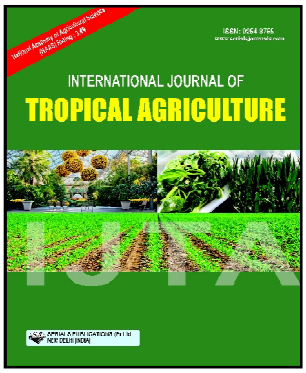

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Enhancement of Amylase Activity and Early Seedling growth in Maize Seeds after Pretreatment by Static Magnetic Field

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Abstract: Maize (*Zea mays* var: Ganga safed) seeds were treated with static magnetic fields (SMF) with magnetic field strength of 200 mT. SMF significantly enhanced the activity of á-amylase enzyme in the seeds compared to the untreated seeds when the seeds were germinated. Maximum enhancement in the activity was obtained after 72 hrs of soaking. The extent of enhancement of amylase by SMF treatment was compared with treatment of seeds with Gibberellic acid (GA) for the same duration of soaking. The enhancement of α-amylase activity by SMF treatment at 200 mT was comparable to the enhancement induced by 0.1 µg/ml GA treatment at 72 hrs. SMF treatment also enhanced the germination percentage, seedling growth and seedling vigour measured after 8 days of seed germination in darkness. SMF treatment can, thus, successfully be used for seed priming to improve germination, seedling growth and early establishment of seedlings. Enhancement in seed germination and seedling vigour by SMF appears to be due to the effect of enhanced amylase activity in the seeds.

Keywords: α-amylase, Gibberellic acid, Magnetopriming, Seed germination.

INTRODUCTION

Seeds are the link between two successive generations of plants. Seed germination is the very first step and determining factor towards the formation of new plant. There are different chemical, biochemical and physical treatments to obtain high quality seeds that subsequently allow early seed germination and also increases their rate of germination [1,2,3]. During seed germination amylase in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots [4,5].

Seed germination process is majorly stimulated and regulated by the plant hormones. Among all the

seed priming techniques treatment of seed with plant hormone increases the rate of cell division, and remove barriers, which adversely affect germination and growth. Gibberellins are such plant hormones. They are tetracyclic diterpenoid growth regulators first isolated as a metabolite from the rice pathogenic fungus, Gibberella sp, and are now widely used in modern agriculture [6,7,8]. Active GAs are responsible for the expression and secretion of alpha amylase from the aleurone layer into the endosperm to catalyze the hydrating reaction of stored starch.

Maize (*Zea mays* L.) is an important crop in the world; it is widely used for feed and industrial raw material. Maize ranks third in the world production following wheat and rice for the area and production. Apart from the chemical and biochemical priming, magnetopriming is the technique which has the ability to improve seed quality and seedling growth as well. Savostin [9] was the first researcher who observed that the growth rate of wheat seedling was increased when seeds were treated with magnetic field. Magnetically treated (MT) wheat seed had a positive effect on seedling growth and crop yield [10]. Aladjadjiyan [11] reported an increase of the germination, fresh weight and shoot length of maize plants, when seeds were exposed to a magnetic field of the intensity 150mT. MF was shown to improve germination under magnetic conditions, in tomato, corn, and rice [12, 13, 14]. The aim of the present study was to quantify the effect of SMF and different concentrations of GA on seedling length, germination percentage, vigour and alpha amylase activity of maize seeds.

MATERIALS AND METHODS

Seed material

The breeder seeds of maize (*Zea mays* var: GSF-2) were collected from Jain seed agency of Indore, M.P., India. Seeds of uniform size and shape without visible defects, and malformation were selected and surface sterilized with 0.01% $HgCl_2$ for 10 minute, washed thoroughly under tap water and finally with distilled water. Seeds were spread on moist filter paper in 15 cm petri dishes and grown in complete darkness at 26 ± 1^0 C for 8 days. After 8 days, germination data were taken.

Magnetic field generation

A Testron EM-20 electromagnetic field generator (Testron Instruments, Delhi, India) was fabricated with a variable horizontal magnetic field strength (50–500 mT) and a gap of 5 cm between pole pieces [15]. The pole pieces were cylindrical in shape with a diameter of 9 cm and a length of 16 cm. The number of turns per coil was 3000 and the resistance of the coil was 16 V. A DC power supply (80 V/10 A) with a continuous variable output current was used for the electromagnet. A digital gauss meter (Model DGM-30, Testron Instrument, Delhi, India), operating on the principle of the Hall Effect, monitored the field strength produced in the pole gap. The probe was made of indium arsenide crystals and encapsulated in a non-magnetic sheet of 5 mm × 4 mm × 1 mm, which could measure 0– 2 T with full-scale range in increments of 5 mT.

Magnetic and chemical Treatment

Maize seeds were exposed to a magnetic field of 200 mT (for 1h) in a cylindrical-shaped sample holder of 42 cm^3 capacity, made from a non-magnetic thin transparent plastic sheet. One hundred visibly sound, mature, healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field and treated for one hour. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. A gauss meter was used to measure the strength of the magnetic field between the poles. The local geomagnetic field was <10 mT. All treatments in the experiments were run simultaneously along with control under similar conditions.

The sterilized seeds were placed in Petri dishes (15 cm diameter) containing filter paper moistened with 10 ml of treatment solutions and incubated at 26 ± 1 ^{0}C in the dark. We investigated the effect of different concentration of GA on untreated control seeds. The treatments were as follows: 0.1µg/ml (GA0.1), 1.0µg/ml (GA1) and 10µg/ml (GA10).

Seed Germination

Three replications with 10 seeds each were spread on moist filter paper in 15 cm Petri dishes. They were placed in a seed germinator (Remi Instruments, Mumbai, India) at 25^{0}C in complete darkness. After 8 days, germinated seeds were grouped as normal seedlings, abnormal seedlings, and dead seeds. Normal seedlings were seedlings with well developed epicotyls, hypocotyls, and radicals whereas abnormal seedlings were seedlings with stunted radicals and weak or no epicotyls. Germination percentage was calculated based on normal seedlings. Subsequently, they were dried overnight in an oven at 60^{0}C for 72 h and their dry weight was measured. Seedling vigor was calculated following Abdul-Baki and Anderson [16] as:

Vigor index I = Germination% × Seedling length (Root + Shoot)

Vigor index II = Germination% × Seedling dry weight (Root + Shoot)

Growth analysis

Growth parameters like shoot length, root length, seedling length were measured after 8 days of seed germination. For dry weight determination, plant parts were dried in an oven at 60^{0}C for 72 h.

Determination of α-amylase content

α-amylase (EC 3.2.1.1) activity was assayed by the method described by Sawhney *et al.* [17] in germinating seeds (100 mg) of maize at 24, 48, 72 and 96h after imbibition. For the enzyme assay, 100mg of seed tissue (after peeling) was ground in a pre-chilled mortar with 5ml of 80% ice-cold acetone. The homogenates were centrifuged at 4^{0}C for 10 min at 10,000rpm and the supernatant was discarded and pellets were dissolved in 0.02M sodium phosphate buffer and then centrifuged at 4^{0}C for 20 min at 12,000rpm. The supernatant was used for enzyme activity assay. All steps in the preparation of the enzyme extract were carried out at 4^{0}C. The reaction mixture in a total volume of 5 ml contained 0.02 M (pH 6.4) sodium phosphate buffer, 0.01 % starch, 0.1N HCl, I_2KI(0.1%) and 200µl enzyme extract. The absorbance was measured at 660 nm.

Statistical analysis

All experiments were conducted using three replicates at least. All of the germination parameters and biochemical parameters were carried out within three independent experiments with three replicated measurements. Data are expressed as a mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc Newman–Keuls multiple comparison test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$) using a Prism 4 software for Windows (GraphPad Software, La Jolla, CA, USA). For the statistical evaluation of the results, significance was defined by a probability level of $P < 0.05$.

RESULTS

Germination Percentage

Germination percentage was significantly enhanced by different treatments including MT (18%), GA0.1 (22%), GA1 (25%), GA10 (27%) as compare to untreated (UT) seeds (Fig. 1).

Seedling length

Seedling length was significantly enhanced by SMF and GA treatments due to increase in root and shoot length. Enhancement of seedling length by SMF,

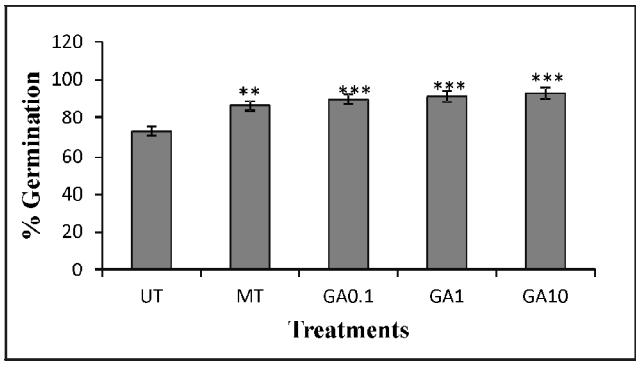

Figure 1: Effect of static magnetic field (200 mT for 1h) treatment (MT) and different concentration of Gibberellic acid including 0.1µg/ml (GA0.1), 1.0µg/ml (GA1) and 10µg/ml (GA10) on germination percentage of maize seeds at 25°C. Vertical line above the bar indicates ± SEM. *, ** and * indicate significance at P < 0.001, 0.01, and 0.05, respectively, compared to control (UT)**

GA0.1, GA1 and GA10 treatments was 30%, 36%, 40% and 48% respectively as compared to untreated (UT) seeds (Fig. 2A,B.C).

Seedling Fresh weight and Dry weight

The effect of SMF and GA treatments were significant on fresh weight and dry weight of seedling compared with control. 28% promotion by SMF and 30%, 34%, and 37% promotion by, GA0.1, GA1 and GA10 mM respectively were recorded for fresh weight (Fig. 3A). Magnitude of enhancement was more in dry weight as compared to the fresh weight of seedlings in MT and GA treated seeds. Dry weight was enhanced by 33% and 45% in SMF and GA (10µg/ml) treated seeds respectively (Fig. 3B).

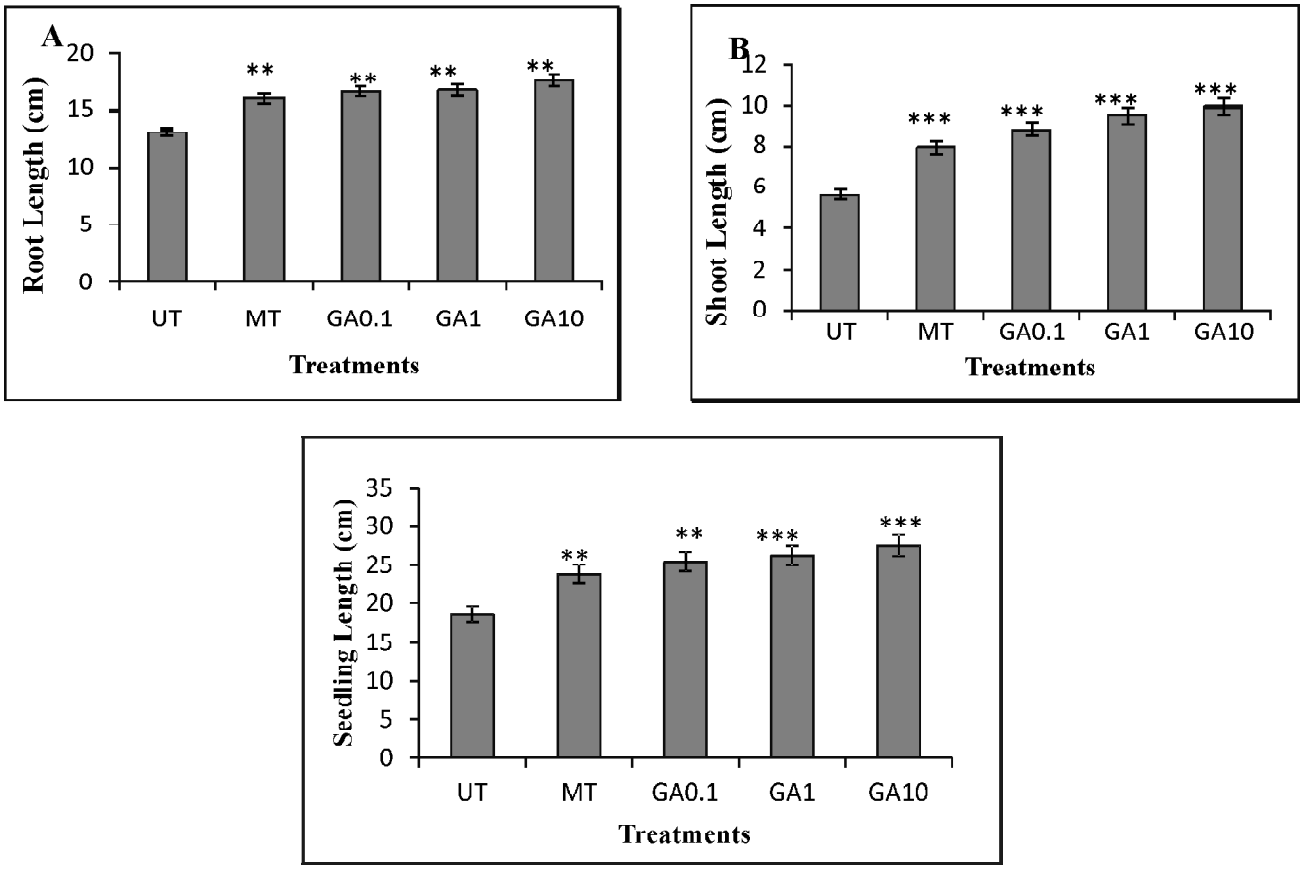

Figure 2: Effect of static magnetic field (200 mT for 1h) treatment (MT) and different concentration of Gibberellic acid including 0.1µg/ml (GA0.1), 1.0µg/ml (GA1) and 10µg/ml (GA10) on root length (A), shoot length (B) and seedling length (C) of maize seeds at 25°C. Vertical line above the bar indicates ± SEM. *, ** and * indicate significance at P < 0.001, 0.01, and 0.05, respectively, compared to control (UT).**

Vigour Index

All the treatments enhanced vigour index I and II. The trend of increase in vigour index I and II was paralleled to the seedling length and dry weight of seedlings (Fig. 3C,D).

Amylase

α-amylase activity was estimated in seeds treated with SMF and GA as compared with untreated controls at different time intervals after imbibition (24-96h). Increased hydrolytic activity was observed in seeds after magnetic field treatment and GA treatment. Amylase activity increased up to 72 h after imbibition in germinating seeds of maize and decreased thereafter. Highest amylase activity was observed after 72 h of imbibitions in GA(10µg/ml) treated seeds. GA (0.1µg/ml) and SMF were showing similar trend in 48 and 72 h of imbibition (Fig. 4).

Statistical analysis

All experiments were conducted using three replicates at least. All of the germination parameters and biochemical parameters were carried out within three independent experiments with three replicated measurements. Data are expressed as a mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc Newman–Keuls multiple comparison test (*P < 0.05, **P < 0.01, ***P < 0.001, #P < 0.05, ##P < 0.01, ###P < 0.001) using a Prism 4 software for Windows

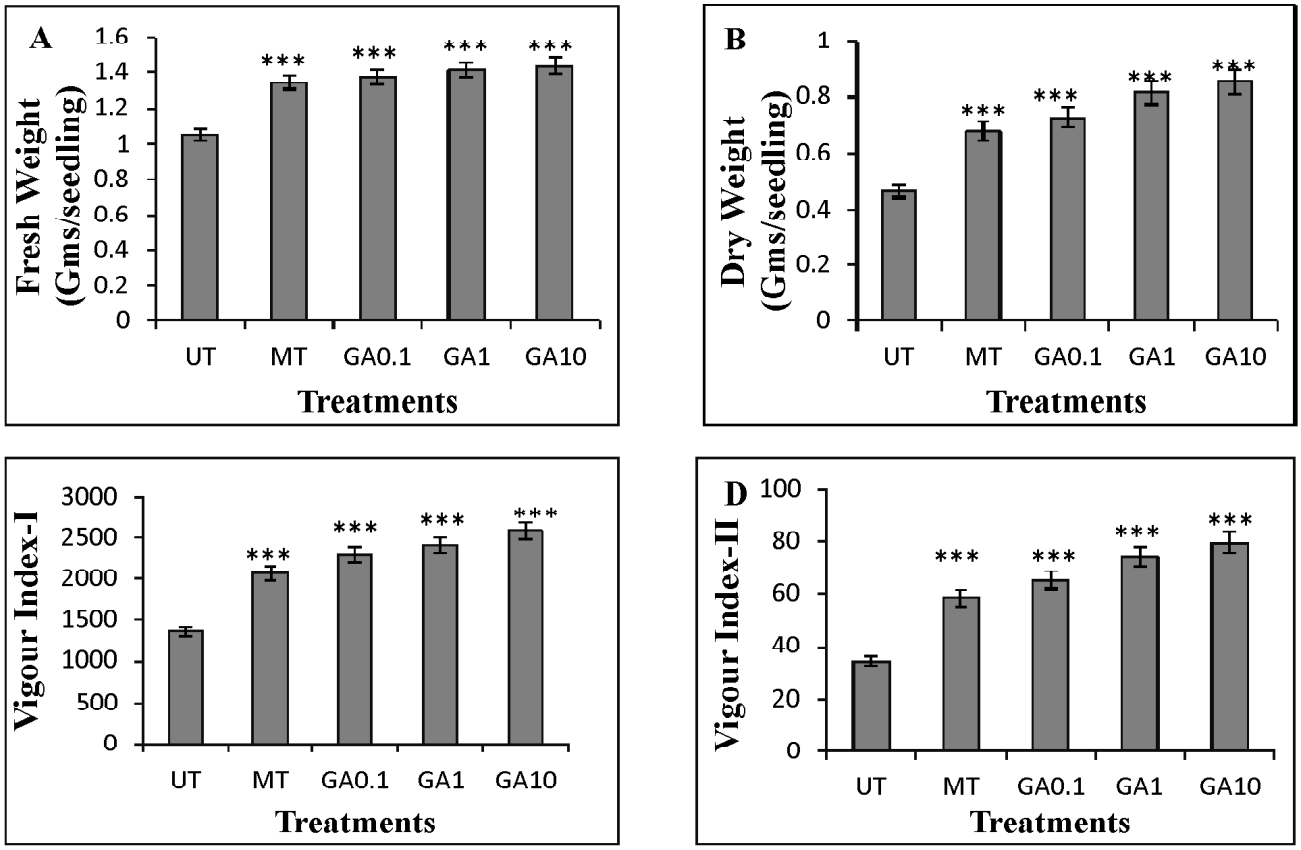

Figure 3: Effect of static magnetic field (200 mT for 1h) treatment (MT) and different concentration of Gibberellic acid including 0.1µg/ml (GA0.1), 1.0µg/ml (GA1) and 10µg/ml (GA10) on seedling fresh weight (A), dry weight (B) vigour index I (C), and vigour index II (D) of maize seeds at 25°C. Vertical line above the bar indicates ± SEM. *, ** and * indicate significance at P < 0.001, 0.01, and 0.05, respectively, compared to control (UT)**

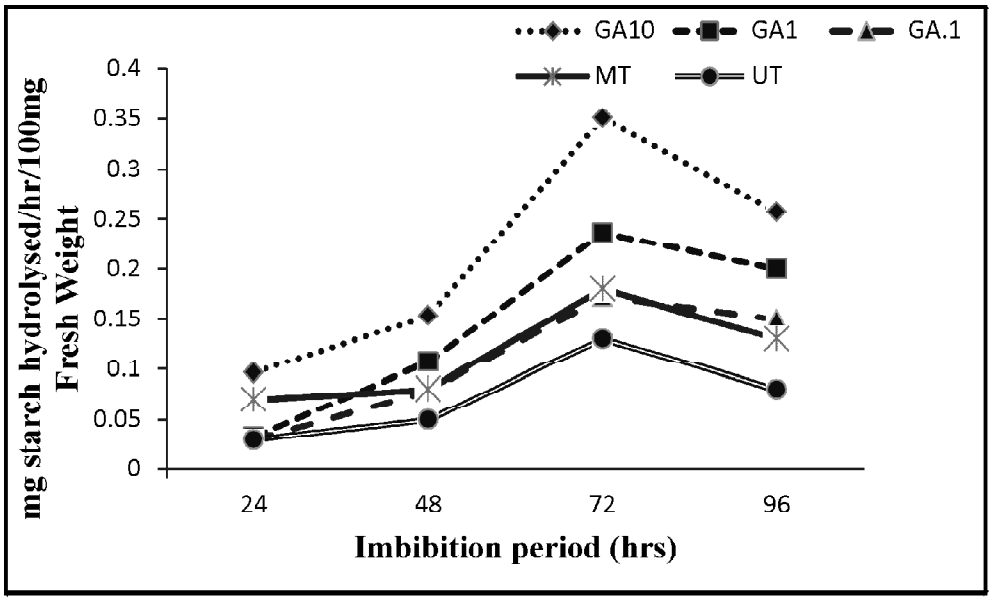

Figure 4: Effect of static magnetic field (200 mT for 1h) treatment (MT) and different concentration of Gibberellic acid including 0.1µg/ml (GA0.1), 1.0µg/ml (GA1) and 10µg/ml (GA10) on á-amylase activity at 24, 48, 72 and 96 h after imbibition at 25°C as compared to control (UT). Data are the means +SE of three independent experiments with three replicated measurements

(GraphPad Software, La Jolla, CA, USA). For the statistical evaluation of the results, significance was defined by a probability level of $P < 0.05$.

DISCUSSION

This research work was conducted to investigate the effect of biochemical priming (i.e. the use of GA) and biophysical priming (i.e. magnetic field, 200 mT 1h) on seedling related parameters of maize seeds. All germination parameters like germination percentage, seedling length, seedling fresh weight, seedling dry weight, vigour index I and vigour index II were determined to indicate how such primed techniques can affect seed germination and seedling growth and in most cases positive effects were observed.

All concentrations of GA used for seed priming had significant effects on seedling length compared with control treatment. GA_{10} was most effective treatment in most measured parameters. Similarly, magnetic field treatment was also shows significant enhancement in all measured parameters. Interestingly, the enhancement effect of least concentration of GA i.e. 0.1µg/ml was comparable to the magnetically treated (200 mT, 1h) seeds.

It is well documented that GA has the germination promoting effect for mature seeds from number of species [18, 19, 20, 21]. Miransari and Smith investigated that exogenously applied gibberellins enhanced the germination of barley seeds [22]. Also, priming of seeds with gibberellins may enhance seed germination in dormant seeds as the biosynthesis of gibberellins is suppressed in dormant seeds compared with non-dormant seeds [23].

SMF treatment positively affects seed germination and consequently induces seedling growth, seed vigour and crop yield [10]. Many researchers found that germination characteristics of maize seeds were enhanced by magnetic field treatment [24, 11, 25, 26]. Effect of magnetic field is depends on magnetic field intensity, exposure time

and the moisture content that finally, increases germination of seeds. Enhanced germination rate and seedling vigour were observed in magnetically treated seeds of groundnut, onion, rice, cucumber and chickpea [27, 28, 29, 30]. Several reports also showed that the magnetic field has the ability to improve quality and sprouting rate of low viability seeds [30, 31]. Pre-sowing seed treatment is secure and inexpensive physical method to enhance the seed germination and seedling growth because, it enhances the concentration of ions, free radicals and electrical charges physically without any degradation/ alteration in the chemical profile of seed and resultantly makes the membranes permeable [32]. Free movement of ions activates the metabolic pathways by enhancing the biochemical and physiological feedback [33]. Level of Ca^{++} ion inside of plant cells increases following exposure to magnetic field. Ca^{++} ions participate in many plant growth processes and responses to stress (heat, salt stress and wounding etc.).

It is assumed that increased accumulation of water in the seeds after magnetic treatment may activate the germinating enzymes, which accelerate the germination in treated seeds [34]. This shows that metabolic events are triggered in MT seeds by faster hydration of associated enzymes.

Data presented here indicate a significant enhancement in germination related parameters in GA treated and magnetically treated seeds as well. Biochemical and biophysical alteration of cucumber seeds using the magnetic fields of 100–250 mT for 1, 2 or 3 h significantly improved seed germination and seedling growth [27]. The most efficient treatment, the 200 mT for 1 h, was selected for the evaluation of water uptake and production of reactive oxygen species and antioxidant enzymes in germinating seeds. ROS production is known to have a beneficial role during seed germination and release of dormancy [35]. Enhancement of ROS in soybean by direct EPR measurement of O_2^- and OH^- radicals in the magneto primed soybean seeds has been demonstrated earlier in our laboratory [36].

In conclusion priming of maize seeds with static magnetic field and GA increased germination percentage, seedling growth and seedling vigour. The results of our germination tests indicated that seed priming significantly increased the final germination percentage and germination related parameters of maize. When magnetic field treatment was compared with different concentration of GA, all concentrations of GA showed greater influence on germination rate however, the effect of GA 0.1µg/ ml and SMF (200mT, 1h) was found to be similar. GA treated seeds was closely associated with their rapid utilization in the synthesis of various amino acids and amides [37], which could be the reason for the increased germination rate whereas enhancements by magnetic field may be because of interaction of magnetic field with ionic current in the plant embryo cell membrane that induces changes in both osmotic pressure and ionic concentrations on both sides of the membrane that finaly alters activity of hydrolyzing enzymes of seeds [38].

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