

## Impact of Pre-Sowing Exposure of Seeds to Stationary Magnetic Field on Nitrogen and Carbon Metabolism in Maize and Soybean

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**ABSTRACT:** A field experiment was conducted to study the impact of pre-sowing exposure of maize (*Zea mays* var. JM 216) and soybean (*Glycine max* var. JS-335) seeds to static magnetic field (200 mT for 1 h) on carbon and nitrogen metabolism. Pre-sowing magnetic field treatment of seeds with 200 mT (1 h) persisted in soybean and maize plants under field conditions till its maturity. Plants that emerged after static magnetic field (SMF) pre-sowing treatment showed enhancement in plant height, leaf area and biomass accumulation in both the crops. Nitrate reductase activity, photosynthetic pigments, net rate of photosynthesis and stomatal conductance were significantly enhanced after SMF pre-sowing treatment as compared to the untreated controls. Thus pre-sowing exposure of seeds to static magnetic field enhanced the carbon and nitrogen metabolism in both the crops. Magnetopriming of dry seeds of maize and soybean can be effectively used as a pre-sowing treatment for improving plant growth and development under field conditions.

**Key Words:** Growth, Biomass, Leaf area, Magnetic field, Nitrate Reductase, Photosynthesis.

### INTRODUCTION

Agricultural sciences take an interest not only in the common and valued crop-forming factors, but also in those less expensive and generally underestimated, though more pro-ecological ones, such as ionizing, laser or ultraviolet radiation, electric and magnetic fields. Magnetic and electromagnetic treatments are being used in agriculture, as a noninvasive technique, to improve the germination of seeds and increase crops yields [1-3]. Magnetic fields (MF) were used widely as presowing seed treatments to increase seed vigour and seedling growth [2-4].

Magnetic field has different effects on plant metabolism [5]. Previous studies indicated that suitable magnetic treatment increased the absorption and assimilation of nutrients [6] and ameliorated photosynthetic activities [7]. Akoyonoglou [8] reported that the activity of carboxydismutase was increased by exposure to MF. Changes in amylase and nitrate reductase activities in germinating seeds treated by electromagnets of different field strengths were also observed [7,9]. Piacentini *et al.* [10] reported that magnetic field pretreatment had a positive effect on stimulating seedling growth and development of

cucumber. An increased number of secondary branches and yield was also observed in safflower [11] by presowing exposure of seeds to 72 mT. Electron paramagnetic resonance spectroscopy study in maize leaves showed that superoxide radical was reduced after magnetic field treatment [4]. Measurement of Chlorophyll *a* fluorescence by plant efficiency analyzer showed that the potential of processing light energy through photosynthetic machinery was enhanced by magnetic field treatment [2].

Growth, development and plants productivity are usually affected by carbon and nitrogen metabolism. Magnetic fields are known to induce biochemical changes and could be used as a stimulator for growth related reactions including affecting photosynthetic pigments and PS II efficiency [2,4]. Previous studies suggested that magnetic field might be involved in the improvement of soybean and maize plant growth due to the better harvesting of light and lower level of free radicals in leaves of plants that emerged from magnetically treated seeds [2,4]. However CO<sub>2</sub> and nitrogen metabolism in maize and soybean has not been studied.

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Nitrate reductase that is located at the junction of two energy-consuming pathways, nitrate assimilation and carbon fixation, results in a controlled response to environmental changes that affect the photosynthesis [12]. The objective of the present work was to investigate the effects of static magnetic fields on the carbon and nitrogen metabolism particularly in two economic crops like maize and soybean. In this regard, in the present study, soybean and maize seeds were exposed to static magnetic field (SMF) of 200 mT for 1h. Furthermore to evaluate the effect of SMF on growth (Plant height, leaf area, fresh weight and dry weight of plants), nitrogen metabolism (nitrate reductase) and carbon metabolism (total chlorophyll, PS II efficiency, rate of photosynthesis, stomatal conductance and internal CO<sub>2</sub> concentration) in both the crop plants maize and soybean under field conditions.

## MATERIAL AND METHODS

### Field Experiment

The breeder seeds of soybean (*Glycine max* var. JS-335) were collected from the Directorate of Soybean Research, Indore, M.P., India and seeds of *Zea mays* var. JM-216 were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara, M.P., India. The experiments were conducted on the terrace of the School of Life Sciences, Devi Ahilya University, Indore, India (latitude 22.48°N) during August'2014 to October'2014 under the ambient environmental conditions. Plants were watered daily until the end of experiments. The experiments were conducted in a randomized block design with three replicates for each treatment. The seeds of uniform size and shape were sown in plastic bags (34 cm H x 34 cm B) filled with a mixture of thoroughly sifted soil, sand and farm-yard manure in the proportion of 2:2:1 by volume.

### Magnetic field generation and Magnetic treatment

An electromagnetic field generator "Testron EM- 20" with variable horizontal magnetic field strength (50–500 mT) with a gap of 5 cm between pole pieces was fabricated (Academy of Embedded Technology, New Delhi, India). Maize and soybean seeds were exposed to a static magnetic field of 200 mT (1 h) in a cylindrical shaped sample holder of 42 cm<sup>3</sup> capacity, made from a non-magnetic thin transparent plastic sheet. Hundred visibly sound, mature healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field

for treatment by the procedure described by Vashisth and Nagarajan [13]. The SMF treatments in the experiments were run simultaneously along with untreated controls under similar conditions.

### Growth Analysis

Growth parameters such as plant height, fresh weight and dry weight of plants were measured in 50 days old plants of soybean and maize. Shoot length was measured from the soil line to shoot tip. The area of third trifoliolate leaf in soybean and third leaf in maize was measured using portable laser leaf area meter CID-202 scanning planimeter (CID Inc., USA). Only plant material above the ground was considered for fresh and dry weight. For dry weight determination, plant parts were dried in an oven at 60°C for 72 h. Five plants from each replica were randomly selected for recording these parameters.

### Determination of Nitrate Reductase Activity (NR)

NR (EC 1.6.6.1) activity was determined by the intact tissue assay method of Jaworski [14] in leaves of 50 days old plants of soybean and maize.

### Photosynthetic pigments and PS II efficiency

The total chlorophyll (Chl) content was determined by dimethyl sulfoxide (DMSO) method as described by Hiscox and Israelstam [15]. Equations of Wellburn and Lichtenthaler [16] were used to calculate the total Chl concentrations in leaves of 50 days old plants of soybean and maize.

Chlorophyll fluorescence characteristics such as maximum quantum yield of primary photochemistry ( $F_v/F_m$ ) which in this terminology is equal to the efficiency by which absorbed photon will be trapped by the PS II reaction center with the resultant reduction of  $Q_A$  to  $Q_A^-$  was measured using a Handy PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments, King's Lynn, Norfolk, UK).

### Gas exchange parameters

Net photosynthesis ( $P_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and internal CO<sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ ) were measured by a portable photosynthetic system (Li- 6200, LI-COR Inc., Lincoln, Nebraska, Serial No. PPS 1332 USA) in intact plants grown in normal sunlight in SMF treated and untreated 50 days old plants of maize and soybean under field conditions at midday between 11.00 to 12:00. Photosynthetic measurements were made under ambient temperature and CO<sub>2</sub> concentration, on clear days, photosynthetic photon flux density

(PPFD) was 1300-1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , air flow (500  $\mu\text{mol s}^{-1}$ ) and  $\text{CO}_2$  concentration (350-380 ppm).

### Statistical analysis

All the data are presented in triplicates; five plants from each replica were taken for the recording of all parameters studied. The data are expressed as means  $\pm$  S.E.M and analyzed by the analysis of variance (ANOVA) followed by post hoc Newman-Keuls Multiple Comparison Test (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) by using Prism 4 software for Windows, Graph Pad Software, Inc, LaJolla, CA, USA.

## RESULTS

### Growth

The plants emerged after SMF pretreatment showed an increase in plant height and leaf area in 50 days old maize and soybean plants under field conditions. The improvement by SMF treatment over the untreated control was 22% and 43% for plant height and 35% and 31% for leaf area respectively in maize and soybean (Fig. 1A, B).

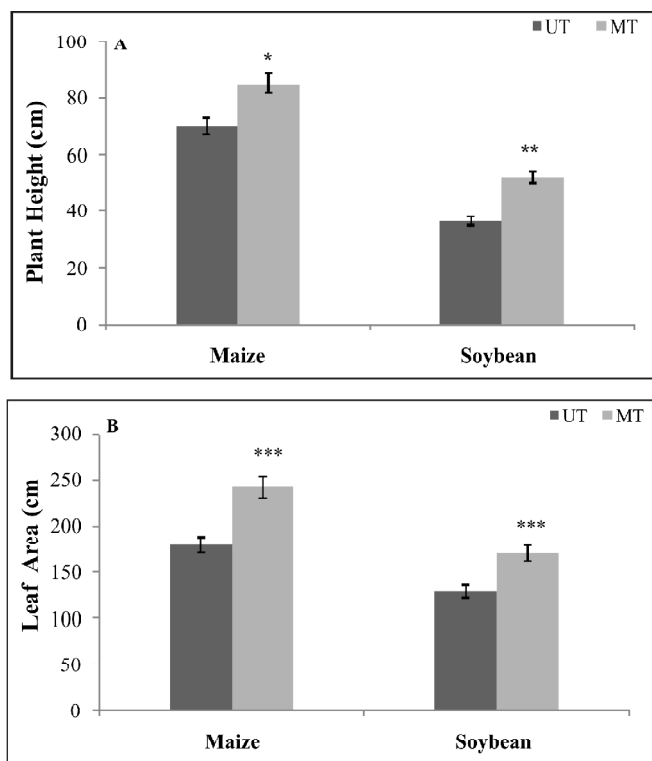


Figure 1: Impact of presowing exposure of seeds to stationary magnetic field (200 mT for 1 h) on Plant height (A) and Leaf area (B) of 50 days old maize and soybean plants. The vertical bar indicates  $\pm$ SE for mean. Values are significantly different at (\* $P < 0.05$  and \*\* $P < 0.01$ ) from untreated control (Newman-Keulis Multiple Comparison Test).

### Plant fresh weight and dry weight

Plant fresh weight and dry weight were also significantly enhanced by static magnetic field treatment as compared to untreated control plants in 50 days old maize and soybean plants. The extent of promotion in plant fresh weight was 51% and 76% and the dry weight was increased by 46% and 105% respectively in maize and soybean (Fig.2A, B).

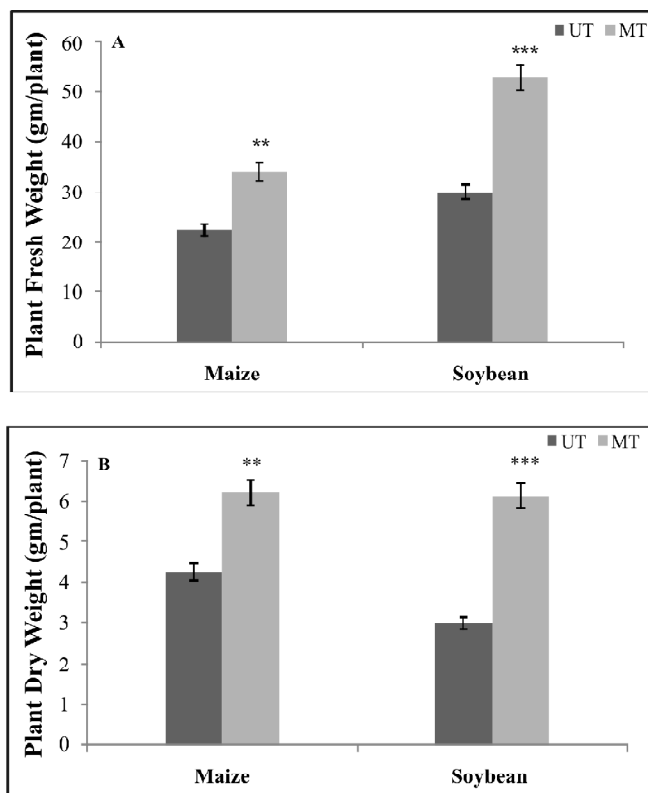


Figure 2: Impact of presowing exposure of seeds to stationary magnetic field (200 mT for 1 h) on Plant fresh weight (A) and Plant dry weight (B) of 50 days old maize and soybean plants.

The vertical bar indicates  $\pm$ SE for mean. Values are significantly different at (\*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ) from untreated control (Newman-Keulis Multiple Comparison Test).

### Nitrate reductase activity (NRA-EC 1.6.6.1)

The reduction of nitrate to nitrite ( $\text{NO}_2^-$ ) is catalyzed by nitrate reductase (NR; EC 1.6.6.4). Plants, emerged from magnetically treated seeds, showed enhanced activity of nitrate reductase; the magnitude of enhancement was more in maize (70%) than soybean (28%) (Fig. 3).

### Chlorophyll Content and PS II efficiency

Enhancement in chlorophyll content was observed in plants that emerged from magnetically treated seeds. On unit fresh weight basis, 17% and 21% increase was

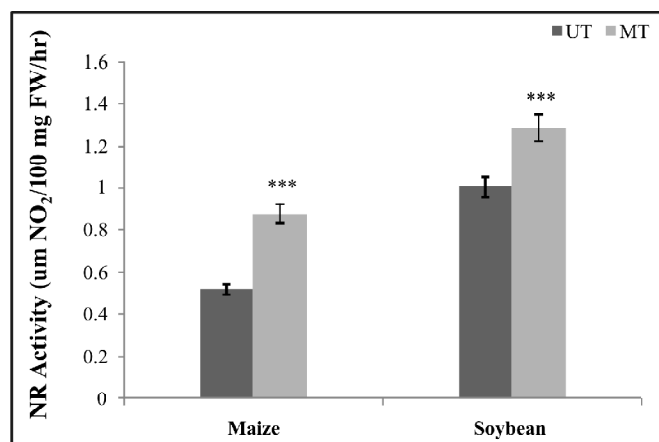


Figure 3: Impact of presowing exposure of seeds to stationary magnetic field (200 mT for 1 h) on Nitrate reductase activity of 50 days old maize and soybean plants. The vertical bar indicates  $\pm$ SE for mean. Values are significantly different at (\*\*\*)  $P < 0.001$  from untreated control (Newman-Keulis Multiple Comparison Test).

recorded in total chlorophyll content respectively in 50 days old maize and soybean plants (Fig. 4A). Enhancement in the level of chlorophyll *b* was higher than chlorophyll *a*. Due to this chlorophyll *a/b* ratio was decreased after magnetic field treatment (data not given). There was also a marginal but significant ( $P < 0.05$ ) increase in maximum quantum yield of primary photochemistry ( $F_v/F_m$ ) in SMF treated plants compared to untreated control plants of maize and soybean (Fig. 4B).

### Photosynthesis

Net rate of photosynthesis in terms of  $\text{CO}_2$  absorbed and stomatal conductance was significantly enhanced while Intercellular  $\text{CO}_2$  concentration was decreased in the 50 days old maize and soybean plants that emerged from magnetically treated seeds as compared to untreated seeds. Net rate of photosynthesis was significantly enhanced by 89% in maize and 22% in soybean (Fig. 5A); stomatal conductance was enhanced by 44% (maize) and 20% (soybean) (Fig.5B); whereas reduction of 30% and 8% was observed in inter-cellular concentration of  $\text{CO}_2$  in maize and soybean plants respectively (Fig. 5C).

### DISCUSSION

The results indicate a positive impact of static magnetic field (SMF) on carbon and nitrogen metabolism. Growth characteristics, such as plant height, leaf area, plant fresh and dry weight have been found to be promoted by pre-sowing SMF treatment (200 mT for 1h) to maize and soybean seeds in the

present study. Similar results of enhanced growth and biomass have also been recorded in soybean [2], maize [4], cucumber [17], cotton [18], sunflower [19] and chickpea [13] along-with increased germination percentage and speed of germination. Magnetic field of 0.15 T strength on maize samples led to an increase of the shoot fresh weight by 72% compared to the control [20] and EMF treatment by 10 mT intensity of wet treated seeds caused increase in fresh and dry biomass weight of maize [21]. Dayal and Singh [22] have found that an increase in height and number of primary branches when tomato seeds were exposed to magnetic fields varying from 15 to 155 mT. Soltani and Kashi [23] revealed that magnetic field increased fresh weight of lettuce root and shoot. Leaf area index, biomass and yield were increased by magnetic field pretreatment as compared to plant raised from untreated control under similar conditions [24].

In the present study, total chlorophyll was increased after treatment with static magnetic field in leaves soybean and maize plants. Effects on MF application on chlorophyll content have been

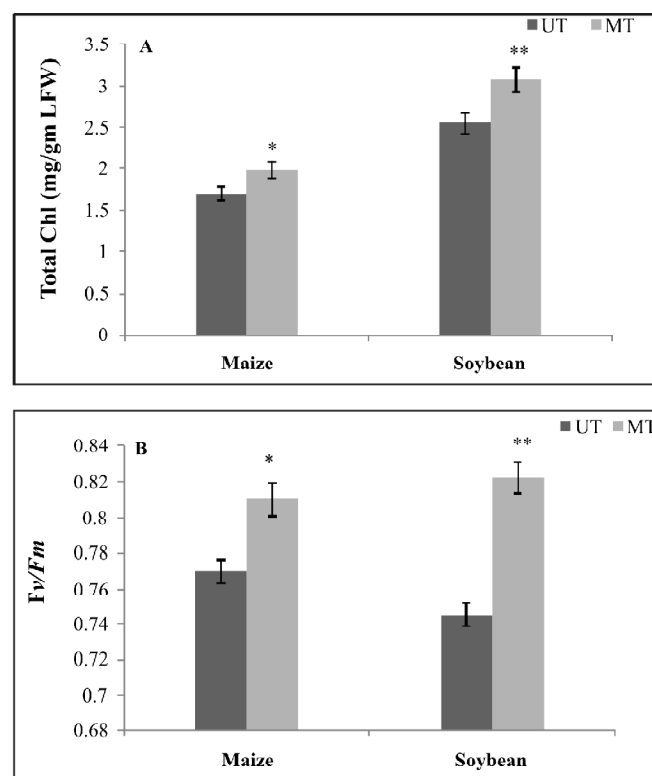


Figure 4: Impact of presowing exposure of seeds to stationary magnetic field (200 mT for 1 h) on Total chlorophyll content (A) and Maximum potential quantum yield of photosynthesis ( $F_v/F_m$ ) (B) of 50 days old maize and soybean plants. The vertical bar indicates  $\pm$ SE for mean. Values are significantly different at ( $P < 0.05$  and \*\*)  $P < 0.01$  from untreated control (Newman-Keulis Multiple Comparison Test).

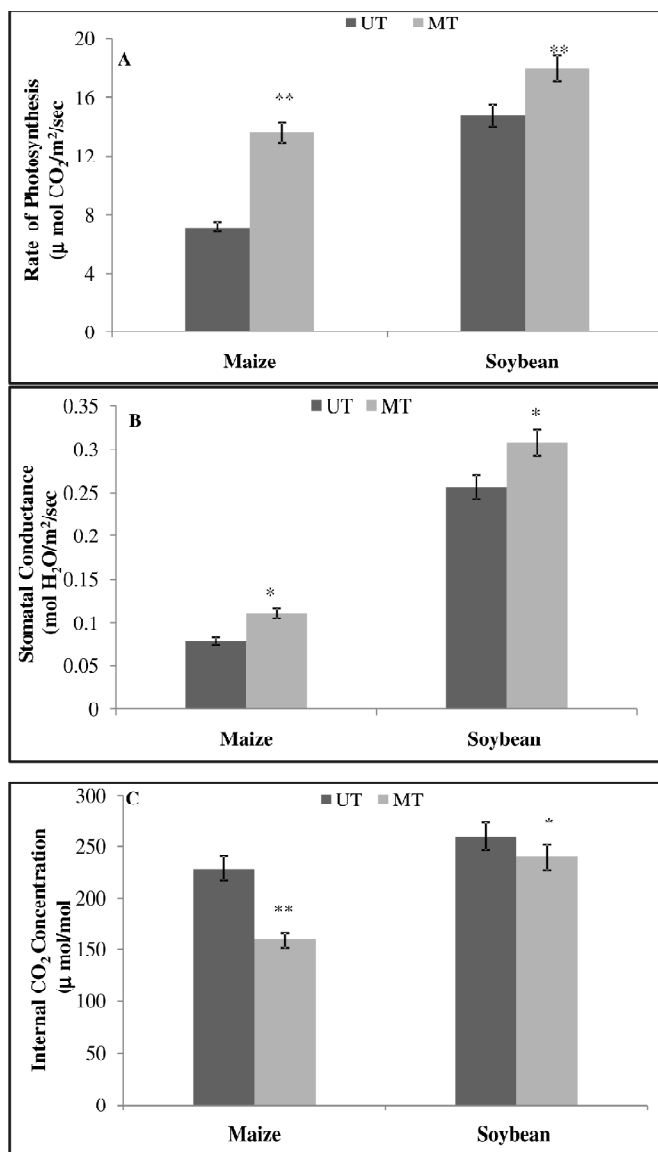


Figure 5: Impact of presowing exposure of seeds to stationary magnetic field (200 mT for 1 h) on Rate of photosynthesis (A), stomatal conductance (B) and the internal CO<sub>2</sub> concentration (C) of 50 days old maize and soybean plants. The vertical bar indicates  $\pm$ SE for mean. Values are significantly different at ( $P < 0.05$  and  $**P < 0.01$ ) from untreated control (Newman-Keuls Multiple Comparison Test).

documented for several plant species [25-27]. Similarly, magnetic fields increased photochemical activities in a unit of chlorophyll molecule resulting in an increase in the green pigment of wheat and bean [28]. Saktheeswari and Subrahmanyam [29] also observed enhancement of chlorophyll content and prevention of chlorosis when fresh paddy seeds were exposed to pulsed magnetic field. Pigments content (chlorophyll *a*, chlorophyll *b*, carotenoids and total pigments) were significantly increased under static magnetic field in date palm [30] and soybean tissue

culture [31]. In onion, a magnetic field exceeding the geomagnetic field by about 13 fold (505  $\mu$ T) stimulate the leaf length by 40% and chlorophyll and protein content by about 70 % [32]. A significant enhancement of the fresh weight and assimilatory pigments level was observed when *Zea mays* were cultivated in 50 mT static magnetic field [33].

The presowing exposure of seeds to stationary magnetic field also showed impact on nitrogen metabolism, as it has been observed through the changes in nitrate reductase enzyme activity. Our results showed significant increase in NR activity in 50 days old maize and soybean plants. Magnetic field treatment enhances the activity of nitrate reductase in leaves of 50 day old plants of soybean; this is particularly significant because nodules activity declines at this stage. Similarly, Radhakrishnan and Kumari [27] also reported enhancement in nitrate reductase in seedlings of soybean that emerged from seeds treated with pulsed magnetic field. Changes in nitrate reductase activities in germinating seeds treated by electromagnets of different field strengths were also observed by Bhatnagar and Deb [9].

Photosynthetic process is the key phase of plant metabolism which is very sensitive to environmental changes. Among the tools used to study the effects of environmental changes on the photosynthetic apparatus, chlorophyll *a* fluorescence is often proposed as a simple, rapid and sensitive method [34] which has been successfully used to monitor the changing physiological states of photosynthetic system. Our results indicate that potential quantum yield of PS II ( $F_v/F_m$ ) had significantly increased in maize and soybean plants. Similarly, chlorophyll *a* fluorescence transients from magnetically treated soybean and maize plants showed a higher fluorescence yield [2,4].

In the present study, plant emerged after SMF pre-treatment showed higher rate of photosynthesis along with increased stomatal conductance and lower intercellular concentration of CO<sub>2</sub> in maize and soybean. Rakosy-Tican *et al.* [35] found that MF of around 4mT had beneficial effects on the growth promotion and enhancement of CO<sub>2</sub> uptake of potato plantlets *in vitro*. Shine *et al.* [2] found an over expression of protein bands corresponds to a larger subunit (53KDa) and smaller sub-unit (14KDa) of Rubisco in the SMF treated plant of soybean. The enhancement in NR activity in the present study may be due to the increase in rate of photosynthesis and it may contribute to the improvement of the crop yield. Since the Nitrogen fixation will be limited by the

availability of photosynthate unless there is a simultaneous increase in the rate of photosynthesis [36]. Results of our study indicate the concomitant increase in NR activity and rate of photosynthesis in both maize and soybean plants.

In conclusion, SMF could be used as a stimulator for growth related reactions. Our results showed that the growth of maize and soybean was influenced after pre-sowing treatment of their seeds with static magnetic field of 200 mT for 1 h. This initial stimulus (SMF treatment) persisted in the plants after differentiation and led to a faster reduction of electron acceptors in the photosynthetic pathway, thereby enhancing the photosynthetic efficiency due to increased concentration of photosynthetic pigment, nitrate reductase activity and rate of photosynthesis. These increases could have important economic implication and be beneficial especially for farmers for increased yield under field conditions. Using magnetic field treatment could be a promising technique for agricultural improvements although extensive research is required at field level.

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