# Pre-treatment of seeds with static magnetic field ameliorates soil water stress in seedlings of maize (*Zea mays* L.)

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The effect of magnetic field (MF) treatments of maize (*Zea mays* L.) var. Ganga Safed 2 seeds on the growth, leaf water status, photosynthesis and antioxidant enzyme system under soil water stress was investigated under greenhouse conditions. The seeds were exposed to static MFs of 100 and 200 mT for 2 and 1 h, respectively. The treated seeds were sown in sand beds for seven days and transplanted in pots that were maintained at -0.03, -0.2 and -0.4 MPa soil water potentials under greenhouse conditions. MF exposure of seeds significantly enhanced all growth parameters, compared to the control seedlings. The significant increase in root parameters in seedlings from magnetically-exposed seeds resulted in maintenance of better leaf water status in terms of increase in leaf water potential, turgor potential and relative water content. Photosynthesis, stomatal conductance and chlorophyll content increased in plants from treated seeds, compared to control under irrigated and mild stress condition. Leaves from plants of magnetically-treated seeds showed decreased levels of hydrogen peroxide and antioxidant defense system enzymes (peroxidases, catalase and superoxide dismutase) under moisture stress conditions, when compared with untreated controls. Mild stress of -0.2 MPa induced a stimulating effect on functional root parameters, especially in 200 mT treated seedlings which can be exploited profitably for rain fed conditions. Our results suggested that MF treatment (100 mT for 2 h and 200 for 1 h) of maize seeds enhanced the seedling growth, leaf water status, photosynthesis rate and lowered the antioxidant defense system of seedlings under soil water stress. Thus, pre sowing static magnetic field treatment of seeds can be effectively used for improving growth under water stress.

**Keywords:** Static magnetic field, Soil moisture stress, Leaf water relations, Antioxidant enzymes

The beneficial effect of both static and oscillating magnetic fields (MFs) of very low to high field intensity has been reported in different plant species. Studies have shown that germination characteristics of the maize seeds exposed to MF are enhanced[1-3]. Wheat seeds exposed to MFs of 50 to 300 mT increase seedling vigor, respiratory quotient and α-amylase activity as compared to control seeds[4]. Growth of the germinated *Vicia faba* seedlings is found to be enhanced by the application of power frequency MFs (100 μ T), as evidenced by mitotic index and $^3$H-thymidine uptake[5]. A review article on currently accepted mechanisms of magnetoreception has reported that the research on magetoresponse in biology has been carried out in an unsystematic manner in the past[6].

Preliminary experiments in our laboratory have reported best combination of magnetic field strength and duration of exposure to get maximum enhancement in germination and field emergence characteristics in maize[7]. From these results, two treatments, namely 100 and 200 mT fields for 2 and 1 h exposure time respectively have been selected for further study. The dramatic two-fold increase in root length and root surface area in one month old plants from treated seeds has prompted us to test the performance of the maize plants raised from magnetically-exposed seeds under controlled moisture stress conditions. In an earlier report, differential response in maize roots and shoots is reported in plants exposed to low ψ (-1.6 MPa). The growth of leaves and stems is found to be rapidly inhibited, but roots continued to elongate to facilitate water uptake from the soil[8].

Abiotic stress also leads to production of reactive oxygen species (ROS) that may cause membrane lipid peroxidation, inactivation of -SH containing enzymes and RNA and DNA damage[9]. Plants have evolved specific protective mechanisms to defend themselves against the overproduction of ROS. The primary scavenger is superoxide dismutase (SOD) which

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converts $O_2^-$ to $H_2O_2$. Catalase (CAT) and a variety of peroxidases catalyze the breakdown of $H_2O_2$. CAT dismutates $H_2O_2$ into water and molecular oxygen, whereas peroxidases decompose $H_2O_2$ by oxidation of co-substrates, such as phenolic compounds and/or antioxidants. Moisture stress may increase the specific activity of these enzymes in order to control the oxidative damage caused by stress.

In the present study, we have investigated the effect of static MF treatment of maize seeds on seedling growth, leaf water status, photosynthetic rate and antioxidant enzymes system of seedlings grown under soil water stress under greenhouse conditions.

## Materials and Methods

### Seed material

The certified maize seeds (cv. Ganga Safed-2) were obtained from National Seed Corporation, New Delhi. Seeds without visible defects, insect damage and malformation were selected and stored in the desiccators having anhydrous calcium chloride. Seed moisture content was 7.6% on a fresh weight (FW) basis before MF treatments and final germination percentage was 100%.

### Electromagnetic field exposure

An electromagnetic field generator "Testron EM-20" with variable MF strength (50 to 500 mT) with a gap of 5 cm between pole pieces was fabricated (Fig. 1). The pole pieces were cylindrical in shape with 9 cm diameter, 16 cm length. The total number of turns of copper coil per pole piece was 3000 and resistance of the coil was 16 Ohm. A DC power supply (80V/10A) with continuously variable output current was used for the electromagnet. A digital gauss meter model DGM-30 operating on the principle of Hall effect monitored the field strength



Fig. 1—Electromagnetic field generator for seed treatment

produced in the pole gap. At low field (50 mT), from center to end of the poles, variation was 0.6% in horizontal direction and 1.6% in vertical direction of the applied field. At high field (300 mT), they were 0.4% and 1.2% of the applied field, respectively. The local geomagnetic field was approx. 6 mT. All treatments in the experiments were run along with control under similar conditions. Seeds were exposed to MF of 100 and 200 mT for 2 and 1 h respectively in a sample holder, cylindrical in shape and made of non-magnetic thin transparent plastic sheet. The temperature during treatment was $25 \pm 1^{\circ}C$. The control seeds were kept away from the magnetic field generator.

### Pot culture experiment

Seeds were sown in sand beds for 7 days and one seedling was transplanted per pot (15 cm in diameter and 15 cm high), which was filled with mixture of sandy loam soil and peat (50:50). The pots were arranged in a completely randomized block design with $3 \times 3$ factorial arrangements (pre-sowing treatments and water regime conditions). The mixture of sandy loam and peat was kept under different tensions in a pressure plate membrane apparatus (NORGEN, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) in triplicate and moisture content under different tensions -0.03 (control), -0.2 and –0.4 MPa was measured. The moisture tensions were maintained by weighing the pots every alternate day and adding the required amount of water calculated from the soil moisture characteristics curve. The pots were kept in greenhouse under natural conditions as ten replicates per treatment. The temperature during the experimental period was mean maximum $35^0C$ and mean minimum $27°C$. All the pots were covered with a plastic sheet around the stem to avoid evaporation from the soil surface. From five randomly selected plants after 40 days of transplanting, the second mature leaf from top was used for measurements of photosynthetic rate and leaf water potential and third leaf was used for relative water content (RWC), chlorophyll, $H_2O_2$ and antioxidant enzymes measurements. The remaining five pots were used for other growth measurements of shoot and root.

### Growth parameters

For each treatment, five remaining seedlings were removed and cut at the root neck to measure shoot parameters, viz. plant height, number of leaves, leaf area (LICOR-100 automatic leaf area meter, Lincoln,
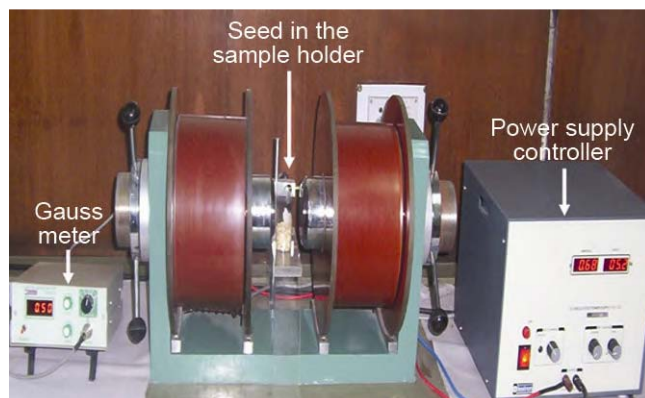
USA) and shoot dry weight (ventilated oven at 85°C until constant weight). Root morphology and architecture measurements (total root length, root surface area, root thickness and root volume) were done by WinRHIZO Root Scanner (LA 1600, Regent Instruments, INC, Canada). Root dry weight (ventilated oven at 85°C until constant weight) was also measured.

**Leaf photosynthesis and stomatal conductance**

Net photosynthetic rate and stomatal conductance were measured in three randomly selected plants from three pots using portable IRGA (LI 6200, LICOR, USA).

**Leaf water status measurements**

Leaf water potential was measured at midday (11 am-12 pm) by taking the same leaves that were used for measurements of photosynthesis. The leaf blade enclosed in a polythene bag to minimize post-excision evaporative losses was detached at its base and inserted with cut end protruding from the pressure chamber[10]. The 'balance' pressure required to force sap to the cut surface was taken as a measure of leaf water potential ($\Psi_w$) with a pressure chamber (S-pms Instruments, New Delhi, India). Immediately after the measurement of $\Psi_w$, the leaves were dipped in liquid nitrogen and stored at –20 °C for the solute potential ($\Psi_s$) determination by thawing and collecting sap. The sap was loaded into the pre-calibrated osmometer (5130 B, Wescor, UT, USA) for $\Psi_s$ measurement. Turgor potential was then calculated from the difference between $\Psi_w$ and $\Psi_s$. RWC was determined by measuring the water deficit in the leaves[11]. Chlorophyll was extracted by non-maceration technique using dimethyl sulfoxide (DMSO) and chlorophyll content was determined spectrophotometrically at 652 nm[12] and expressed on dry weight basis. There were five replications for all measurements.

**$H_2O_2$ measurement**

One g of third leaf was ground in 10 ml of cold acetone in a cold room (10°C). Mixture was filtered with Whatman No.1 filter paper, followed by addition of 4 ml titanium reagent and 5 ml ammonium solution to precipitate the titanium–hydroperoxide complex. Reaction mixture was centrifuged at 10,000 x g for 10 min. The precipitate was dissolved in 10 ml 2 M $H_2SO_4$ and re-centrifuged. $H_2O_2$ was estimated in the supernatant at 415 nm as the formation of titanium-hydroperoxide[13].

**Antioxidant enzymes extraction and assays**

Leaves (1.0 g) were cut into small segments and crushed into a fine powder in a mortar and pestle with liquid $N_2$. Soluble protein was extracted by homogenizing the powder in 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone with the addition of 0.2 mM ascorbate in case of ascorbate peroxidase assay to protect APX activity. The homogenate was centrifuged at 10,000 x g for 30 min at $0^0$C and supernatant was used for the following assays. Total soluble protein was measured using BSA as a standard[14]. The specific activity of all enzymes was expressed as nmol product mg$^{-1}$ protein min$^{-1}$.

Ascorbate peroxidase activity (APOX) (EC. 1.11.1.11) was estimated by recording the decrease in absorbance at 290 nm ($\varepsilon = 2.8$ mM$^{-1}$ cm$^{-1}$) for 1 min in 3 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM $H_2O_2$ and 0.1 ml enzyme extract. The reaction was started by the enzyme extract. Correction was done for the low, non-enzymatic oxidation of ascorbic acid by $H_2O_2$[15].

Catalase activity (CAT) (E.C.1.11.1.6) was determined by following the consumption of $H_2O_2$ ($\varepsilon = 39.4$ mM$^{-1}$ cm$^{-1}$) at 240 nm absorbance for 3 min[16]. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM $H_2O_2$ and 0.1 ml of enzyme extract in a 3 ml volume.

Peroxidase activity (POX) (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 mM$^{-1}$cm$^{-1}$) from guaiacol[17]. The reaction solution (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2mM $H_2O_2$ and 20 µl enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 s.

Superoxide dismutase (SOD) (EC 1.15.1.1) was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT)[18]. The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 25 mM NBT, 2 µM riboflavin, 0.1 mM EDTA, 50 mM sodium carbonate and 0.1 mL enzyme extract. The reaction mixture was illuminated for 15 min at a light intensity of 3600 lux. One unit of SOD was defined as the amount of enzyme required for causing 50% inhibition of the reduction of NBT as monitored at 560 nm.

**Statistical analysis**

Statistical analysis of data was performed using statistical software package MSTAT C. The results were subjected to two-way ANOVA using MF and soil water potential as two factors to detect differences between parameters. Means were compared using Duncan's multiple range test at P<0.05. Values of leaf moisture and relative water contents were transformed using arcsine transformation.

**Results**

The effect of magnetic treatment was significant for plant height, number of leaves, leaf area, shoot dry weight, root-shoot ratio, longest root length, total root length, root surface area, mean root diameter, leaf water potential, turgor potential and RWC, irrespective of soil water potential (Tables 1 and 2). The 100 and 200 mT MFs seedlings showed greater root-shoot ratio (70.7 and 50.3%), RWC (1.7% and 3.5%) and leaf water potential (15.3% and 20.6%) respectively than that of the control (P<0.05).

However, mean root diameter (16% for 100 mT and 21% for 200 mT) was markedly less than those shown by the controls (P<0.05). Leaf moisture content and osmotic potential were unaffected (P<0.05) by magnetic treatments (Tables 1 and 2). Leaf chlorophyll content on dry weight basis showed significant increase due to MF only for 200 mT exposure (Fig. 2A).

On the other hand, the soil water potential had a marked response on root-shoot ratio, mean root diameter, leaf water potential and RWC, irrespective of magnetic treatments (Tables 1 and 2). Root-shoot ratio and RWC (28% and 2.1%, respectively) were significantly increased at -0.4 MPa as compared to the control seedlings. However, mean root diameter and leaf water potential (11.2% and 63.7.0% respectively) were noticeably decreased at -0.4 MPa as compared to the control seedlings (Tables 1 and 2). Net rate of photosynthesis decreased drastically, irrespective of magnetic treatments (Fig. 2B). $H_2O_2$ level increased by more than 3% in water- stressed plants (Fig. 3). APOX activity increased under severe water stress condition by 16-45% (Fig. 4A). A tremendous increase in CAT and total POX activity was observed under this stress level (Fig. 4 B-C). SOD activity increased by 13-16% in water stressed plants compared to irrigated plants (Fig. 4D).

Interaction of magnetic treatments and soil water potentials had a significant effect (P<0.05) on plant height, number of leaves, leaf area and shoot and root dry weights. Seedlings from 100 mT showed an increase of 47% for -0.2 MPa and 29% for -0.4 MPa in plant height, while 200 mT MF seedlings showed an increase of 64% for -0.2 MPa and 34% for -0.4 MPa,
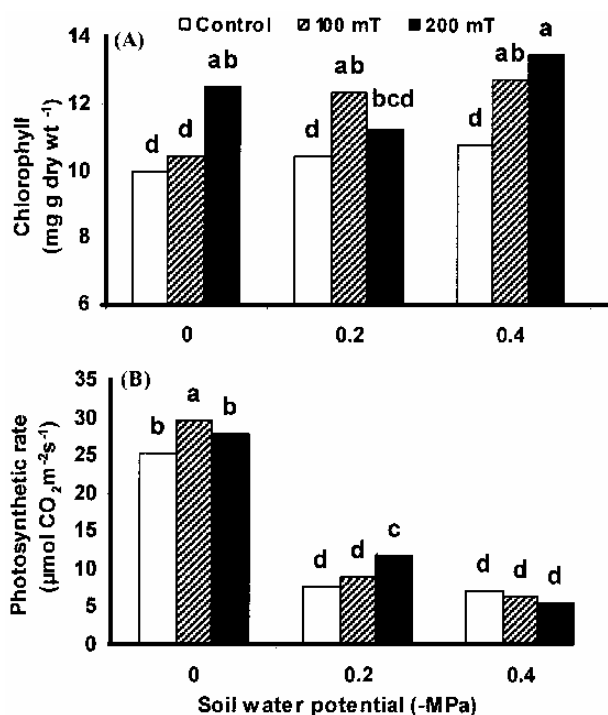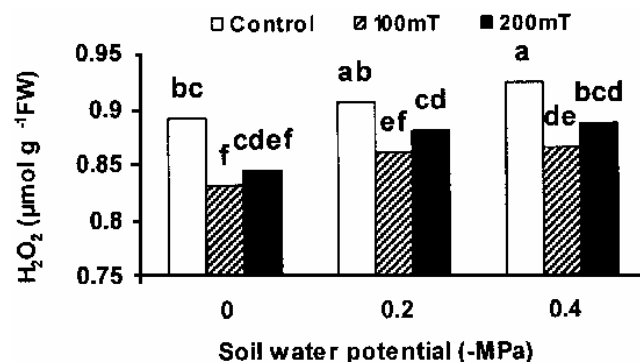


Fig. 2—Effect of maize (var. Ganga Safed-2) seeds exposure to static MF of 100 mT (for 2 h) and 200 mT (for 1 h) on (a): leaf chlorophyll content, (b): net photosynthesis of 45 days old potted plants subjected to -0.03, -0.2 and -0.4 M Pa soil moisture tensions [The bars indicate the means of 4 seedlings per treatment. Different alphabets on bars indicate significant differences at P<0.05 according to Duncan's multiple range test between the MF treatments and soil moisture tensions]



Fig. 3—Effect of maize (var. Ganga Safed-2) seeds exposure to static MF of 100 mT (for 2 h) and 200 mT (for 1 h) on $H_2O_2$ production in leaves of 45 days old potted plants subjected to -0.03, -0.2 and -0.4 M Pa soil moisture tensions [The bars indicate the means of 4 seedlings per treatment. Different alphabets on bars indicate significant differences at P<0.05 according to Duncan's multiple range test between the MF treatments and soil moisture tensions]

Table 1—Effect of MF and soil water potentials on growth parameters of 45 day old plants of maize (cv. Ganga Safed-2)
[Values are mean ± SE (n = 4)].

| | Plant height (cm) | No. of leaves | Leaf area (cm$^2$) | Shoot dry wt. (g) | Root dry wt. (g) | Root/shoot ratio | Length of longest root (cm) | Total root length (cm) | Root surface area (cm$^2$) | Mean root diameter (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Magnetic treatment** | | | | | | | | | | |
| Control | 10.8±1.3[b] | 8.3±1.0[b] | 112.0±30.0[b] | 0.428±0.06[b] | 0.072±0.01[b] | 0.171±0.05[b] | 20.2±2.9[b] | 123.9±28.0[b] | 21.86±5.4[b] | 0.499±0.04[a] |
| 100 mT | 14.2±1.3[a] | 10.3±1.3[a] | 365.8±97.0[a] | 1.072±0.32[a] | 0.307±0.08[a] | 0.292±0.04[a] | 29.9±4.5[a] | 623.8±94.5[a] | 86.18±19.5[a] | 0.419±0.03[b] |
| 200 mT | 14.5±1.7[a] | 10.4±1.2[a] | 404.7±134.4[a] | 1.367±0.52[a] | 0.347±0.1[a] | 0.257±0.04[a] | 31.7±6.6[a] | 687.1±162.3[a] | 89.89±25.1[a] | 0.394±0.03[b] |
| **Soil water potential** | | | | | | | | | | |
| - 0.03 M Pa | 13.2±1.5[ab] | 10.4±1.2[a] | 312.7±151.9[b] | 0.993±0.42[b] | 0.239±0.14[b] | 0.216±0.07[b] | 24.4±5.5[c] | 384.7±131.7[b] | 55.40±30.3[b] | 0.465±0.07[a] |
| - 0.2 M Pa | 13.9±3.0[a] | 10.2±1.6[a] | 356.3±194.3[a] | 1.177±0.71[a] | 0.281±0.18[a] | 0.227±0.06[a] | 30.4±9.2[a] | 598.1±292.3[a] | 82.66±42.2[a] | 0.434±0.05[ab] |
| - 0.4 M Pa | 12.4±1.7[b] | 8.4±0.9[b] | 213.5±104.9[c] | 0.697±0.26[c] | 0.201±0.09[b] | 0.277±0.06[a] | 27.1±4.6[b] | 452.0±141.4[b] | 59.87±28.4[b] | 0.413±0.05[b] |

Different alphabets indicate significant differences at P<0.05 according to Duncan's multiple range test.

Table 2—Effect of MF and soil water potentials on water relations of 45 days old plants of maize (cv. Ganga Safed 2).
[Values are mean + SE (n = 4). Values in parentheses are arcsine values]

| | Leaf water potential (MPa) | Osmotic potential (MPa) | Turgor potential (MPa) | Leaf moisture content (%) | Relative water content (%) |
|---|---|---|---|---|---|
| **Magnetic treatment** | | | | | |
| Control | -1.283±0.27[a] | -1.892±0.14[a] | 0.599±0.27[b] | 86.6 (68.6)±0.9[a] | 87.9 (69.7)±2.8[b] |
| 100 mT | -1.086±0. 26[b] | -1.929±0.14[a] | 0.843±0.14[a] | 87.8 (69.5)±0.9[a] | 89.6 (72.7)±1.5[a] |
| 200 mT | -1.018±0.25[b] | -1.900±0.26[a] | 0.880±0.11[a] | 89.0 (70.8)±3.1[a] | 91.4 (73.0)±1.8[a] |
| **Soil water potential** | | | | | |
| - 0.03MPa | -0.853±0.17[c] | -1.747±0.18[b] | 0.893±0.12[a] | 88.4 (70.1)±0.6[a] | 87.9 (69.6)±1.2[c] |
| - 0.2 MPa | -1.138±0.08[b] | -1.950±0.12[a] | 0.813±0.15[a] | 88.3 (69.1)±2.9[a] | 91.0 (72.7)±2.5[a] |
| - 0.4 MPa | -1.397±0.22[a] | -2.024±0.13[a] | 0.616±0.27[b] | 86.7 (69.0)±0.8[a] | 90.0 (71.7)±2.3[b] |

Different letters indicate significant differences at P<0.05 according to Duncan's multiple range test.

Table 3—Effect of maize (var. Ganga Safed-2) seeds exposure to static magnetic fields of 100 mT (for 2 h) and 200 mT (for 1 h) on growth parameters of root and shoot of 45 days old potted plants subjected to -0.03, -0.2 and -0.4 MPa soil moisture tensions
[Values are mean ± SE (n = 4)]

| | Plant height (cm) | No. of leaves | Leaf area (cm$^2$) | Shoot dry wt. (g) | Root dry wt. (g) | Root/shoot ratio | Length of longest root (cm) | Total root length (cm) | Root surface area (cm$^2$) | Mean root diameter (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | | | | | | | | | | |
| -0.03 MPa | 12.12 ±0.65[d] | 9.25 ±0.47[d] | 130.9 ±12.1[f] | 0.435 ±0.02[e] | 0.062 ±0.006[f] | 0.127 ±0.007[f] | 17.75 ±1.23[d] | 112.0 ±15.7[f] | 22.8 ±2.5[e] | 0.536 ±0.040[a] |
| -0.2 MPa | 10.12 ±0.42[e] | 8.25 ±0.25[e] | 127.1 ±9.5[f] | 0.428 ±0.03[f] | 0.067 ±0.003[f] | 0.160 ±0.008[e] | 20.25 ±0.78[d] | 110.6 ±17.0[f] | 17.2 ±1.9[e] | 0.487 ±0.004[a] |
| -0.4 MPa | 10.25 ±0.44[e] | 7.50 ±0.28[f] | 77.8 ±1.1[f] | 0.383 ±0.01[f] | 0.087 ±0.007[f] | 0.225 ±0.010[cd] | 22.37 ±0.14[cd] | 149.0 ±6.1[f] | 25.5 ±3.2[e] | 0.474 ±0.008[b] |
| **100 mT** | | | | | | | | | | |
| -0.03 MPa | 14.50 ±0.86[b] | 11.20 ±0.47[ab] | 462.1 ±40.2[b] | 1.429 ±0.08[b] | 0.377 ±0.02[b] | 0.265 ±0.004[bcd] | 27.50 ±1.51[b] | 634.3 ±46.8[b] | 91.8 ±1.5[b] | 0.435 ±0.012[bcde] |
| -0.2 MPa | 14.48 ±0.51[a] | 10.70 ±0.47[a] | 370.1 ±14.2[c] | 1.060 ±0.06[c] | 0.313 ±0.03[b] | 0.292 ±0.010[a] | 30.70 ±3.22[b] | 704.0 ±36.3[b] | 99.8 ±12.05[b] | 0.435 ±0.005[bc] |
| -0.4 MPa | 13.22 ±0.34[bcd] | 8.75 ±0.25[e] | 265.1 ±17.6[d] | 0.716 ±0.99[d] | 0.229 ±0.02[d] | 0.320 ±0.020[a] | 31.50 ±1.67[b] | 533.0 ±31.6[cd] | 66.9 ±7.9[c] | 0.387 ±0.010[ef] |
| **200 mT** | | | | | | | | | | |
| -0.03 MPa | 13.07 ±0.14[c] | 10.70 ±0.25[c] | 345.0 ±24.2[c] | 1.070 ±0.04[c] | 0.276 ±0.01[c] | 0.257 ±0.018[bcd] | 28.00 ±1.40[b] | 407.6 ±23.1[d] | 51.5 ±3.6[d] | 0.424 ±0.0173[cdef] |
| -0.2 MPa | 16.55 ±0.60[a] | 11.50 ±0.28[a] | 571.5 ±36.1[a] | 2.030 ±0.15[a] | 0.461 ±0.03[a] | 0.227 ±0.002[cd] | 39.80 ±2.00[a] | 979.6 ±80.2[a] | 130.9 ±5.7[a] | 0.387 ±0.016[ef] |
| -0.4 MPa | 13.72 ±0.11[bcd] | 9.00 ±0.40[bcd] | 297.6 ±19.9[c] | 0.990 ±0.03[c] | 0.286 ±0.02[c] | 0.287 ±0.021[ab] | 27.30 ±0.90[b] | 674.0 ±53.5[b] | 87.1 ±3.7[b] | 0.376 ±0.010[f] |

Different alphabets indicate significant differences at P<0.05 according to Duncan's multiple range test.

respectively compared to the control. Except root diameter, all other root traits registered a significant increase in treated plants as compared to untreated controls at –0.2 M Pa potential for 200 mT treatment (Table 3). The effect of 200 mT MF was significantly higher than those of 100 mT MF in most of the growth parameters at -0.2 MPa soil water potential.

Leaf water relations as measured by leaf water potential, osmotic potential, turgor potential and RWC were greater in treated plants both in well watered and stressed conditions (Fig. 5 A-D). Photosynthesis and chlorophyll content (expressed on dry weight basis) were higher in plants from magnetically-treated seeds at different soil water
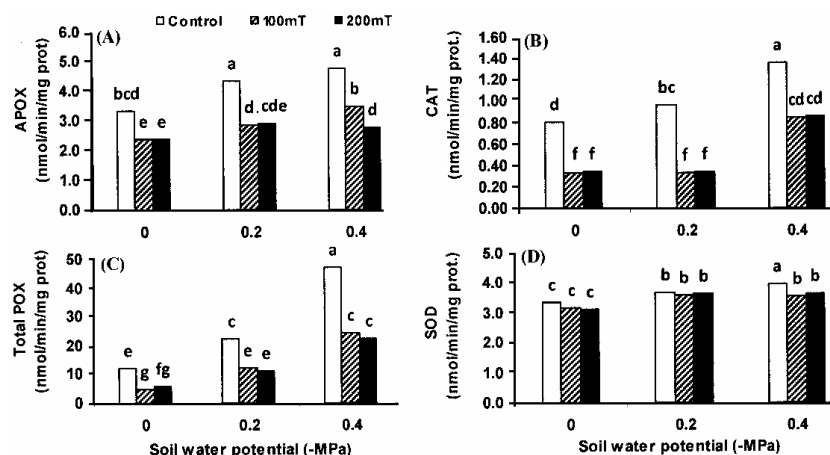


Fig. 4— Effect of maize (var. Ganga Safed-2) seeds exposure to static MF of 100 mT (for 2 h) and 200 mT (for 1 h) on (A) APOX (B) CAT (C) total POX (D) SOD specific activity in leaves of 45 days old potted plants subjected to -0.03, -0.2 and -0.4 M Pa soil moisture tensions [The bars indicate the means of 4 seedlings per treatment. Different alphabets on bars indicate significant differences at P<0.05 according to Duncan's multiple range test between the MF treatments and soil moisture tensions]
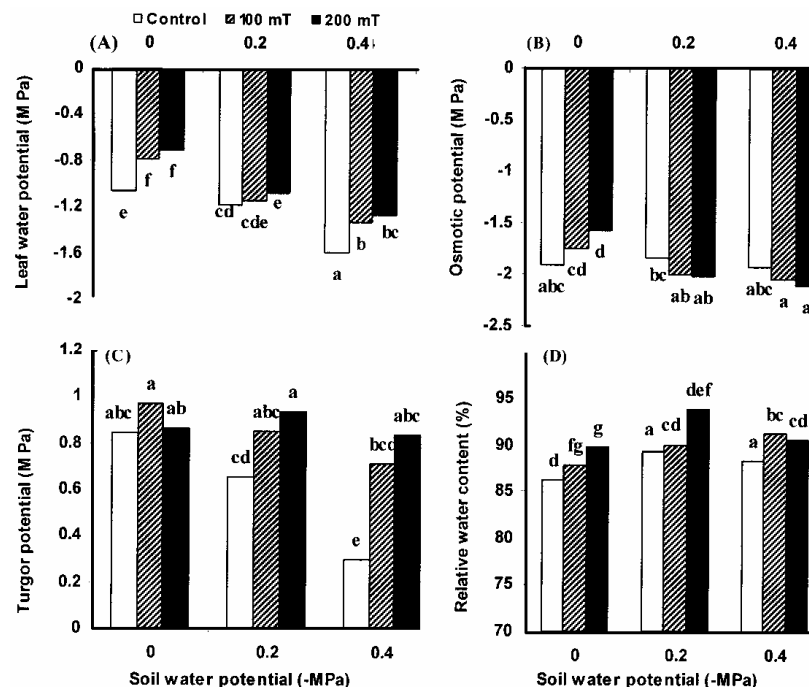


Fig. 5—Effect of maize (var. Ganga Safed-2) seeds exposure to static magnetic fields of 100 mT (for 2 h) and 200 mT (for 1 h) on water relations of 45 days old potted plants subjected to -0.03, -0.2 and -0.4 MPa soil moisture tensions [(A): Leaf water potential, (B): osmotic potential, (C): turgor potential, and (D): relative water content. The bars indicate the means of 4 seedlings per treatment. Different alphabets on bars indicate significant differences at P<0.05 according to Duncan's multiple range test between the MF treatments and soil moisture tensions]

potential (Fig. 2 A-B). There was 3-7% reduction in $H_2O_2$ in treated plants as compared to untreated plants under all the moisture regimes (Fig. 3).

Antioxidant defense system enzymes showed lower activity in magnetically-treated plants. APOX activity was found to be 29-40% less in treated plants compared to control, irrespective of stress treatment (Fig. 4A). Significant reduction in CAT activity was observed in treated plants as compared to untreated controls (Fig. 4B) Total POX activity also showed the similar pattern in magnetically-treated plants as it decreased by 44 and 58% in –0.2 MPa and irrigated plants respectively compared to untreated control (Fig. 4C). SOD activity reduced by 8-10% in treated plants as compared to untreated control at –0.4 MPa (Fig. 4D).

## Discussion

The results of exposure of maize seeds to 100 and 200 mT static MFs for 2 and 1 h led to a considerable improvement in seedling growth and biological response to soil water stress of seedlings. Mild stress (-0.2 MPa) had a stimulating effect on both shoot and root traits and water status in plants from 200 mT treatment. Alleviation of inhibitory effects of heat and drought stress[19] and increased saline-alkali tolerance[20] in magnetically-treated plants has been reported.

The noticeable increase of root growth in terms of length of longest root, total root length, root surface area, mean root diameter and root-shoot ratio promoted by magnetic exposure indicated the alterations in the growth pattern of seedlings might lead to their adaptation to water stress conditions. The low availability of water in soil affects the root-shoot ratio and root continues its growth in search of water in deeper soil areas, while shoot stops to grow by water stress[8,21]. Maize seedlings have been found to adapt to low water potential by making the walls in the apical part of the root more extensible that is partly accomplished by increase in expansin activity and partly by other complex changes in the cell wall. In addition, ephemeral conditions in root cell wall established by transmembrane proteins, such as plasma membrane electron transport system, ion channels and $H^+$-ATPases modulate both the activity of wall enzymes and physical properties of wall[22].

The plants from magnetically-treated seeds in our study might have greater modulation of both the activity of wall enzymes and physical properties of wall matrix and, therefore, better root length and root surface area. In studies with rice seeds exposed to

pulsed MF, increased $Ca^{2+}$ ions uptake in rice seedlings results in better growth of leaf, meristematic tissues in stems and roots. It was postulated that ion-cyclotron resonance may interfere with the $Ca^{2+}$ ion sequestering, thereby enabling the rise in free $Ca^{2+}$ concentration in the system. The increased $Ca^{2+}$ concentration may signal the cell to enter into early mitotic cycle[23]. In our study also, a resonance like phenomenon might have induced a remarkable increase in plant height, number of leaves, leaf area, shoot and root dry weight in seedlings derived from magnetically-treated seeds.

ROS are formed in normal cell metabolism and their regulation is a normal cellular event. ROS produced at lower levels by cell wall NADPH oxidase, peroxidase, amino oxidase or flavin containing oxidase may act as signaling molecules[24,25]. Abiotic stress condition increases the probability of ROS generation in chloroplasts, leading to oxidative damage[26]. The plants have adapted to live with ROS and there is a fine balance between their production and scavenging under different environments enabling them to perform signaling or protective role. The reduced rate of photosynthesis under both moisture stress levels, in our experiments might be due to decreased availability of $NADP^+$ to accept electrons from photosystem I. This might have resulted in more generation of activated oxygen species under moisture stress conditions.

A significant increase in free radicals has been observed in pea seeds exposed to MF. This brings about initial changes during development as seedlings are reported to have a longer hypocotyls, roots and thus greater vigor. However, no effect of stimulation is observed in organs of young pea plants[27]. Our results contradicted these findings, as we observed a decrease in $H_2O_2$ production and scavenging enzymes in leaves from treated seeds. This suggested that MF ameliorated the adverse effect of stress by restricting the production of free radicals. The metabolic energy that would have been utilized for scavenging these free radicals was now efficiently utilized towards maintaining growth of the plant under stressed condition.

The plants from magnetically-exposed seeds showed a non-significant increase in chlorophyll content and an increase in rate of photosynthesis over the plants from untreated seeds at well watered and mild stress levels. Exposure to MF has shown to increase photochemical activities in a unit of chlorophyll molecule, resulting in increase in green

pigment of wheat and bean[28]. MF treatment also increases the cell membrane permeability and ion transport in the ion channels which then affects some metabolic pathways activity[29,30], leading to improved growth as observed in our results on different growth parameters. However, in grown-up plants, complexity of the role of reduced ROS under magnetic treatment, resulting in improved growth under normal and stressed environment still needs to be explored.

In conclusion, our study demonstrated that pre-sowing MF treatment enhanced seedling growth, leaf water status, photosynthesis rate of seedlings under soil water stress in maize. Decreased free radicals production and antioxidant enzymes activity in leaves from 45 days old plants of MF-treated seeds indicated that MF treatment ameliorated water stress, so that the plants do not have to divert their metabolic energy in detoxification of free radicals that are generally produced under stress conditions. Thus, presowing MF treatment can be effectively used for alleviation of adverse effects of water stress in crop plants.

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