Seed pretreatment with magnetic field alters the storage proteins and lipid profiles in harvested soybean seeds

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Received: 2 July 2017 / Revised: 21 December 2017 / Accepted: 2 January 2018 / Published online: 17 January 2018
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Abstract The increase in crop productivity is an urgent need of the time to reduce scarcity of food in underdeveloped countries. Several biological, chemical and physical methods have been applied to promote crop yield. Application of magnetic field (MF) is an emerging physical method used to increase plant growth and yield. The reports on MF pretreatment-induced nutritional changes in harvested seeds are scarce. We previously identified the optimal frequency of MF to improve plant growth and yield as 1500 nT at 10.0 Hz. This study was aimed to investigate the effect of MF treatment on storage proteins and fatty acids in harvested soybean seeds. The results showed that MF triggered globulin production and suppressed prolamin production. However, lipid content in seeds increased, because MF exposure caused an elevation of several fatty acids including caprylic acid, palmitic acid, heptadecanoic acid, linoleic acid, lignoceric acid and eicosapentaenoic acid. This is the first report to reveal the seed pretreated MF on nutritional values of harvested seeds. This study suggests that MF treatment improves seed quality by regulating the metabolism of storage proteins and fatty acids.

Keywords Fatty acids · Magnetic field · Soybean · Storage proteins

Introduction

The research on the effect of electromagnetic fields on biological systems has gained rapid interest in the past few years. Applications of the magnetic field (MF) are being developed in several areas, particularly in agricultural science. Pulsed magnetic field (PMF) technology is beneficial because it is affordable to farmers, and pre-sowing seed treatment using MF can improve crop productivity (Leelapriya et al. 2003; Vashisth and Nagaraja 2010). The effects of MF on seed germination, plant growth, biochemical parameters, hormonal changes, and yield have been studied intensely (Radhakrishnan and Ranjitha-Kumari 2012).

Soybean plant growth is improved by microbes and MF treatments in adverse salinity stress conditions (Baghel et al. 2016; Kim et al. 2017). The use of PMF is an alternative method to chemical fertilizers to increase soybean yield. In our previous study, soybean seeds were exposed to 1500 nT at 0.1, 1.0, 10.0 and 100.0 Hz of PMF for 5 h per day for 20 days. This treatment significantly improved the seed germination rate, plant height, biomass, and pods and seeds, as well as proteins and the intensity of protein polypeptides, activities of various enzymes, and uptake of nutrients in PMF-treated soybean seedlings compared with PMF-untreated controls (Radhakrishnan and Ranjitha-Kumari 2012, 2013). Several studies have confirmed that the growth of agricultural plants is enhanced under specific MF conditions; however, a systematic and extensive study identifying the usability of applying MF is required. Soybean is a rich source of nutrients, such as proteins (40%); oils (18–22%); and carbohydrates, minerals, and other phytochemicals (35%). Seed storage proteins and fatty acids are important in the human diet. The storage proteins are classified as albumins, globulins, prolamins, and

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glutelins. The consumption of seeds provides essential and nonessential amino acids. Similarly, various fatty acids in soybean also play an important role in the diet and health. Therefore, this study was aimed to understand the effect of MF on the nutritional value of harvested soybean seeds.

Materials and methods

In our previous study, MF (1500 nT at 10.0 Hz) was used to improve plant growth and yield (Radhakrishnan and Ranjitha-Kumari 2012). In this study, the qualitative and quantitative changes in storage proteins and fatty acids were analyzed in harvested seeds of controls and MF pretreated plants to understand the effect of MF on the nutritional value of soybean. Seed samples were finely ground using a mortar and pestle for quantifying storage proteins. The obtained powder was dissolved in 10 mL of petroleum ether to remove fats. The solvent was removed after 12 h, and the flour was air-dried for complete solvent evaporation (Kalpana and Rao 1997). Fat-free flour was used to analyze the content of storage proteins according to the Osborne method (1907). Sodium chloride (0.5 M, 10 mL) was added to the seed flour and kept for 2 h at 4 °C with mild shaking at periodical intervals. The suspension from each sample was centrifuged at 5000 rpm for 15 min. The supernatant was removed and the residue was again extracted with 40 mL of distilled water for 30 min. The obtained extract was dialyzed against cold distilled water for 24 h. After centrifugation, the precipitated salt-soluble globulins were separated and the water-soluble albumins remained in the supernatant. The residual pellet was extracted with 70% aqueous ethanol to obtain the prolamin fraction. Glutelins in the remaining residue were extracted using 0.1 N sodium hydroxide. The protein fractions were quantified individually by the Bradford method (1976).

Control and MF-exposed seeds (5 g) were ground with 25 mL of a 2:1 mixture of chloroform:methanol. The extract was filtered through a filter paper. About one-third volume of double-distilled water was added to the filtrate and vortexed thoroughly to remove water-soluble impurities. A small amount of sodium sulfate was added to the filtrate to remove moisture. The lipid filtrate was dried at room temperature and its weight was measured. To understand the pattern of fatty acid variation, 1 mL of saponification reagent was added to the lipid filtrate and boiled for 30 min. Methylation reagent (2 mL) was added to the above mixture and boiled again at 80 °C for 20 min. Extraction solvent (1.25 mL) was added to the mixture after cooling at room temperature, and the tube was tightly closed and rotated for 10 min. The lower aqueous phase was removed, and 3 mL of bare wash was added. From the upper phase, a two-third portion of the organic extract was

transferred to a vial. The analysis of individual fatty acids was performed by gas chromatography (Perkin Elmer Clarus 500 Model) using an instrument equipped with a flame ionization detector and capillary column. Nitrogen was used as the carrier gas at a flow rate of 1 mL per min. The column temperature was 40–250 °C. The obtained peaks were identified by comparison with authentic standards, and each fatty acid was quantified by determining the integrated peak areas. The experiments were repeated three times, and the obtained data were analyzed by one-way analysis of variance using Duncan's multiple-range test at a 5% level of significance (SPSS Inc., Chicago, Illinois, USA).

Results and discussion

Crop productivity can be promoted during the exposure of magnetic field. MF pretreated seeds or MF based irrigation practices play important role to enhance the seed germination, early and late growth of plant through regulating several biochemical and physiological process (Asghar et al. 2016, 2017; Haq et al. 2016; Iqbal et al. 2016; Abbas et al. 2017; Urva et al. 2017). An increase of soybean production would be useful because proteins and oils are major components of soybean seeds. Trugo et al. (2000) reported that protein content can be increased in germinated soybean seeds after heat treatment. We previously reported that protein content in soybean seedlings can be increased by the MF-pretreatment of seeds (Radhakrishnan and Ranjitha-Kumari 2012). Moreover, MF pretreatment plays a significant role in regulating the nutritional content of soybean. There is no report on MF-induced increased nutritional value in harvested seeds. In this study, the variation in the content of storage proteins, such as albumins, globulins, prolamins, and glutelins, was observed in harvested soybean seeds subjected to an MF (Table 1). Albumins were found to be present in the highest quantity (47.10–47.95 mg/g) in the seeds of both MF- and non-MF-treated plants, and their content was not significantly affected by MF treatment. The globulin content enhanced by fivefold after the MF treatment, but the prolamin

Table 1 Effect of MF on seed storage proteins in soybean seeds

Storage proteins	Control seeds	MF treated seeds
Albumin	47.95 ± 1.10 ^a	47.1 ± 2.87 ^a
Globulin	1.25 ± 0.096 ^b	6.01 ± 0.450 ^a
Prolamin	0.94 ± 0.026 ^a	0.44 ± 0.001 ^b
Glutelin	19.65 ± 0.072 ^a	19.77 ± 0.024 ^a

Means followed by the same letter (a, b) are not significantly different ($p \leq 0.05$) as determined by DMRT

Table 2 Influence of MF on fatty acid profile of harvested soybean seeds

Fatty acids	Control seeds	MF treated seeds
Butyric acid (C4:0)	42.58 ± 2.0	–
Caprylic acid (C8:0)	0.03 ± 0.001 ^b	0.45 ± 0.012 ^a
Capric acid (C10:0)	0.88 ± 0.034	–
Undecanoic acid (C11:0)	0.11 ± 0.002	–
Lauric acid (C12:0)	0.27 ± 0.015	–
Tridecanoic acid (C13:0)	0.03 ± 0.002	–
Myristic acid (C14:0)	0.06 ± 0.003	–
Pentadecanoic acid (C15:0)	0.06 ± 0.003	–
Palmitic acid (C16:0)	0.08 ± 0.004 ^b	1.01 ± 0.06 ^a
Heptadecanoic acid (C17:0)	0.08 ± 0.001 ^b	0.29 ± 0.016 ^a
Stearic acid (C18:0)	0.44 ± 0.018 ^b	1.37 ± 0.008 ^a
Elaidic acid (C18:1nt)	1.02 ± 0.062 ^b	2.31 ± 0.152 ^a
Linolic acid (C18:2n6)	0.07 ± 0.005 ^b	0.26 ± 0.014 ^a
Behenic acid (C22:0)	–	0.35 ± 0.021
Arachidonic acid (C20:4n6)	1.17 ± 0.0071 ^b	3.66 ± 0.013 ^a
Lignoceric acid (C24:0)	0.18 ± 0.003 ^b	0.77 ± 0.024 ^a
Eicosapentaenoic acid (C20:5n3)	1.51 ± 0.002 ^b	5.65 ± 0.079 ^a
Nervonic acid (C24:1)	0.73 ± 0.019 ^a	0.77 ± 0.042 ^a

Means followed by the same letter (a, b) are not significantly different ($p \leq 0.05$) as determined by DMRT

content declined from 0.94 to 0.44 mg/g in the seeds. However, the glutelin content was unaffected by MF treatment. The percentage of storage proteins varies depending on the plant species. For example, moth bean (*Vigna aconitifolia*) seeds contain 63.3% of globulins, 27.83% of glutelins, 5.06% of albumins, and 3.17% of prolamins (Sathe and Venkatachalam 2007) and *Teramnus labialis* seeds contain 49.0% of globulins, 10.83% of glutelins, 32.7% of albumins, and 7.37% of prolamins (Viswanathan et al. 1999). A previous study (Bhardwaj et al. 1999) revealed that the genotype of soybean and the locality of soybean cultivation influence the protein and oil content in seeds. For example, the protein and oil content in the seeds of BARC-9 cultivar was 494 and 173 g/kg, respectively, whereas that in the seeds of Hutcheson cultivar was 426 and 217 g/kg, respectively, at four localities. An increase in the protein content in soybean seeds is desirable for enhancing the quality of tofu—a soybean product (Bhardwaj et al. 2004). Environmental or physical stimulants increase or decrease plant yield. Grunwald and Endress (1988) tested the effect of sulfur dioxide and ozone treatments on soybean plant growth and yield. An exposure to sulfur dioxide enhanced the protein content in harvested seeds but did not affect the oil content. Similarly, an exposure to ozone caused plants to produce seeds that had lower oil content without altering the protein content.

Primomo et al. (2002) investigated lipids and fatty acids in soybean seeds at diverse environments to understand the

stability of fatty acids and found that the synthesis of oleic and linolenic acids was more sensitive to environmental changes compared with that of other fatty acids. The authors suggested that environmental factors, including temperature and precipitation, play a significant role in fatty acid synthesis. However, other factors such as planting date, cultural practices, soil type, weed, disease, and insects also affect the fatty acid content (Wilcox and Cavins 1992; Schnebly and Fehr 1993). In this study, an alteration in the lipid and fatty acid profiles of MF-treated plant seeds was observed. The lipid content of MF-exposed plant seeds increased by 6 mg/g compared with control seeds. Hence, the fatty acid profile is regulated by MF in harvested soybean seeds (Table 2). Behenic acid was present only in MF-treated seeds. The content of caprylic acid (15%), palmitic acid (13%), heptadecanoic acid (7%), stearic acid (3%), elaidic acid (2%), linoleic acid (4%), arachidonic acid (3%), lignoceric acid (4%), eicosapentaenoic acid (4%), and nervonic acid (1%) increased, and some fatty acids (butyric acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, and pentadecanoic acid) were not detected in seeds harvested from MF-pretreated plants compared with seeds from control plants. Proplastids in seeds carry out lipid biosynthesis (Stumpf 1980). The synthesis and elongation of fatty acids in seeds are controlled by several loci on chromosomes (Kunst et al. 1992). However, external or environmental stimuli can influence fatty acid synthesis in seeds.

Bhardwaj et al. (2004) showed significant differences in the fatty acid profile of soybean seeds produced at three locations. Similarly, some studies documented the effect of years and locations on the fatty acid profile of soybean because of changes in weather patterns (Cherry et al. 1985; Schnebly and Fehr 1993). In addition, atmospheric temperature plays a major role in fatty acid metabolism. Extreme high temperature reduced linoleic and the linolenic acid content, but enhanced oleic acid synthesis (Howell and Collins 1957; Dornbos and Mullen 1992). Similarly, Rennie and Tanner (1989) reported that high temperature reduced the linolenic acid content but elevated the stearic acid content. The changes in the fatty acid profile in MF-treated soybean seeds might have the prolonged effects of MF-induced energy in fatty acid metabolism in plants.

Our results show that the pretreatment of seeds with MF influences the productivity of soybean seeds and alters the content of reserve materials such as storage proteins and fatty acids. The results suggest that the application of MF would be an alternative energy source to stimulate crop production in sustainable agriculture. More intensive genomics and proteomics studies are required to elucidate the mechanism of MF interaction on plant nutrition. Although, MF therapy is an acceptable cure to some illness in humans, the future studies are required on animal nutritional benefits of MF exposed crop seeds to commercialize the MF based agricultural products.

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