The effect of gamma radiation and magnetic field on seed germination andseedling growth at low temperature in sorghum x sudangrass hybrids

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Abstract

This study was aimed to provide seed germination and seedling growth of sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids at low temperature by using gamma radiation and magnetic field strength. Seeds of two hybrids ("Aneto" and "Sugar Graze") were treated with different gamma doses (0-control, 100 Gy) and magnetic field strengths (0-control and 75 mT) for 72 hours while some were applied with both gamma radiation + magnetic field. Germination trials were carried out for 10 days in an incubator at 15°C temperature. Germinated seeds from each treatment were transferred to pots (5 pots with 4 seeds each) and cultured for 28 days in growth chamber at 15°C temperature. In this study, germination percentage, seedling growth percentage, seedling height, root length, seedling fresh and dry weights, and water content of seedling were determined. Lowest results in all parameters in both hybrids were observed in the control treatment without gamma radiation and magnetic field strength application. The highest values in all parameters studied were recorded in seeds treated both by gamma radiation+magnetic field strength applied together in "Aneto", and in seeds treated with magnetic field strength in "Sugar Graze". As a result of the research, gamma radiation and magnetic field strength applied to seed increased seed germination and seedling growth at a low temperature of 15°C.

Introduction

Sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids produce quality forage that grows rapidly, tolerates heat and drought and high yielding. Sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids are the plant most adapted to high temperature climate (Zerbini and Thomas, 2003; Ali et al, 2009; Borghi et al, 2013). Seed germination and plant growth take place between 25-35°C. Sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids are generally sensitive to low-temperature stress (range of 20°C to about 0°C) (Yu et al, 2004). Sorghum suffers chilling injury when subjected to non-freezing temperatures below 10–15°C (Peacock, 1982). Maize, as an allied species of sorghum, is more tolerant to low temperature. However, several studies have been reported to improve maize tolerance against low-temperature (or cold stress). Thus, cold tolerance is required to be studied in sorghum.

Gamma irradiation along with other high energy rays is a type of electromagnet waves which owe high penetration capacity into molecules and can bring about ionization of the subject material by removing their electrons (UNSC, 2000). Exposure of plant materials specifically seeds to gamma irradiation results in mutagenic changes in living cells by several means. Since gamma radiation owes high energy and greater penetrability into exposed cells and tissues, DNA of the subject material may undergo severe alterations.. These alterations can either result from direct physical strikes of gamma irradiation on DNA or due to production of reactive O2 species such as hydrogen peroxides, hydroxyl ions and other active atomic oxygen which can further interact with DNA and other cellular components and biomolecules resulting in the ionization, functional changes of proteins and enzymes and overall metabolic activities (Mittler, 2002; Wi et al, 2005). Gamma-rays can be employed to develop new varieties that can be readily adapted in a short period in different locations with varying agro-climatic and growing conditions, and where available resources are limited. Low doses of gamma rays have been reported to increase cell proliferation, germination, cell growth, enzyme activity, stress resistance, and crop yields (Baek et al, 2005; Kim et al, 2005).

All organisms live under the influence of the Earth's magnetic field (MF), also termed the geo MF (GMF), (5×10^{-6} Tesla (T)) with geographical variations in its intensity ranges from 25 to 65 μT, its inclination, and declination (Maus et al, 2010). Biophysical methods like magnetic and electromagnetic stimulation can be promising and environmentally friendly methods in the future of agriculture. Since the stimulation of plants with magnetic field to increase the quantity and quality of seed germination, seedling development and yields of different species by influencing physiological and biochemical mechanisms has caught the interest of many scientists all over the world.

Many studies have reported the positive impact of gamma irradiation and magnetic field strength on seed germination and seedling growth in rice (Florez et al, 2004), maize (Florez et al, 2007), *Festuca arundinaceae* Schreb. and *Lolium perenne* L. (Carbonell et al, 2008), *Asparagus officinalis* L. (Soltani et al., 2006) for growing under adverse environmental conditions (Javed et al, 2011; Baghel et al, 2018; Radhakrishnan 2019; Majeed et al, 2018; Beyaz, 2020). There are limited reports on the effects of low-temparature on seed germination and seedling growth in sorghum hybrids (Ercoli et al, 2004; Yu et al, 2004; Maulana and Tesso, 2013; Tari et al, 2013; Antony et al, 2019). Therefore, this study was aimed to investigate the effects of gamma irradiation and magnetic field strength applied alone or together on seed germination and seedling growth in sorghum hybrids at 15°C temperature.

Material and methods

Plant Materials

Seeds of two sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids ("Aneto" and "Sugar Graze") were used as plant material in this study. The seeds were obtained from the Field Crops Central Research Institute (Ankara, Turkey) and Ulusoy Seed Company (Ankara, Turkey).

Gamma irradiation

Seeds were irradiated with different doses (0-control and 1000 Gy) of ^{60}Co γ rays at 0.8 kGy h^{-1} at the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Sarayköy, Ankara. Fricke and alanine dosimeters were used for dose mapping and determination of dose rates of gamma souce. Seeds were irradiated along with a dosimeter for 75 Gy to be sure that ionization was uniform. The seeds were placed on a plate and rotated 360 degrees in a cylindrical radiation field for the gamma irradiation treatment

Magnetic field generation

A MF system consists of two Helmholtz coils forming an electromagnet mounted on a wooden frame. The number of turns of copper wire per coil was 3000. The mean MF in the center of the coils ranged from 50 to 500 mT. Coils which were placed horizantall, were connected to a power supply (0-12 A, ref. 13506-93, PHYWE, Gottingen, Germany). Moreover, intensity through the coils was measured by an ampermeter. GD anode was placed between coils. The accuracy and uniformity of MF strengths were detected by a digital teslameter (ref. 13610-93, PHYWE, Gottingen, Germany).

Magnetic field treatment

Seeds of two hybrids ("Aneto" and "Sugar graze") placed in the middle of the gap between the coils were exposed to 75 mT MF strength for 72 h. The MF strength used in the study (75 mT) were determined according to the protocols described by Aycan et.al. (2018) and Bahadir et al (2018). All trials were carried out in laboratory condition where temperature and humidity were controlled

Growth conditions and morphological observations

Seeds of two hybrids ("Aneto" and "Sugar Graze") were treated with different gamma doses (0-control, 100 Gy) and magnetic field strengths (0-control and 75 mT) for 72 hours. One hundred seeds were treated with gamma and magnetic field seperately while 100 seeds were applied with gamma radiation + magnetic field strength together. Five replicates of 25 seeds for each treatment for both hybrids were placed between filter papers that were watered with 20 ml of tap water, and put in plastic bags. Water content of the filter papers were controlled every day and they were watered with the same amount of water when it was needed. Plastic bags were incubated at 15°C in the dark for 10 days for seed germination evaluation. Germinated seeds from each treatment were transferred to pots (5 pots with 4 seeds each) and cultured for 28 days in growth chamber at 15°C temperature. Seed germination (%), seedling growth (%), seedling height (cm), root lenght (cm), fresh and dry weight seedling contents (g), and seedling water content in weight and percentage were recorded on the 28th day after germinated seeds were transferred to soil. Dry weights were measured after drying samples at 105°C for 2.5 h.

Statistical Analysis

The experiment was conducted according to the "Completely Randomized Block Design" concept. Five replicates were tested, and each replicate contai-

Table 1 - The effects of gamma radiation and magnetic field strength on seed germination and seedling growth at a low temperature (15°C) in sorghum x sudangrass hybrids

Hybrids	Treatments	Germination (%)	*Seedling Growth (%)	Seedling Height (cm)	Seedling Root Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)	Seedling Water Content (g) – Percent (%)
"Aneto"	Control	43.33±1.7c	38.33±1.7b	13.27±1.6b	5.50±0.3b	0.11±0.009c	0.013±0.003b	0.10±0.007c – 91.00
	Gamma	46.67±1.7bc	38.33±1.7b	16.50±1.8a	7.83±0.8a	0.17±0.006b	0.017±0.003b	0.15±0.007ab - 88.00
	Magnetic Field	53.33±1.6b	43.33±1.6b	16.50±0.3a	9.00±1.2a	0.17±0.003b	0.023±0.003ab	0.15±0.006ab – 88.00
	Gamma + Magnetic Field	75.00±2.9a	68.33±3.3a	17.67±1.2a	9.33±1.2a	0.21±0.009a	0.033±0.003a	0.18±0.009a – 86.00
"Sugar Graze"	Control	48.33±1.7d	41.67±1.7d	13.33±1.7c	4.50±0.3c	0.08±0.01c	0.01±0.00c	0.07±0.009c – 87.50
	Gamma	65.00±2.9c	63.33±1.7c	14.83±0.7c	5.83±0.4c	0.12±0.003b	0.02±0.003c	0.10±0.006b – 83.33
	Magnetic Field	86.67±3.3a	81.67±1.7a	21.67±0.4a	11.33±0.7a	0.18±0.007a	0.04±0.003a	0.14±0.009a – 77.78
	Gamma + Magnetic Field	76.67±1.7b	71.67±3.3b	18.33±0.6b	7.50±0.3b	0.13±0.006b	0.03±0.003b	0.10±0.007b – 77.00

Each value is the mean of 3 replications. The study was set in two parallels. Values represent mean ±standard error of the mean Values within a column followed by different letters are significantly different at the 0.01 level for each cultivar *Seedling growth percentage means developed seedlings out of total germinated seed

ned 25 seeds for seed germination percentage, and 4 seedlings for seedling growth percentage, seedling height, root length, fresh and dry weights of seedling, and seedling water content evaluations. The experiments were repeated twice. In all statistical analyses, 'SPSS Statistics ver. 22 (IBM Corp, Chicago, IL, USA)' was used. Analysis of Variance (One-way ANOVA) was applied for each experiment, followed by Duncan's multiple range test at the 1% level to find significant differences among treatments (Snedecor and Cochran, 1967).

Results and discussion

The effects of gamma radiation and magnetic field strength on seed germination percentage, seedling growth percentage, seedling height, root length, fresh and dry weights of seedling, and water content of seedling of two sorghum x sudangrass hybrids ("Aneto" and "Sugar Graze") are reported in Table 1. The differences in all parameters among treatments (control, gamma irradiation, magnetic field strength and gamma+magnetic field strength) in both hybrids were statistically siginificant ($p < 0.01$) (Table 1).

In hybrid "Aneto", the lowest data for all parameters were recorded in control treatment where there were no gamma irradiation and magnetic field strength. On the other hand, the highest data were observed in the treatment of gamma radiation + magnetic field strength applied to seeds together. The highest values recorded were 75.00%, 68.33%, 17.67 cm, 9.33 cm, 0.21 g, 0.033 g and 0.18 g in germination percentage, seedling growth percentage, seedling height, root length, fresh and dry weights of seedling, and water content of seedling, respectively (Table 1). The effect of magnetic field strength on seed germination percentage, seedling growth percentage, root length and dry weight of seedling resulted higher than that observed by gamma radiation. For other parameters (seedling height, fresh weight of seedling and water content of seedling), values were similar in both gamma radiation and magnetic field strength treatments as 16.50 cm, 0.17 g and 0.15 g, respectively. Water content in percentage in the treatment of gamma radiation + magnetic field strength applied to seeds together was found as 86.00% which is the lower than other treatments (Table 1).

In hybrid "Sugar Graze", the lowest results in all parameters were again obtained from control treatment. The highest values were obtained from the treatment of magnetic field strength as 86.67%, 81.67%, 21.67 cm, 11.33 cm, 0.18 g, 0.04 g and 0.14 g in germination percentage, seedling growth percentage, seedling height, root length, fresh and dry weight of seedling, and water content of seedling, respectively. Water content in percentage in the treatment of magnetic field strength was recorded as 77.78.00% which is the lower than control and gamma treatments but a little bit higher than gamma radiation + magnetic field strength treatment.

The lower results in water content in percentage showed that the highest metabolic activity and mitotic cell division were in seedlings developed from gamma + magnetic field treatment.

In our study, the highest results were obtained from treated seeds (by gamma radiation and magnetic field alone or together) in all parameters in both hybrids. Seed germination and seedling growth are the critical

Fig. 1 - Seedlings of hybrid "Aneto" developed from seeds treated by gamma radiation + magnetic field (on the left) and from non-treated seeds (control) (on the right)

and/or main indicators for the resistance of plants to adverse environmental conditions. Beyaz et al (2016) and Bahadır et al (2018) confirmed the positive effect of gamma irradiation and magnetic stimulation on seed germination and seedling growth in *Lathyrus chrysanthus* Boiss. Payez et al (2013) reported that electromagnetic field increase seed germination and growth of wheat seedlings. Shine et al (2011) reported the beneficial effect of pre-sowing magnetic treatment for improving germination parameters and biomass accumulation in soybean. It has also been reported that decreased germination and seedling growth in stressed rice seedlings is due to decreased mobilization of starch and α-amylase activity (Lin and Kao, 1995).

From our findings, it can be concluded that the lower levels of all parameters of non-treated (control) seeds were directly due to a decreased water uptake from the soil and consequently, a reduced mobilization of nutritions in the plant. Treatment of seeds with gamma radiation and magnetic field either alone or together increased permeability of the seed coat and so enabled water, all solutes and nutrients to transfer into the tissue more easily, providing all cells with high metabolic activity. In our case, increases in fresh and dry weights of seedlings developed from treated seeds at the end of the study were chiefly due to an increase in the absorption of water and other components from the soil. In our study, seedlings from non-treated seeds (control) were found smaller than the ones from treated ones in both hybrids. Dale (1988) states that fresh weight increase is mainly due to cell enlargement by water absorption, cell vacuolation, and turgor-deriven wall expansion. The increase in dry weight is closely related to cell division and new material synthesis (Sunderland, 1960). Dry weight increase in seedlings developed from treated seeds is due to an increase in carbohydrate metabolism resulting from increased water uptake.

It has been stated that water stress alters the level of plant hormones (Morgan, 1990). The decreased cytokinin and gibberellic acid and increased abscisic acid content under stress conditions (Boucaud and Unger, 1976) suggest that growth reduction under stress conditions could be the result of the stress-induced changes in membrane permeability and water uptake due to altered endogenous hormonal levels (Ilan, 1971). Hsia (1973) has reported that the inhibition of growth under water stress conditions is the result of inhibition of cell division. Baghel et al (2017) reported that static magnetic field pretreatment (SMF) mitigated the adverse effects of water stress in soybean. Beyaz (2020) noted that low-dose of gamma radiation eliminating negative effects both salt and drought stress in common vetch (*Vicia sativa* L.). Wang et al (2018) also revealed that gamma ray pretreatment could improve the salt tolerance of barley seedlings. Selim and El-Nady (2011) stated that magnetic treatments act as a protective factors against water deficit in tomato.

Rochalska and Orzeszko-Rywka (2005) reported that a low frequency MF (16 Hz) can be used as a method of post-harvest seed improvement for different plant species, especially for seeds of temperature sensitive species germinating at low temperatures.

In the present study, magnetic field treatment alone was seen more effective than gamma radiation on seed germination and seedling growth in sorghum x sudangrass (*Sorghum bicolor* (L.) Moench) hybrids at a low temperature of 15°C. Lower results in gamma radiation treatment could be attributed to the dose applied. A dose-dependent enhancement of germination capacity of seeds and seedling growth by magnetic field treatment has also been reported in wheat seeds (Shine et al 2011). Singh et al (2012) showed that seed germination and seedling growth increased with gamma radiation in a dose-dependant manner in wheat plant. Bahadır et al (2018) reported that magnetic field (MF) strength and different exposure time periods have different effect on seed germination and seedling growth. Morover, Marcu et al (2013) and Majeed et al (2017) reported that germination and growth response of different plants to gamma radiation treatment generally depends on the quantity of radiation dose, exposure time and plant species.

In the current study, the results showed that magnetic field + gamma radiation applied together also gave positive effects on seed germination and seedling growth in both hybrids. Our results were verified with the study of Alikamanoğlu et al 2007) who reported that magnetic field changes the effect of gamma radiation in a positive way as a result of combined treatment of magnetic field and gamma radiation in *Paulowinia tomentosa* tissue culture. Furthermore, Pietruszewski et al (2017) reported that the application of electromagnetic field (EMF) and electromagnetic radiation (EMR) stimulation could be a good tool for improving the yield and plant growth for agricultural production.

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To our knowledge, this is the first study reporting that seed germination and seedling growth at low temperature in sorghum x sudangrass hybrids could be increased by gamma radiation and magnetic field treatments using either alone or both treatments. The effects of gamma radiation and magnetic field on other adverse environmental conditions such as salinity and drought in sorghum hybrids should be addressed in future studies.

Conflict of interest

Authors declare no conflict of interest

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