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Influence of pulsed magnetic field on soybean (*Glycine max* L.) seed germination, seedling growth and soil microbial population

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The effects of pulsed magnetic field (PMF) treatment of soybean (*Glycine max* L. *cv* CO3) seeds were investigated on rate of seed germination, seedling growth, physico-chemical properties of seed leachates and soil microbial population under laboratory conditions. Seeds were exposed to PMF of 1500 nT at 0.1, 1.0 10.0 and 100.0 Hz for 5 h per day for 20 days, induced by enclosure coil systems. Non-treated seeds were considered as controls. All PMF treatments significantly increased the rate of seed germination, while 10 and 100 Hz PMFs showed the most effective response. The 1.0 and 10 Hz PMFs remarkably improved the fresh weight of shoots and roots, leaf area and plant height from seedlings from magnetically-exposed seeds compared to the control, while 10 Hz PMF increased the total soluble sugar, total protein and phenol contents. The leaf chlorophyll a, b and total chlorophyll were higher in PMF (10 and 100 Hz) pretreated plants, as compared to other treatments. In addition, activities of α-amylase, acid phosphatase, alkaline phosphatase, nitrate reductase, peroxidase and polyphenoloxidase were increased, while β-amylase and protease activities were declined in PMF (10 Hz)-exposed soybean plants. Similarly, the capacity of absorbance of water by seeds and electrical conductivity of seed leachates were significantly enhanced by 10 Hz PMF exposure, whereas PMF (10 Hz) pretreated plants did not affect the microbial population in rhizosphere soil. The results suggested the potential of 10 Hz PMF treatment to enhance the germination and seedling growth of soybean.

Keywords: Soybean, *Glycine max* L., Bacteria, Biochemicals, Fungi, PMF frequencies, Seed germination, Seed leachates

In the evolution process, earth's magnetic field (about 50 μT) was a natural component of the environment for living organisms. The effects of magnetic fields on seed germination, plant growth, biochemical changes and yield have been the subject of intense research in recent years, but a systematic study is necessary to locate the mechanisms of magnetic action in tissues and to identify its useful applications. Savostin[1] first showed significant increase in the elongation of the wheat seedlings exposed with magnetic field. The external electric and magnetic fields are reported to influence the activation of ions and polarization of dipoles in living cells[2].

Pre-treatment of seeds with static magnetic field has been found to increase the maize seedling growth against water stress[3]. The effects of either continuous or pulsed magnetic field (PMF) on plant growth and development have been investigated in a large number of plant species[4]. The increase in height and number of primary branches is reported in tomato seeds exposed to different magnetic field strengths varying from 15-155 mT[5]. In addition, exposure to a 150 mT magnetic field has been shown to stimulate an increase of germination and leads to improvement in length and fresh weight of shoot in maize[6]. The magnetic field pre-treatment has also been found to have positive effect on cucumber seedlings, such as stimulating seedling growth and development[7].

The various effects of magnetic fields on seed germination and growth of plants depend on a complex way of magnetic flux densities, frequencies, pre-treatment of plant material and treatment duration. In addition, electric or magnetic treatments enhance seed vigour by influencing the biochemical processes, which stimulate the activity of proteins and enzymes[8]. To the best of our knowledge, studies are lacking on the efficacy of PMF with different frequencies on the soybean improvement. Thus, in the present study, we have investigated the influence of extremely low frequency PMFs on seed germination, seedling growth, physico-chemical

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properties of seed leachates, enzymatic and biochemical activity in leaves on soybean plant and soil microbial population under laboratory conditions.

Materials and Methods

PMF treatment of seeds

Seeds of soybean (*Glycine max* L. *cv.* CO-3) were obtained from Tamilnadu Agricultural University, Coimbatore, India. The dry seeds (moisture content, 8.6%) were exposed to PMFs as per the method described by Radhakrishnan and Ranjithakumari[9]. The healthy uniform size seeds were selected and each batch of 100 g of seeds were exposed to 0.1, 1, 10 and 100 Hz PMFs of 1500 nT for 5 h per day for 20 days to determine optimal frequency. Control seeds were kept under the similar condition in the absence of PMF.

Seed germination, plant growth and biochemical parameters in PMF-treated plants

PMF-exposed and control seeds were surface sterilized with 0.1% mercuric chloride solution for 5 min and washed thoroughly 5-times with distilled water. The 30 seeds were germinated in clay pots (17 × 12 cm) containing clay and sand mixture (3:1). The pots were maintained under natural photoperiod with 35% (w/w) soil moisture content. After 3 days, the seed germination was observed.

Influence of PMF exposure was observed by the measurement of height of the plants at 10 days after sowing. Twenty-two days later, the length of shoots, fresh weight of shoots and roots and leaf area were measured. The leaves were harvested and analyzed for chlorophyll a, b, total chlorophyll, protein, total soluble sugar and phenol in PMF-treated and untreated plants. The photosynthetic pigments, such as chlorophyll a, b and total chlorophyll were extracted and estimated as described previously[10]. For determination of protein, the leaves were homogenized using 70% ethanol and subsequently precipitated by adding 20% (w/v) TCA. The precipitate was then dissolved in 1% (w/v) NaOH solution and the protein was estimated by the method of Bradford[11]. In addition, plant leaves were ground in 80% (w/v) ethanol and centrifuged at 5000 rpm for 10 min. The supernatant was collected and was used for the analysis of total soluble sugar[12] and phenol[13].

Enzyme activity in PMF-treated plants

Activity of α-amylase (EC 3.2.1.1) and β-amylase (EC 3.2.1.2) was determined by the previously described procedure[14]. Seedlings (10 days old) were ground in distilled water and centrifuged at 10000 rpm for 15 min at 4°C to collect the supernatant, which was used to study the above enzymes activity. In addition, fresh seedlings (10 days old) were homogenized in 0.1 M acetate buffer and after centrifugation at 10000 rpm for 15 min at 4°C, the supernatant was used for analysis of acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1)[15] activities. Activity of protease (EC 3.4.24.4) was also estimated[16]. Seedlings homogenized with 0.1 M potassium phosphate buffer containing 1% PVP were centrifuged at 10000 rpm for 15 min at 4°C and supernatant was used to measure the activity of polyphenol oxidase (EC 1.14.18.1)[17]. Nitrate reductase (EC 1.6.6.2) activity was also assayed[18]. The enzyme activities were expressed as optical density (OD)/g fwt.

Microbial population

The soil samples were collected from the rhizosphere of PMF pre-treated soybean plants, dissolved in sterile distilled water and then inoculated with nutrient agar plate for bacterial counts and potato dextrose agar plate for fungal counts. After 2 days, the bacterial and fungal colonies were counted and expressed as colony forming unit (cfu) per g of fresh soil sample.

Physico-chemical changes in seed leachates

For each treatment, 50 seeds were soaked in 20 mL of distilled water at 25°C for 8 h. Thereafter, the fresh and dry weight and water content of the seeds were measured. The pH (Model 335 pH meter, Systronics, India) and electrical conductivity (Model 374 electrical conductivity meter, Systronics, India) were measured from soaked water (seed leachates) of both control and PMF-treated seeds.

Statistical analysis

Data of seed germination, plant growth, biochemical parameters, physicochemical properties of seed leachates and soil microbial population of control and PMF-treated plants were expressed as a mean ± SE and analyzed by one-way analysis of variance (ANOVA) to find out the significance of differences among the treatment means. The treatment means were compared using Duncan's multiple range test at a 5% level of significance by SPSS 11 software package.

Results and Discussion

Effects of PMF on seed germination, plant growth and biochemical changes

Seed germination

Exposure of soybean seeds to various PMF frequencies significantly increased the rate of germination, as compared to the untreated (control) seeds (Fig. 1). The seed germination gradually increased due to the exposure of PMF from 0.1 to 10 Hz (96.2%). However, higher dose of PMF (100 Hz) slightly declined the germination than 10 Hz, which might be due to the biochemical changes or altered enzyme activity[19]. Earlier, it is reported that exposure of static magnetic fields significantly increases germination, speed of germination, length and dry weight of sunflower seedling, as compared to the unexposed control[20]. Magnetic fields also significantly enhance komatsuna plant growth and also increase germination (20%)[21]. Significant enhancement of seed germination under static and weak magnetic fields is also been reported in chickpea, sunflower and maize[8,19,22]. It is suggested that higher rate of maize

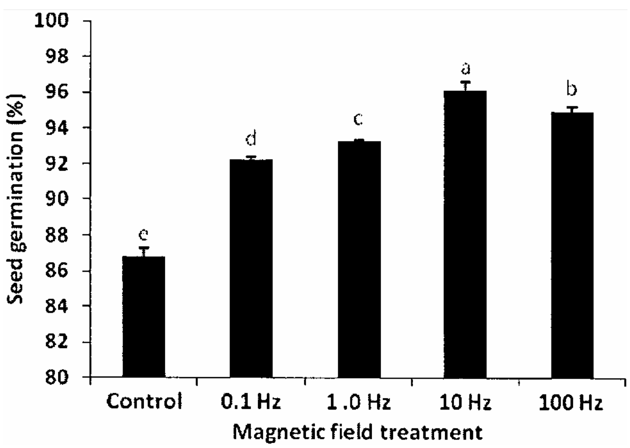

Fig. 1—Effect of PMF on soybean seed germination [Values represent means of 30 replicates with SE and values marked with the different letters (a, b, c ,d and e) were significantly differ according to Duncan's multiple range test (P ≤ 0.05)]

seed germination and seed vigour under the treatment of magnetic field influence the biochemical processes that involve free radicals formation and stimulate the activity of proteins and enzymes. The enhancement of seed vigour might be due to paramagnetic properties of chloroplasts[21].

Plant growth

Figure 2 shows the effect of PMF-induced changes in plant growth of 10 days old seedlings. The maximum plant height was observed at 10 Hz treatment. In addition, PMF enhanced the shoot length, as compared to control (Table 1) and significant improvement was observed in 22 days old plants treated with 10 Hz. Earlier, the positive influence of 10 Hz PMF in shoot length and biomass of 8 days old soybean plants is reported[9]. The fresh weight of shoot was significantly higher (23%) in 10 Hz-exposed plants as compared to control, while doses of 0.1, 1.0 and 100.0 Hz increased the rates by 11%, 18% and 8%, respectively. Moreover, significant enhancement of root fresh weight (84%)

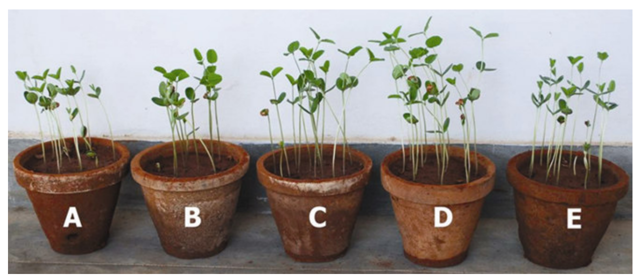

Fig. 2—Soybean seedling growth under the different frequencies of PMFs [A, Control; B, 0.1; C, 1.0; D, 10 and E, 100 Hz PMF treatments]

Table 1—Effects of PMF on morphological variations, total protein, total soluble sugar, phenol, seed weight, absorbed water content of seeds and physicochemical characters of seed leachates

Parameters	Control	0.1 Hz	1.0 Hz	10 Hz	100 Hz
Shoot length (cm/plant)	31.6 ± 0.10^{cd}	31.9 ± 0.11^{c}	36.4 ± 0.10^{b}	37.2 ± 0.10^{a}	31.1 ± 0.27^{d}
Fresh weight of shoot (g/plant)	8.02 ± 0.1^{d}	8.93 ± 0.21^{c}	9.48 ± 0.14^{b}	9.89 ± 0.2^{a}	8.69 ± 0.11^{cd}
Fresh weight of root (g/plant)	3.62 ± 0.07^{d}	4.28 ± 0.05^{c}	5.43 ± 0.03^{b}	6.66 ± 0.15^{a}	5.26 ± 0.15^{bc}
Leaf area (cm^2/plant)	14.4 ± 0.14^{d}	16.1 ± 0.07^{b}	15.3 ± 0.06^{c}	17.2 ± 0.07^{a}	12.4 ± 0.21^{e}
Total soluble sugar (mg/g fw)	78.47 ± 1.7^{b}	76.55 ± 0.70^{d}	77.54 ± 1.1^{c}	79.19 ± 0.37^{a}	75.30 ± 0.55^{e}
Total protein (mg/g fw)	5.5 ± 0.05^{e}	5.7 ± 0.05^{d}	6.1 ± 0.04^{c}	8.6 ± 0.05^{a}	6.5 ± 0.00^{b}
Phenol (mg/g fw)	4.81 ± 0.05^{b}	4.73 ± 0.12^{cd}	4.79 ± 0.18^{c}	4.36 ± 0.13^{d}	5.26 ± 0.14^{a}
Weight of water soaked seeds (mg/seed)	276.08 ±1.2^{b}	278.87 ± 1.7^{cd}	273.48 ± 2.8^{d}	298.43 ± 0.6^{a}	291.47 ± 0.1^{c}
Dry weight of seeds (mg/seed)	111.7 ± 0.1^{b}	115.68 ± 0.1^{c}	117.18 ± 0.4^{e}	118.89 ± 0.1^{a}	118.21 ± 0.2^{d}
Absorbed water content of seed (mg/seed)	164.38 ± 1.1^{b}	163.19 ± 1.6^{d}	156.3 ± 2.4^{e}	179.54 ± 0.5^{a}	173.26 ± 0.0^{c}
pH of seed leachates	7.00 ± 0.07^{a}	6.10 ± 0.23^{b}	6.72 ± 0.06^{abc}	6.76 ± 0.04^{ab}	6.65 ± 0.03^{cd}
EC of seed leachates (mS/cm)	18.37 ± 0.10^{c}	21.2 ± 0.12^{b}	13.3 ± 0.23^{d}	22.6 ± 0.28^{a}	22.4 ± 0.31^{ab}

Values represent means of 12 replicates and values marked with the different letters (a, b, c, d) significantly differ according to Duncan's multiple range test (P ≤ 0.05) was observed in 10 Hz-treated plants. The PMF exposure of 0.1, 1.0 and 10.0 Hz also influenced leaf area, as compared to 100 Hz. The plants derived from seeds treated with magnetic field showed a greater leaf area than the control (Table 1). This effect might be due to the increased photosynthetic rates, greater interception of light and greater amount of assimilates available for vegetative growth[23]. Our results also suggested that PMF treatment could be useful in enhancing the germination of seeds and in promoting the plant growth.

Earlier, the higher plant height and increase in fresh weight is reported in maize subjected with magnetic field[19]. Enhancement of height and number of primary branches is also found in tomato seeds pretreated with 15 to 155 mT[4]. Significant increase in shoot and root fresh weight is reported in sunflower seedlings exposed to magnetic field[20]. Magnetic field also enhances above ground biomass and leaf area of cucumber plants[7].

Biochemical changes

Chlorophyll content in plants was higher in some of the treatments of PMF. In comparison with control, 100.0 Hz-exposed plants showed chlorophyll a content nearly (40.6 mg^{-1} fw^{-1}) equal to control, whereas 0.1 and 1.0 Hz treatments degraded 5% and 3% chlorophyll a content, respectively (Fig. 3). The significant increase of chlorophyll a (6%) was observed in 10 Hz-exposed plants. Similarly, the enhanced amount of chlorophyll b (22%, 10%, 2.0% and 0.5%) was found in plants on treatment with 100.0, 10.0, 1.0 and 0.1 Hz PMF. In addition, PMF of 10.0 and 100.0 Hz also increased the concentration of total chlorophyll. Earlier, an increase of chlorophyll

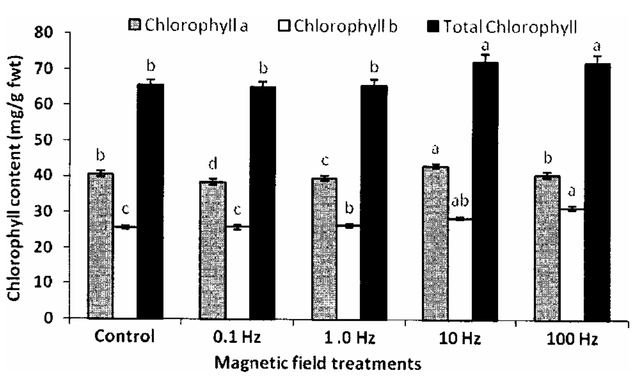

Fig. 3—Effect of magnetic field on chlorophyll a, chlorophyll b and total chlorophyll content in leaves of soybean plant [Values represent means of 4 replicates and values marked with the different letters (a, b, c and d) were significantly differ according to Duncan's multiple range test (P ≤ 0.05)]

content due to the application of magnetic field has been reported in rice[24], cotton[25] and potato[26].

The total soluble sugar was slightly lower in plants at different frequencies of PMF, except 10 Hz (Table 1). However, leaf protein content was higher in plants at all PMF treatments and especially 10 Hz PMF significantly increased the amount of protein (56%). PMF application could induce the protein synthesis in plants and it might be the reason of more accumulation of protein[9]. The enhanced growth and yield of soybean might be due to the significant increase in subunits of Rubisco enzyme by PMF treatment[9]. Earlier study has also reported higher protein content in magnetic field exposed *Cucumis sativus* seedlings[27]. PMF also influenced the phenolic content, depending in its frequencies. A gradual increase of phenol accumulation was noted in plants treated with 0.1 to 100 Hz, except 10 Hz and a marked increase (9%) was observed at 100 Hz PMF.

The 10 Hz magnetic field increased α-amylase (50%) and decreased β-amylase (26%) activity in 10 days old soybean plants (Fig. 4). Amylase activity plays an important role in hydrolyzing the starch into simple sugars, which provide the energy for the growth of roots and shoots[27]. PMF application enhanced the seedling growth by the stimulation of starch degradation, thus might be responsible for improvement of soybean plant growth.

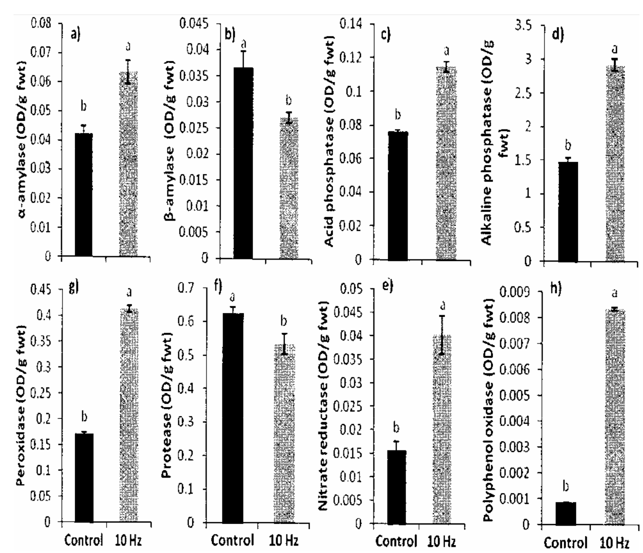

Fig. 4—Effect of 10 Hz magnetic field on (a) α–amylase, (b) β-amylase, (c) acid phosphatase, (d) alkaline phosphatase, (e) nitrate reductase, (f) protease, (g) peroxidase and (h) polyphenoloxidase activities of soybean plant [Values represent means of 4 replicates and values marked with the different letters (a and b) were significantly differ according to Duncan's multiple range test (P ≤ 0.05)]

The increased activities of acid phosphatase (51%) and alkaline phosphatase (97%) were found in seedlings subjected with 10 Hz PMF. Acid phosphatase implicates in hydrolysis and absorption of organic phosphate compounds from soil and also plays a role in tissue differentiation[28]. Increase of acid phosphatase activity could be related to either *de nova* synthesis of protein or activation of protein[29].

Enhanced activities of nitrate reductase, peroxidase and polyphenol oxidase (158%, 866% and 139%, respectively) in comparison with control and a decline in protease activity (15%) were observed in PMF-exposed seedlings. The increased nitrate reductase activity might be due to the higher concentration of Ca in seedling tissues[30]. An increase in the peroxidase activity in plants pretreated with magnetic field has been observed in tomato plants[31]. Over-production of polyphenol oxidase activity indicates the tolerance of plants[32]. PMF application could either induce the synthesis of proteins or prevent the degradation of proteins by the inhibition of protease activity. In the current study, PMF improved the seedlings growth by the regulation of enzyme activities.

PMF did not affect the bacterial and fungal colonies of rhizosphere of soybean plants (Fig. 5). Bacterial and fungal population was 2.6 and 6.5 cfu/g of soil in soybean rhizosphere, respectively. PMF pre-treatment of seeds improved the plant growth and did not affect the soil biota, suggesting that it was eco-friendly to soybean plants and soil microbes.

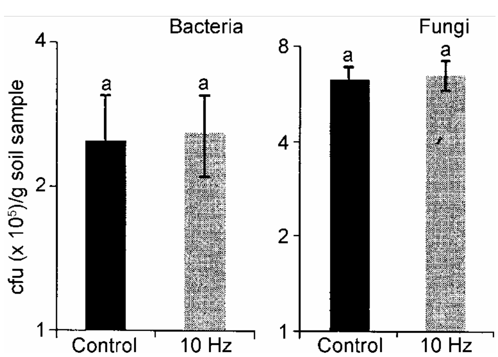

Fig. 5—Soil bacterial and fungal population from control and MF (10 Hz) treated plants grown soil [Values represent means of 6 replicates and values marked with the same letters (a) was not significantly differ according to Duncan's multiple range test ($P \leq 0.05$)]

Influence of PMF on physico-chemical properties of seed leachates

Seed weight and water content

The weight of water-soaked and dry seeds and absorbed water content was measured in control and PMF-treated seeds. The 10 Hz PMF increased the weight of water-absorbed seeds (11%) and dried seeds (6%) than control and other PMF treatments (Table 1). The absorbed water content of seeds was higher (6%) at 10 and 100 Hz, whereas 1.0 Hz reduced the water absorbability of seeds.

pH and EC of seed leachates

The gradual increase of pH towards acid to neutral range was observed in PMF-treated seed leachates (Table 1). The pH of different frequencies of PMF-treated seed leachates was acidic in nature, ranging from 6.10 to 6.76, but pH (7.0) of control seeds soaked water did not change. The leachates of PMF pre-treated seeds had slightly acidic pH (6.10-6.76) due to some of the ions released from the seeds, which also a reason to increase the conductivity of seed leachates[33]. In addition, electrical conductivity of seed leachates was higher at 0.1, 10 and 100 Hz treatments than the control. Earlier, the weak magnetic field-induced higher water uptake and conductivity of cellular membrane have been reported in *Lactuca sativa* seeds[34]. The reduced conductivity of leachates is also reported in sunflower seeds pretreated with 50, 200 and 250 mT[8], while an increase in electrolyte leakage is observed in IAA pretreated seed leachates[35]. In the present study, PMF treatment might have rearranged the membrane contents, leading to increase the water absorption and ionic currents in seeds.

Conclusion

MF treatment influences several signaling pathways and gene expression in plant cells[36], for example, it can alter the electromagnetic properties of biological molecules[37] and membranes[38] which might be a reason for enhancing the plant growth. The present study showed that 10 Hz PMF treatment enhanced seed germination, length of shoots, fresh weight of shoot and roots, leaf area, chlorophyll, protein, total soluble sugar and enzyme activities, as compared to the control plants. In addition, water absorbance of seeds and electrical conductivity of seed leachates were also higher in 10 Hz PMF-exposed plants than other treatments. PMF treatment did not affect to soil microbial population in rhizosphere soil. Thus, 10 Hz PMF treatment positively influenced the soybean plant growth and was eco-friendly to soil microbes. However, further molecular level study is needed to illustrate the mechanism of PMF on soybean crop improvement.

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