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Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean

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ABSTRACT

Maize and Soybean seeds were pre-treated with static magnetic field (SMF) of 200 mT for 1 h to evaluate the effect of magnetopriming on germination and early growth characteristics of seeds under saline conditions. The adverse effect of NaCl induced salt stress was found on percentage germination and germination related parameters. Enhanced percentage germination and early seedling growth parameters (root and shoot length, and vigour indices) under different salinity levels (0–100 mM NaCl) indicated that magnetopriming was more effective in alleviating salinity stress at early seedling stage of both maize and soybean as compared to untreated seeds. α- amylase and protease activities were also higher in SMF treated seeds under both non-saline and saline conditions. This could have resulted in faster hydration of enzymes in SMF treated seeds leading to higher rate of germination. Increased levels of superoxide radical and hydrogen peroxide was found in germinating magnetoprimed seeds of maize and soybean, under both the growing conditions. Enhancement in seed germination and seedling vigour under both the growing conditions by SMF treatment may be due to the combined effect of enhanced α- amylase and protease activities and enhanced levels of free radicals in the seeds.

1. Introduction

Salt stress imposes a major environmental threat to agriculture, therefore the efforts to increase salt tolerance of crop plants bear remarkable importance to sustainable agriculture on marginal lands and could potentially improve crop yield overall. Salinity leads to various metabolic disturbances resulting in general suppression of seed germination, plant growth and yield (Sharma and Saran, 1994; Chandrasekar and Sandhayarani, 1996). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri et al., 2001). Crop establishment depends on an interaction between seedbed environment and seed quality (Khajeh-Hosseini et al., 2003). Factors adversely affecting seed germination may include sensitivity to drought stress (Wilson et al., 1985), and salt tolerance (Perry, 1984; Sadeghian and Yavari, 2004). Earlier growth stages are more sensitive to salinity than subsequent ones (Lal, 1985). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri et al., 2001). The adverse effects of salinity like low yield of crops have been reported in many crops like wheat (Afzal et al., 2008), maize (El-Tayeb, 2005), soybean (Essa, 2002) and cotton (Sattar et al., 2010).

To induce tolerance in different plants under saline conditions different strategies are employed recently. Among these, an important strategy to mitigate the adverse effects of salinity is considered as seed priming (Jisha et al., 2013). In various crops hydropriming, $CaCl_2$, KCl, NaCl and Kinetin were reported as effective priming agents to alleviate the adverse effects of salinity (Afzal et al., 2008, 2012). These techniques should have a low environmental impact, and at the same time contribute for the increase of yield in crops. Magnetic and electromagnetic treatments are being used in Agriculture, as a non invasive technique, to improve the germination of seeds and increase crop yields. Previous reports summarized the beneficial effects observed on seedlings magnetically treated under different conditions, which depend on the specific magnetic treatment applied such as time of exposure, magnetic field strength, stationary or alternating, frequency etc. Poinapen et al. (2013) evaluated the role and contribution of different environmental factors on the seed germination of tomato and they found that seed orientation and SMF strengths influenced seed imbibition more than relative humidity and exposure time. Magnetic fields (MF) promoted the germination ratios of bean and wheat seeds and moreover the treated plants grew faster than control (Cakmak et al., 2010). The influence of MF treatment in two pea varieties proved favorable on the emergence, growth, development and the final seed yield (Podlesny et al., 2005). Furthermore, the electromagnetic stimulation of amaranth seeds resulted in the increase of essential fatty acids and a decrease in most of the saturated fatty acids (Sujak and

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Dziwulska-Hunek, 2010). A pre-sowing MF treatment of tomato seeds enhanced the growth and yield of tomatoes (De Souza et al., 2006) and germination and early growth characteristics in chickpea (Vashisth and Nagarajan, 2008), beans and wheat (Cakmak et al., 2010), soybean (Shine et al., 2011) and maize (Shine and Guruprasad, 2012).

Some earlier studies confirmed that MF pretreatment might ameliorate inhibition of other stresses in the plants. MF protects plants from drought and heat stress (Ruzic and Jerman, 2002; Selim and El-Nady, 2011). MF changes water properties; magnetized water is the best treatment for improving the harmful effects of drought stress (Tian et al., 1989; Selim and El-Nady, 2011). Exposure to the SMF mitigates the adverse effects of salinity at seed germination and early seedling growth of chick pea (Thomas et al., 2013) and enhance organogenesis, growth, biomass accumulation and yield of crop plants under salt stress (Radhakrishnan and Kumari, 2013; Baghel et al., 2016; Rathod and Anand, 2016). These studies have suggested that the use of SMFs could be useful in the alleviation of abiotic stress *in vitro* and in the field conditions also. However, similar studies under stressed environment are not available in literature for soybean and maize. Soybean (*Glycine max;* a dicot crop) is one of the most important food crops in the world and it is salt sensitive (Joshi and Nimbalkar, 1983). Maize (*Zea mays;* a monocot crop) is an important cereal crop, providing basic food and oil for human consumption, as well as feed for livestock throughout the world and also classified as moderately sensitive to salinity (Katerji et al., 1994). Keeping in mind the significant effect of SMF, the objective of the present study was to examine whether the pre-sowing seed treatment with SMF could alleviate the adverse effects of salt stress on germination and various germination related attributes in soybean and maize plants. Under laboratory condition, pre-standardized SMF strength of 200 mT (1 h) was proven best for improving different seedling parameters of soybean and maize (Shine et al., 2011; Shine and Guruprasad, 2012) and were used for present study. We hypothesized that SMF pretreatment of 200 mT (1 h) will ameliorate the salinity stress in maize and soybean seedlings which ultimately improved the seed vigour.

2. Materials and methods

The breeder seeds of soybean (*Glycine max* (L.) var. JS-335) were collected from the Directorate of Soybean Research, Indore, M.P., India and seeds of maize (*Zea mays* (L) var. JM-216) were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara, M.P., India.

2.1. Static magnetic field (SMF) pre-treatment to seeds

An electromagnetic field generator (Testron EM-60, Electronic and Scientific Devices, India) with variable horizontal magnetic field strength (50–300 mT) with a gap of 5 cm between pole pieces was fabricated [for the detail of the instrument refer to Vashisth and Nagarajan (2008)]. The pole pieces were cylindrical in shape with 9 cm diameter, and 16 cm length. The number of turns per coil was 3000 and the resistance of the coil was 16 Ω. A 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 is made up of Indium Arsenide crystal encapsulated by non-magnetic sheet of 5 mm × 4 mm × 1 mm; probe could measure 0–2 T with full scale range in increments of 5 mT.

Maize and soybean seeds were exposed to SMF of 200 mT for 1 h in a cylindrical-shaped sample holder of 42 cm^3 capacities, made of a non-magnetic thin transparent plastic sheet. The dose was selected on the basis of seed invigoration studies in soybean and maize reported previously from our laboratory (Shine et al., 2011; Shine and Guruprasad, 2012). Gauss meter was used to measure the strength of the magnetic field between the poles. 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. At low fields (50 mT), from the center to the 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 fields (300 mT), they were 0.4% and 1.2% in the horizontal and vertical direction, respectively. The temperature during the course of seed exposure was 25 ± 5 °C. For parallel control, seeds from the same lot used for magnetic field exposure were kept under conditions which had no influence of the induced magnetic field.

2.2. Germination percentage under SMF and saline conditions

Germination of the seeds was determined by using “between papers” method (ISTA, 1985). One hundred seeds in four replications of 25 seeds each were placed between two layers of moist germination papers using distilled water in control (with no salt solution, 0 mM NaCl) and respective saline solution in other treatments. These were wrapped in a sheet of wax paper to reduce surface evaporation of moisture and placed in saline solutions of 25, 50, 75,100 mM NaCl in the germination incubator at 25 °C in an upright position. After 8 days, seeds were evaluated for normal, abnormal seedling, ungerminated and dead seeds. Germination percentage was worked out on the basis of normal seedling only. The data obtained from the optimization experiments are expressed as mean ± S.E. of four replicates (n = 4) of 25 seeds from each replica for germination percentage.

2.3. Seedling growth and vigour indices under SMF and saline conditions

Ten normal seedlings from each replicate were randomly selected to measure shoot and root length. The seedlings were subsequently dried at 80 °C in an oven and weighed together to obtain seedling dry weight. The data expressed as mean ± S.E. of four replicates (n = 4) of 10 seedlings from each replica for early seedling characteristics like root and shoot length, seedling dry weight, vigour index I and II.

Seedling vigour was calculated following Abdul-Baki and Anderson (1973) as given below:

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

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

2.4. Seed water uptake under SMF and saline conditions

The water uptake (WU) by seeds during the different hours of imbibition was determined under non-saline as well as saline (0, 25 and 50 mM NaCl) conditions. Weighed seeds were placed between water-saturated cotton in a plastic box and incubated at 25 °C for 96 h. At intervals of 24 h till 96 h, all the seeds were removed, blotted dry, weighed, and immediately returned to the moist cotton to continue imbibition. The change in weight due to imbibition was expressed as the amount of water absorbed per unit seed dry weight. The data expressed as mean ± S.E. of four replicates (n = 4) of 25 seeds from each replica for water uptake.

2.5. Assay of enzymes involved in germination under SMF and saline conditions

α- Amylase and Protease activities were measured in the seedlings emerged from SMF treated and untreated seeds of maize and soybean after different hours of imbibitions (24–96 h). All the data are presented as mean ± S.E. of triplicates (n = 3); five seedlings from each replica were taken for the recording of these enzymes.

2.5.1. α- Amylase activity

100 mg germinating seeds of soybean and maize were grounded in 5 ml chilled 80% acetone by mortar and pestle and centrifuged at