF wywierało stymulujący wpływ także na napięcie fotosyntezy oraz przewodnictwo szparkowe, co skutkowało wyższym plonowaniem roślin kukurydzy w warunkach zasolenia. Liście roślin pochodzących z nasion traktowanych SMF charakteryzował obniżony poziom nadtlenu wodoru (H₂O₂) w porównaniu z nasionami nietraktowanymi, zarówno w warunkach zasolenia jak i jego braku. SMF łagodzi niekorzystny wpływ stresu solnego na rośliny kukurydzy redukując poziom H₂O₂ oraz wpływając korzystnie na wzrost, wydajność fotosyntetyczną i plonowanie w warunkach stresu solnego. Magnetyczna stymulacja suchych nasion kukurydzy może być skutecznie stosowana jako metoda ich przedsewnej obróbki w celu zwiększenia tolerancji roślin na zasolenie.