Effect of static magnetic field pretreatment on growth, photosynthetic performance and yield of soybean under water stress

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Abstract

In order to evaluate the effect of static magnetic field (SMF) on morphological and physiological responses of soybean to water stress, plants were grown under well-watered (WW) and water-stress (WS) conditions. The adverse effects of WS given at different growth stages was found on growth, yield, and various physiological attributes, but WS at the flowering stage severely decreased all of above parameters in soybean. The result indicated that SMF pretreatment to the seeds significantly increased the plant growth attributes, biomass accumulation, and photosynthetic performance under both WW and WS conditions. Chlorophyll *a* fluorescence transient from SMF-treated plants gave a higher fluorescence yield at J–I–P phase. Photosynthetic pigments, efficiency of PSII, performance index based on absorption of light energy, photosynthesis, and nitrate reductase activity were also higher in plants emerged from SMF-pretreated seeds which resulted in an improved yield of soybean. Thus SMF pretreatment mitigated the adverse effects of water stress in soybean.

Additional key words: carbon metabolism; dry mass; magneto-priming; nitrogen metabolism; PSII efficiency.

Introduction

In persistently changing environment, there are many adverse stress conditions, such as cold, drought, salinity, and UV-B, which influence plant growth and crop production greatly (Shao *et al.* 2007). Drought is a worldwide problem that seriously affects crop production and quality (Passioura 2007). Water deficiency has a profound impact on ecological and agricultural systems and is a limiting factor in the initial phase of plant growth and establishment (Shao *et al.* 2009). Water deficit at certain times of the growing season can be compensated after rewatering so that crop growth and yield are sustained (Shan and Zhang 2006).

Under stress conditions, the plants present a series of changes in their morphology, physiology, and biochemistry, negatively affecting their growth and productivity. In various parts of the world, the impacts of water stress on crop plants can reduce productivity by 50% (Lisar *et al.* 2012). According to Gerten and Rost (2010), twothirds of world food production through cultivation occurs under water stress. The stress induced by water deficits in plants depends on the conditions provided by the environment, varying according to the intensity and duration of water deficits, the rate of water-deficit induction, and the stage of plant development when drought occurs (Pinheiro and Chaves 2011, Bertolli *et al.* 2012).

Magneto-priming is one of the techniques of modern agronomic practices to improve seed vigour, growth, and yield of plants; it is an efficient and eco-friendly technology based on physical treatments of seeds. SMF is an inevitable environmental factor for plants on the Earth. In several plant species, the speed of germination and seedling growth increases by pre-germination exposure of the dry seed to an introduced magnetic field (Shine *et al.* 2011, Krawiec *et al.* 2013, Mahajan and Pandey 2014). Several previous studies also demonstrated that SMF pretreatment could alleviate the harmful effect of other stresses, such as heat (Ružič and Jerman 2002), cadmium (Chen *et al.* 2011), and salinity (Thomas *et al.* 2013, Baghel *et al.* 2016).

Climate change results in increased drought stress

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Abbreviations: Car – carotenoids; Chl – chlorophyll; DAE – days after emergence; DM – dry mass; FS – flowering stage; PI_{abs} – performance index based on absorption of light energy; P_N – net photosynthetic rate; PFS – pod-filling stage; PLS – pod lengthening; ROS – reactive oxygen species; SMF – static magnetic field; UT – untreated seeds; VS – vegetative stage; WW – well watered; WS – water stress.

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and water scarcity is already the abiotic stress most limiting crop production (Wallace 2000); it is particularly pertinent to the drought-sensitive legumes (Liu *et al.* 2005). For example, a moderate level of water deficit can reduce soybean production by approximately 40% (Manavalan *et al.* 2009), thus limiting the production of soybean vegetable oil, protein feed or biodiesel (Friedman and Brandon 2001). Soybean production generally depends on natural rainfall, but uneven distribution of rainfall (too much or too little) causes year-to-year yield variation in the same field. Soybean (*Glycine max* L. Merrill) has excelled as a major oilseed crop. It is one of the major crops of the world meeting half the global

Materials and methods

Plant material: The seeds of soybean (Glycine max L. Merrill cv. JS 335) were obtained from the Directorate of Soybean Research, Indore, India. The experiments were conducted on the terrace of Department of Life Sciences, Devi Ahilya University, Indore, India (latitude 22°43'N) during August - November 2015. The SMF-treated and untreated moistened seeds of soybean var. JS-335 were also treated with recommended fungicides, i.e., Bevistin and Diathane M at 2 mg kg⁻¹(seed), and then the seeds were inoculated with powder of Rhizobium culture (National Fertilizer Limited, New-Delhi, India) with 3 g kg⁻¹(seed) before sowing. The *Rhizobium* (*R. japonicum*) culture was sprinkled well to the moistened soybean seeds so that all the seeds were properly coated with it. The seeds of uniform size and shape were sown in plastic nursery bags $(34 \times 34 \text{ cm})$ filled with a mixture of thoroughly sieved soil, sand, and farm-yard manure in the proportion of 2:2:1 by volume. Five plants of uniform size were maintained in each bag.

Magnetic field pretreatment to seeds: An electromagnetic field generator Testron EM-20 (Testron Instruments, Delhi, India) with variable horizontal magnetic field strength (50–500 mT) with a gap of 5 cm between pole pieces was fabricated (Vashisth and Nagarajan 2008). The pole pieces were cylindrical in shape with 9 cm in diameter and 16 cm of length. The number of turns per coil was 3,000 and the resistance of the coil was 16 Ohm. A direct current (DC) power supply (80 V/10 A) with continuous variable output current was used for the electromagnet. A digital Gauss meter model DGM-30 (Testron Instruments, Delhi, India) operating on the principle of Hall effect monitored the field strength produced in the pole gap. The probe made up of indium arsenide crystal encapsulated by nonmagnetic sheet of $5 \times 4 \times 1$ mm; this probe can measure SMF from 0–2 T with a full scale range in increments of 5 mT.

Soybean seeds were exposed to a SMF of 200 mT (for 1 h) in a cylindrical-shaped sample holder of 42 cm^3 capacity, made from a nonmagnetic thin transparent plastic

demand for vegetable oils and proteins. Keeping in mind the significant effect of SMF treatment, the objective of the present study was to examine whether the pretreatment of seeds with SMF could alleviate the adverse effects of water stress on growth, various physiological attributes, and yield in soybean plants. No information is available in this respect for soybean under water stress. To the best of our knowledge this is the first study on the SMF effects on growth, dry mass accumulation, photosynthesis together with chlorophyll (Chl) fluorescence-related parameters and yield in soybean under water stress given at different growth stages.

sheet. For SMF treatment, hundred apparently healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field. By regulating the current in the coils of the electromagnet, the required strength of the magnetic field was obtained. A Gauss meter was used to measure the strength of the magnetic field between the poles. At low field (50 mT), from centre to end of the poles, the variation was 0.6% in the horizontal direction and 1.6% in the vertical direction of the applied field. At high field (500 mT), they were 0.4 and 1.2% of the applied field, respectively. The local geomagnetic field was less than 6 mT. Treatment by 200 mT (for 1 h) was found to be the most beneficial dose of SMF for improving physiological characteristics in soybean (Shine et al. 2011) under normal ambient conditions, so in the present study we used such a treatment for soybean seeds under drought stress. All treatments were run simultaneously along with control under similar conditions.

Effect of SMF pretreatment of soybean seeds under water stress (WS) under field conditions: The irrigation was ceased during four stages, such as vegetative, reproductive, grain filling, and maturity stage. The climate was characterized by a mean yearly air temperature of 25°C and a total annual rainfall of 1,062 mm. During the study period the mean air temperature was 24.5°C, total rainfall was 605 mm.

Stage		DAE	Time of stress aplication
VS	Vegetative	30	Four fully expanded leaves
FS	Reproductive	40	At the beginning of flowering
PLS	Grain filling	50	The first pod appeared
PFS	Maturity	60	The seed formation started

For all these stages, the irrigation was stopped for 5 d. Then the plants were rewatered untill their maturity. Another group of control plants was watered daily and termed as well-watered plants (WW). **Plant growth**: All growth attributes were measured in plants emerged from SMF (200 mT for 1 h)-pretreated and untreated seeds (UT) under WW and WS conditions at different stages of their development. The aboveground growth attributes of soybean, such as plant height and leaf area, were measured at VS, FS, PLS, and PFS. The plants were removed from the plastic nursery bags along with the soil and adhering soil particles were gently removed. Plant height was measured from the soil to shoot tip level. The area of third trifoliate leaf was measured using portable laser leaf area meter *CID 202* scanning planimeter (*CID Bio-Science*, NW Camas, WA).

The belowground growth attributes of soybean, such as root length and root dry mass (DM) per plant, were taken at VS, FS, PLS, and PFS. Roots were taken out carefully, washed, and the root length was measured. For root DM, plant roots with nodules were dried at 60°C for 72 h and weighed.

Pigment content: Photosynthetic pigments were extracted by dimethyl sulfoxide (DMSO) (Merck, Mumbai, India) (Hiscox and Israelstam 1979) from the third trifoliate leaves of 30 and 40-d-old soybean plants emerged from SMF and UT seeds under WW and WS conditions (at VS and FS on 30 and 40 DAE, respectively). For the extraction of Chl and carotenoids (Car), 25 mg well-cleaned fresh leaf discs were transferred in to 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 measured at 470, 649, and 665 nm with Shimadzu UV/VIS 1601 spectrophotometer (Kyoto, Japan). Equations of Wellburn and Lichtenthaler (1984) were used to calculate the Chl a, Chl b, total Chl, and Car concentrations. Chl content was expressed as mg $g^{-1}(\text{leaf FM})$. The analysis of pigments was done in triplicates; five leaves from each replicate were taken for the extraction of the pigments.

Chl a fluorescence transient exhibited by dark-adapted (30 min) leaves was measured by a Handy PEA fluorimeter (Hansatech Instruments, Pentney, King's Lynn, UK) in the third trifoliate leaves of 30 and 40-d-old soybean plants that emerged from SMF (200 mT for 1 h)-pretreated and UT seeds under WW and WS conditions (at VS and FS on 30 and 40 DAE, respectively). The transients were induced by red light (peak at 650 nm) of 600 W m⁻² (3,200 μ E $m^{-2} s^{-1}$) 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 μ s for the first 2 ms and every 1 ms thereafter (Strasser *et al.* 1995). All the measurements were recorded at $25 \pm 1^{\circ}$ C. The Chl a fluorescent transient, when plotted on a logarithmic scale clearly showed a polyphasic fluorescence rise kinetics (O-J-I-P phase). The fluorescence intensity at 20 µs was considered as the intensity F_o (O phase) when all reaction centers are open, the fluorescence intensity at 2 ms was J phase, 30 ms was I phase, and the maximum fluorescence (F_m) was the P phase. (F_p equals here to F_m since the excitation intensity is high enough to ensure the closure of all reaction centers of PSII).

We calculated the quantum efficiencies, such as maximum quantum yield of PSII photochemistry (F_v/F_m), which is equal to the efficiency by which absorbed photon will be trapped by the PSII reaction center with the resultant reduction of Q_A to Q_A^- (TR_o/ABS), phenomenological fluxes, such as electron transport per leaf CS (ET_o/CS_m), and the performance index based on absorption of light energy (PI_{abs}). All of these parameters were measured using the software *Biolyzer HP 3* (Chl fluorescence analyzing program by *Bioenergetics Laboratory*, University of Geneva, Switzerland).

Leaf gas exchange: Gas-exchange parameters, *i.e.*, CO₂ assimilation (P_N), intracellular CO₂ concentration (C_i), stomatal conductance (g_s), and rate of transpiration (E) were recorded in each treatment by using a portable infra red gas analyzer (*L1-6200*, *L1COR*, Lincoln, Nebraska) on third trifoliate leaves of intact plants that emerged from SMF (200 mT for 1 h)-pretreated and UT seeds under WW and WS conditions (at VS and FS on 30 and 40 DAE, respectively) at midday between 11:00 and 12:00 h. The gas-exchange estimations were measured on the same leaves for which O–J–I–P test parameters were calculated. Photosynthetic measurements were made under ambient temperature and CO₂ concentration on clear days in each treatment, PPFD was 1,300–1,600 µmol m⁻² s⁻¹, air flow of 500 µmol s⁻¹, and CO₂ concentration of 350–380 ppm.

Nitrate reductase (NR) activity and crop yield: Nitrate reductase (EC 1.6.6.1) activity was determined by the intact tissue assay method of Jaworski (1971) in the third trifoliate leaves of soybean plants emerged from SMF (200 mT for 1 h)-pretreated and UT seeds under WW and WS conditions (at VS and FS on 30 and 40 DAE, respectively). Chopped leaf pieces (100 mg) were incubated for 2 h at 30°C in a 10 ml of reaction mixture, which contained 2.5 ml of 0.1 M phosphate buffer, 0.5 ml of 0.2 M potassium nitrate, and 2.5 ml of 5% isopropanol (*Merck*, Mumbai, India). The nitrite formed subsequently was determined at 540 nm after azocoupling with sulphanilamide (*HiMedia*, Mumbai, India) and naphthylenediamine dihydrochloride (*Sigma Aldrich*, Mumbai, India). The NR activity was expressed as $nM(NO_2) g^{-1}(FM) h^{-1}$.

All yield attributes were measured at harvest maturity (120 DAE) of soybean plants emerged from SMF (200 mT for 1 h)-pretreated and UT seeds under WW and WS conditions at VS, FS, PLS, PDS at 30, 40, 50, and 60 DAE, respectively. Yield attributes including total dry mass (DM) accumulation, number of pods per plant, seed mass per plant, and 100-seed mass were taken at the crop maturity in three replicates of five plants each.

Statistical analysis: All the data are presented in triplicates (n = 3), five plants from each replica were taken for the recording of all parameters studied. The data are

expressed as means \pm SE and analyzed by the *Student*'s *t*-test by using *Microsoft Excel*.

Results

Growth and DM: All the aboveground and belowground growth attributes of soybean were measured at different stages of WS and under WW conditions. Pre-sowing treatment with SMF was effective in improving all the measured aboveground and belowground growth parameters in both WW and WS conditions.

The severe reduction in aboveground and belowground growth attributes was found when WS was given at FS (40 DAE) as compared with WW (Fig. 1A-C) in the plants emerged from UT seeds. It caused 51% reduction in plant height and 38% reduction in leaf area as compared with WW conditions in plants, which emerged from UT seeds (Fig. 1A,B), while the plants that emerged from SMF-pretreated seeds showed an enhancement in all these parameters as compared with UT seeds at different stages and WS. Plant height was enhanced by 49, 36, 78, and 51% at VS, FS, PLS, and PFS, respectively, by SMF as compared with UT seeds (Fig. 1A). Leaf area was enhanced by 31, 42, 53, and 50% at VS, FS, PLS, and PFS, respectively (Fig. 1B).

Belowground growth attributes, *i.e.*, root length and root DM, also decreased in plants emerged from UT seeds

under WS conditions at all the stages VS, FS, PLS, and PFS as compared with WW conditions. The significant reduction of 25% in root length and 32% in root DM was found when WS was given at FS stage as compared with WW conditions in the plants emerged from UT seeds (Fig. 1*C*,*D*), while SMF treatment increased both the root length and root DM as compared with UT seeds under WS as well as WW conditions. Root length of SMF-treated seeds was enhanced by 48, 55, 59, and 45% at VS, FS, PLS, and PFS conditions, respectively (Fig. 1*C*). Root DM was also enhanced by 38, 47, 56, and 41% in SMF-treated seeds at VS, FS, PLS, and PFS, respectively, as compared with UT seeds (Fig. 1*D*).

Pigment content: Enhancement in Chl contents was observed in plants that emerged from SMF-treated seeds under WS conditions as well as WW conditions. The SMF treatment enhanced the total Chl content by 40% at FS as compared with UT seeds under WW conditions (Fig. 2*C*). This enhancement at FS in the content of total Chl occurred due to the greater increase in Chl *b* by 82% rather than due to Chl *a* (25%) (Fig. 2*A*,*B*).



Fig. 1. Impact of prior-sowing exposure of soybean seeds to static magnetic field (200 mT for 1 h) on plant height (*A*), leaf area (*B*), root length (*C*), and root dry mass (*D*) under water stress at different stages [vegetative stage (VS), flowering stage (FS), pod-lengthening stage (PLS), and pod-filling stage (PFS)]. The vertical bar indicates \pm SE for mean analyzed by the *Student's t*-test: $^{\#\#}p<0.001$; $^{\#}p<0.01$; $^{\#}p<0.05$ denote significant differences between well watered control with water stress given at different growth stages of soybean plants that emerged from untreated seeds and $^{***}p<0.001$; $^{**}p<0.05$ denote statistically significant differences between SMF-pretreated and untreated seedlings grown under the same stage of water stress in well-watered as well as water-stress conditions.



Fig. 2. Impact of prior-sowing exposure of soybean seeds to static magnetic field (200 mT for 1 h) on chlorophyll (Chl) *a* (*A*), Chl *b* (*B*), total Chl (*C*), and total carotenoids (Car, *D*) under water stress at VS and FS on 30 d after emergence (DAE) and 40 DAE, respectively. The vertical bar indicates \pm SE for mean analyzed by the *Student*'s *t*-test. ###p<0.001; #p<0.01; #p<0.05 denote significant differences between control and water stress given at differences between SMF-pretreated and untreated seedlings grown under the same stage of water stress in well-watered as well as water-stress conditions.



Fig. 3. Changes in polyphasic chlorophyll *a* fluorescence (O-J-I-P) transient curves in soybean leaves from untreated seeds and after pretreatment of seeds with static magnetic field (200 mT for 1 h) under water stress at VS on 30 d after emergence (DAE) (*A*) and FS on 40 DAE (*B*). (O–J–I–P are fluorescence yield at 20 µs, 2 ms, 30 ms, and maximum fluorescence, respectively).

Under WS conditions SMF treatment showed 72% promotion at VS and 34% promotion at FS in total Chl as compared with UT plants. The same trend was obtained in Car; after SMF treatment, 39% promotion was found when WS was given at VS on 30 DAE and 25% promotion was found when WS was given at FS on 40 DAE (Fig. 2*D*).

Chl *a* fluorescence: Polyphasic Chl *a* fluorescence transient was measured to evaluate the effect of presowing exposure of SMF on photochemical efficiency of PSII under water stress conditions. The time course of fluorescence yield in dark-adapted intact leaves of soybean was plotted on logarithmic time scale (Fig. 3A, B); it clearly showed the separation of O–J–I–P phase in plants emerged from UT and SMF-treated seeds under WW and WS at VS on 30 DAE (Fig. 3A). However, the O–J–I–P phase was not at all distinguished when water stress was given at FS on 40 DAE in leaves of soybean plants emerged from



B Water stress given at Flowering Stage [FS] on 40 DAE

Fig. 4. Leaf model showing phenomenological energy fluxes per excited cross section (CS) of soybean leaves from untreated seeds (*A*) and after pretreatment with static magnetic field (200 mT for 1 h) (*B*). ABS/CS_m – absorption flux per CS, approximately F_m ; TR_o/CSM – trapped energy per CS; ET_o/CS_m – electron transport flux per CS; DI_o/CSM – dissipated energy per CS. Each relative value is represented by the size of the proper parameters (*arrow*), *empty circles* represent reducing Q_A reaction centres (active), *full circles* represent nonreducing Q_A reaction centres (inactive or silent).

UT seeds (Fig. 3*B*). While at the same stage of WS, the damage was very small in the plants emerged from SMF-treated seeds (Fig. 3*B*) as compared with the plants emerged from UT seeds. Thus, fluorescence yield at J, I, and P phase increased in the plants emerged from SMF-treated seeds as compared with the plants emerged from UT seeds under WS at FS on 40 DAE (Fig. 3*B*).

A phenomenological leaf model generated by *Biolyzer HP* 3 software depicted more active reaction centers per unit of area of the leaf in the plants after SMF treatment. In this model, open circles represent the active reaction center; the plants after SMF treatment had more active reaction centers combined with higher efficiency of electron transport indicated by broader width of the arrow in the leaf models under WW as well as WS at VS and FS (Fig. 4*A*,*B*).

The maximal quantum efficiency of PSII (calculated from F_v/F_m) was only marginally decreased at VS (Fig. 5*A*), while it was drastically reduced when the WS was given at FS on 40 DAE (Fig. 5*A*). The F_v/F_m did not show any significant difference between SMF-treated plants and UT plants in WW as well as WS conditions at VS (Fig. 5*A*). On the other hand, the SMF-treated plants recovered from WS as it was shown by significant increase in F_v/F_m as compared with UT plants under WS at VS and FS (Fig. 5*A*).

Under WS, the electron transport per leaf CS (ET_o/CS_m) decreased more at FS in leaves of UT plants, while

SMF treatment caused significant promotion of 43% at FS (Fig. 5*B*). The PI_{abs}, which is an indicator of sample vitality, decreased drastically by 78% when WS was given at FS in leaves of the plants, which emerged from UT seeds (Fig. 5*C*). PI_{abs} was significantly enhanced by SMF treatment; the enhancement of 189% was found under WS given at FS as compared with UT plants (Fig. 5*C*).

Leaf gas exchange: The P_N , g_s , and E in SMF-treated plants were repeatedly higher in comparison to UT plants in WW as well as WS conditions at VS and FS (Fig. 6*A*–*C*). C_i was found to be slightly higher in the plants from UT seeds as compared to SMF-treated seeds (Fig. 6*D*).

An increase of 51 and 49 % (VS and FS, respectively) in $P_{\rm N}$ and 38 and 50% enhancement in $g_{\rm s}$ (VS and FS, respectively) was recorded in the plants that emerged from SMF-treated seeds as compared with UT seeds in WW conditions (Fig. 6*A*,*B*). WS reduced these parameters as compared to WW conditions. WS given at VS caused 82 and 62% reduction in $P_{\rm N}$ at VS and FS, respectively, as compared with WW conditions (Fig. 6*A*).

The SMF treatment caused 38 and 92% in P_N , 29 and 81% enhancement in g_s , and 35 and 26% enhancement in E under WS conditions at VS and FS conditions, respectively, as compared to their UT plants under WW (Fig. 6*A*–*C*). However, the SMF treatment caused a slight reduction of 12 and 16% in C_i at VS and FS stage, respectively, as compared with UT plants (Fig. 6*D*).





Fig. 5. Impact of prior-sowing exposure of soybean seeds to static magnetic field (SMF) (200 mT for 1 h) on Fv/Fm (*A*), ETo/CSm (*B*), and PI_{abs} (*C*) under water stress at VS on 30 d after emergence (DAE) and FS on 40 DAE. The vertical bar indicates \pm SE for mean analyzed by the *Student*'s *t*-test: ###p<0.001; #p<0.05 denote significant differences between control and water stress given at different growth stages of soybean plants that emerged from untreated seeds and ***p<0.001; *p<0.01; *p<0.05 denote setuce setuce statistically significant differences between SMF-pretreated and untreated seedlings grown under the same stage of water stress in well-watered as well as water-stress conditions.

Fig. 6. Impact of prior-sowing exposure of soybean seeds to static magnetic field (SMF) (200 mT for 1 h) on net photosynthetic rate (P_N, A) , stomatal conductance (g_s, B) , transpiration rate (E, C), and intracellular CO₂ concentration (C_i, D) under water stress at VS on 30 d after emergence (DAE) and FS on 40 DAE. The vertical bar indicates \pm SE for mean analyzed by the *Student*'s t-test: *### p*<0.001; ## p < 0.01; # p < 0.05 denote significant differences between control and water stress given at different growth stages of soybean plants that emerged from untreated seeds and *p<0.001; **p<0.01; *p<0.05 denote statistically significant differences between SMFpretreated and untreated seedlings grown under the same stage of water stress in wellwatered as well as water-stress conditions.

STATIC MAGNETIC FIELD AMELIORATES WATER STRESS IN SOYBEAN



Fig. 7. Impact of prior-sowing exposure of soybean seeds to static magnetic field (SMF) (200 mT for 1 h) on NR activity under water stress at VS on 30 d after emergence (DAE) and FS on 40 DAE. The vertical bar indicates \pm SE for mean analyzed by the *Student*'s *t*-test: ###p< 0.001; ##p< 0.01; #p<0.05 denote significant differences between control and water stress given at different growth stages of soybean plants that emerged from untreated seeds and ***p<0.001; **p<0.01; *p<0.05 denote statistically significant differences between SMF-pretreated and untreated seedlings grown under the same stage of water stress in well-watered as well as water stress conditions.



Fig. 8. Effect of prior-sowing exposure of soybean seeds to static magnetic field (SMF) (200 mT for 1 h) on total dry mass [vegetative stage (VS), flowering stage (FS), pod-lengthening stage (PLS), and pod-filling stage (PFS)]. The vertical bar indicates \pm SE for mean analyzed by the *Student*'s *t*-test: ### p<0.01; ## p<0.01; # p<0.05 denote significant differences between control and water stress given at different growth stages of soybean plants that emerged from untreated seeds and ***p<0.001; ** p<0.01; *p<0.05 denote statistically significant differences between SMF-pretreated and untreated seedlings grown under the same stage of water stress in well-watered as well as water-stress conditions.

NR activity and yield: NR activity was measured in the leaves of soybean, when water stress was given at VS on 30 and FS on 40 DAE. Plants emerged from UT seeds showed lower NR activity which was significantly decreased (38%) under WS at FS, whereas the plants that emerged from SMF-treated seeds showed higher NR activity under WS as well as under WW conditions (Fig. 7). Plants emerged from SMF-treated seeds showed 29 and 31% increase in NR activity under WS given at VS on 30 and at FS on 40 DAE, respectively, as compared to their UT counterparts at both the stages (Fig. 7).

All the yield parameters, such as number of pods, number of seeds per plant, seed mass per plant, 100-seed mass, and total DM, were significantly reduced under WS conditions given at all the stages (VS, FS, PLS, and PFS) in soybean plants from UT seeds (Fig. 8), while the plants

Discussion

Water stress is an important environmental factor that could influence the physiological and biochemical characteristics of plants (Ren et al. 2007). Water stress retards the growth and metabolic activity in soybean genotypes (Sepanlo et al. 2014, Khan et al. 2016). Our results also showed that water stress given at different growth stages (VS, FS, PLS, and PFS) caused reduction in growth, photosynthetic efficiency, and yield; it was also observed when the water stress was given at FS stage on 40 DAE - then it caused severe inhibitory effects on growth and yield of soybean. The effect of water deficit during reproductive growth was more pronounced than that during vegetative growth in rapeseed (Ghobadi et al. 2006). The inhibitory effect of the drought stress was reported on the rate of photosynthesis, growth, and particularly the seed yield of soybean plants was severely affected (Heidarzade et al. 2016).

Result of the present study indicated a positive impact of SMF of 200 mT for 1 h on plant growth, PSII efficiency, photosynthesis, nitrogen metabolism, and yield under WW as well as WS conditions. The positive effect of SMF on the germination, growth, and yield of soybean was clearly evident under both normal and saline conditions (Thomas *et al.* 2013, Baghel *et al.* 2016). Under WS conditions, magnetic field treatment induced growth improvement in chickpea (Vashisth and Nagarajan 2010) and maize (Javed *et al.* 2011, Anand *et al.* 2012). However, nitrogen metabolism and yield attributes along with photosynthetic performance were not explored in the earlier studies on the effect of magnetic field under drought stress in maize seedlings (Javed *et al.* 2011, Anand *et al.* 2012).

In the present study, Chl *a*, Chl *b*, and total Chl decreased under water stress in all developmental stages in accordance with Loggini *et al.* (1999), Younis *et al.* (2000), and Elsheery and Cao (2008). Considerable reduction in Chl content due to water deficit was reported in some crop species (Ashraf *et al.* 1994, Garg *et al.* 1998). The decrease in Chl under drought stress occurred mainly

from SMF pretreatment showed a significant enhancement in all of these parameters under WS as well as WW conditions (Fig. 8).

The enhancement in number of pods by SMF treatment was 23, 97, 42, and 51% as compared with UT at VS, FS, PLS, and PFS stages of WS, respectively (Fig. 8*B*). Promotion recorded in seed mass per plant by SMF treatments was 56, 116, 24, and 23%, respectively, at VS, FS, PLS, and PFS stages of WS as compared with UT controls (Fig. 8*C*). The enhancement in a 100-seed mass by SMF treatment was 50, 112, 50, and 43% compared with UT control at VS, FS, PLS and PFS stages of WS, respectively (Fig. 8*D*). Similarly, total DM (above- and belowground parts of plants) was enhanced by SMF treatment as compared with UT under WS as well as WW conditions (Fig. 8*A*).

because of damage to chloroplasts by active oxygen species (Smirnoff 1995). Our data also showed that the total Chl content was enhanced in leaves of soybean plants emerged from SMF treatment under WW as well as WS conditions (at VS and FS). Chl *b* significantly increased, while Chl *a* increased to a lesser extent in plants emerged after SMF pretreatment. Similarly, SMF treatment enhanced Chl content in soybean and maize leaves (Kataria *et al.* 2015, Baghel *et al.* 2016)

Chl a fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis (Kalaji et al. 2016). The JIP test gives adequate information about the structure, conformation, and function of the photosynthetic apparatus. Since shape changes in O-J-I-P fluorescence transients can be translated to quantitative changes of several parameters, JIP parameters are very useful for in vivo investigation of photosynthetic apparatus, especially of PSII (Strasser et al. 2004). The time course of fluorescence yield in darkadapted intact third trifoliate leaves (under WW and WS conditions at VS and FS) plotted on logarithmic time scale showed the separation of O-J-I-P phase (Fig. 3A,B). Polyphasic Chl a fluorescence (O–J–I–P) transients of the leaves in plants emerged from SMF treatment gave a higher fluorescence yield at I and P phase when the WS was given at FS stage as compared with the plants emerged from untreated seeds (Fig. 3B). The J step represents the momentary maximum of QA-, and I is suggested to be related to a heterogeneity in the filling up of the plastoquinone pool (Govindjee 1995, Strasser et al. 1995). The I-P phase seems to be related to the content of PSI reaction centers (Ceppi et al. 2012). However, a close link between PSI kinetics and the OJIP rise can also be explained on the basis of the PSII conformational change hypothesis (Schansker et al. 2014).

Results on Chl *a* fluorescence reported in present study revealed that leaves of soybean plants that emerged from UT seeds under WS (both at VS and FS) showed lower efficiency of electron transport per cross section of leaf with higher inactive reaction centers, while on other hand, plants emerged from SMF-treated seeds had higher reducing power with higher efficiency of electron transport and more active reaction centers under WS conditions at VS and FS as well as WW conditions. Lazár *et al.* (2013) found that exogenous melatonin increased quantum yield of photochemistry of PSII due to the amount of open reaction centres of PSII and suggested that more open reaction centres reflect better functionality of all photosynthetic transport chain constituents.

Pretreatment of corn seeds with different electromagnetic treatments, particularly by 100 and 150 mT for 10 min, significantly alleviated the drought-induced adverse effects on growth by improving efficiency of PSII, $P_{\rm N}$, E, and $g_{\rm s}$ (Javed et al. 2011). In a similar experiment under greenhouse conditions, it was found that photosynthesis, g_s, and Chl content increased in maize plants exposed to SMFs of 100 mT for 2 h and 200 mT for 1 h, compared with control under irrigated and mild-stress conditions (Anand et al. 2012). The present study differs from these previous studies on effect of SMF treatment under water stress (Javed et al. 2011), because here the water stress was given at different growth stages of soybean (VS, FS, PLS, PFS), contrary to corn seedlings, where water stress was given at one stage only. O-J-I-P transient curves and JIP-test are the key indicators for studying the response of plants to environmental stress. In our results, drought stress at FS stage in particular changed the characteristic sites of the O-J-I-P transient and reduced the flourescence intensity. Meng et al. (2016) reported that drought stress considerably decreased the fluorescence characteristics of PSII in leaves of Plectranthus scutellarioides via inhibition of electron transport and decrease of PSII photochemical activity. The parameters, which were affected most by water and salt stress, were the time needed to reach the maximal Chl fluorescence (F_m), and the F_v/F_o, and PI_{abs} (Kalaji et al. 2011, Meng et al. 2016).

Depending on the intensity and duration of water stress, metabolic limitations are frequently correlated with decrease in ATP (Parry *et al.* 2002). This reduction in ATP synthesis is due to the decrease in electron transport and photophosphorylation (Catuchi *et al.* 2011). The thylakoid membranes may suffer structural damage caused by decreasing the water content and ROS overproduction (Lawlor and Cornic 2002), reducing the efficiency of the photochemical apparatus.

In the present study, water stress at VS did not influence the F_v/F_m measured in dark-adapted leaves of soybean, while water stress at FS drastically reduced the F_v/F_m . On the other hand, SMF pretreatment enhanced F_v/F_m enormously under water stress at FS as compared with untreated controls. Plant vitality could be characterized by PI_{abs} (Strasser *et al.* 2000). This integrative parameter includes three independent parameters: (1) density of fully active reaction centers (RCs); (2) efficiency of electron movement by trapped exciton into the electron transport chain beyond the Q_A ; and (*3*) the probability that an absorbed photon will be trapped by RCs. Strasser *et al.* (2004) reported that PI_{abs} reflects the functionality of both PSI and PSII and gives the quantitative information on the current state of plant performance under stress conditions. In the present study, PI_{abs} significantly increased by the SMF treatment in the soybean plants grown under WW and WS conditions. PI is often found to be a very sensitive parameter in different crops (Strasser *et al.* 2004, Jiang *et al.* 2006, Christen *et al.* 2007, Baghel *et al.* 2016, Kataria *et al.* 2017), which is in accordance with our results achieved on soybean plants under water stress.

In the current study, water stress conditions significantly decreased the rate of photosynthesis and g_s of leaves at both the stages studied (VS and FS), while SMF treatment enhanced both under WS and WW conditions (Fig. 6). Water stress reduced the g_s , limiting the entry of CO₂ into the substomatal chambers and thus reducing the diffusion of carbon to the site of carboxylation, resulting in significant decreases in carbon assimilation (Yu *et al.* 2004). Decreased g_s due to water stress was also observed in soybean leaves by Makbul *et al.* (2011).

NR is a key enzyme of N metabolism, which converts nitrate (NO₃⁻) into nitrite (NO₂⁻) on the metabolic pathway leading to the formation of amino acids (Cánovas *et al.* 2007). In the present study, SMF treatment led to an induction in NR activity in leaves of soybean plants when compared to the untreated controls. The extent of promotion was greater at FS stage and WS. Similarly, the NR activity increased in soybean plants grown under salinity stress by SMF treatment (Baghel *et al.* 2016). The increase in NR activity by SMF treatment in the present study may be due to the increase of ATP supply through the enhancement in the activity of PSII under WS as well as WW conditions.

In the present study, the plant height, leaf area, total dry mass accumulation, efficiency of PSII, and rate of photosynthesis was promoted by SMF treatment. Due to the enhanced rate of photosynthesis and NR activity the yield of soybean improved by SMF treatment. The increase in yield was measured in term of number of pods, seed mass, and 100-seed mass.

Our results are in agreement with the earlier reports showing the increase in the yield of soybean by the SMF treatment under normal and salt stress conditions (Baghel *et al.* 2016). According to our results, water stress given at FS (40 DAE) severely decreased the number of pods, seed mass, and 100-seed mass as compared to the stress given at VS, PLS and PFS. Our data showed that if water limitation occurred during the critical stage of growth, especially, at the time of FS, then due to water deficiency, the seed yield of soybean was reduced drastically (81%) as compared with WW controls. Heidarzade *et al.* (2016) also observed that reproductive stage was most sensitive to water limitation and resulted in high reduction in pod numbers and yield loss.

The flowering and pod-setting stages were found to be the most sensitive stages to water stress in chickpea (Nayyar *et al.* 2006) and soybean (Liu *et al.* 2003).

On the other hand, the plants emerged from SMF treatment showed enhancement of 116% in the grain yield of soybean as compared to the plants emerged from untreated seeds under water stress at FS stage. Thus, SMF treatment to the soybean seeds was effective in alleviating the harmful effect of water stress. Similarly Radhakrishnan et al. (2012) reported that pulsed magnetic field treatment of soybean alleviated the harmful effect of salinity on calli growth by improving primary and secondary metabolism under salt-stress conditions. Thomas et al. (2013) reported the activity of total amylase, protease, and dehydrogenase increased by magneto-priming under both nonsaline and saline conditions. The magneto-priming of dry seeds of soybean can ameliorate the harmful effects of salt and UV-B stress on growth and yield (Baghel et al. 2016, Kataria *et al.* 2017). Increased uptake of Ca^{2+} ions in rice seedlings grown from seeds exposed to pulsed magnetic fields were responsible for greater leaf growth and meristematic tissues in stems and roots (Saktheeswari and Subrahmanyam 1989). It appears that membraneassociated Ca²⁺ transport represents a crucial step at which magnetic fields could come into play (Çelik et al. 2009, da Silva and Dobránszki 2016).

Two mechanisms of magneto-reception are currently receiving attention: (1) the "radical-pair mechanism" consisting of modulation of singlet-triplet interconversion rates of a radical pair by weak magnetic fields, (2) the "ion cyclotron resonance" that revolves around the fact that ions should circulate in a plane perpendicular to an external magnetic field with their Lamor frequencies,

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which can interfere with an alternating electromagnetic field (Galland and Pazur 2005, Maffei 2014, da Silva and Dobranszki 2016). Related to radical pair model, cryptochromes have recently been reported to supposedly participate in the magneto-reception of plants, serving as sensors since they can form radical pairs (Xu *et al.* 2014, Maffei 2014).

In summary, the water stress at each stage of the growth may lead to a reduction in the yield of soybean. Our data showed that when water stress occurred during the critical stage of growth, especially, at the time of FS, then the seed yield was reduced drastically due to water deficiency in soybean. Possible causes for more stressful influence at the reproductive stages are remobilization of nitrogen and carbohydrates from leaf to leaf with increasing age, degradation of chlorophyll and light harvesting complexes (Momeni 2010). We found the significant changes in morphological and physiological characteristics of soybean plants after pretreatment with SMF under water-deficit conditions, which underline the susceptibility of this crop to drought stress. Based on the present results, we can conclude that water stress retarded the growth, metabolic activity, and the yield of soybean, whereas SMF-treated seeds showed a significant promotion in all the studied parameters under wellwatered as well as water-stress conditions. SMF pretreatment to the soybean seeds (200 mT for 1 h) significantly alleviated the water stress-induced adverse effects on growth, PSII efficiency, NR activity, P_N, and yield of soybean. This study may help to understand some adaptive mechanisms developed by soybean genotypes and contribute to identify useful traits for soybean breeding programmes under abiotic stresses.

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