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Influence of Ammonium Molybdate and magnetized water as a physical pre-sowing seed priming on sorghum (*Sorghum bicolor* L. Moench) germination and Seedling Growth

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***Abstract**: In this study, a pot experiment was carried out to study the response of some parameters of seed germination and seedling growth of presowing sorghum (Sorghum bicolor L. Moench) seed treatments to four types of seed priming and three types of irrigation water. Seed priming treatments have been treated separately for soaking 12h in distilled water (Hydro- priming, DW), 500 ppm solution of Ammonium Molybdate (Mo), and magnetized water (physical priming, MW), while dry seeds representing unprimed seeds, as a control. While irrigation treatments involve three types of water: normal tap water (ITW), distilled water (IDW), and magnetized water (IMW). The analysis of the results indicated that seed priming and irrigation treatments significantly affected final germination percentage (FGP), mean germination time (MGT), leaf area (LA), shoot length (SL), root length (RL), and seedling vigor index (SVI). The best values for FGP (92.22, 88.89%), MGT (3.07, 3.2 day), LA (10.35, 9.95 cm2), SL (25.11, 24.43 cm), RL (14.14, 13.23 cm), and SVI (3760, 3373) were observed in priming with MW and Mo, respectively. Whereas, the best values for FGP (91.67%), MGT (3.09 day), LA (10.22 cm2), SL (25.47 cm), RL (14.14 cm), and SVI (3575) have recorded in IMW treatment. The interaction of different type of priming, and irrigation water have also recorded a significant effect for FGP (100, 93.33%), LA (13.26, 12.96 cm2), SL (28.80, 28.27 cm), and RL (17.03, 15.17 cm) in the interaction of treatments IMW×MW and IMW×Mo, respectively. Overall, the results show that seed priming with magnetized water or Ammonium Molybdate and irrigation with magnetized water could be a promising technique for improving sorghum seedling germination and growth.*

***Keywords**: Sorghum, Ammonium Molybdate, Magnetized water, Irrigation*

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I. Introduction

Seed sorghum (*Sorghum bicolor L. Moench*) ranking the fifth most economically important crop among cereals in the world after wheat, maize, rice, and barley due to their high nutritional quality. It is one of the principal sources of energy and protein. Thus, it is an important staple food crop in the world. Furthermore, sorghum provides the raw material for the supply of starch, fiber, biofuels, and other products (Teressa *et al*., 2021). Seed priming is one of the most major progress to help rapid and uniform germination and the emergence of seeds and vigorous seedlings in different crops. Seed priming is considered an easy, highly successful, low-cost, and low-risk technique intervention appropriate to the farmers' needs (Pawar and Laware, 2018). On the other hand pre-sowing seed treatments (seed priming) including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), biopriming (hydration using biological compounds), and magneto-priming (Sarraf *et al*., 2021; Salehzade *et al*., 2009; Ashraf and Foolad, 2005). Although seed priming occurs in many forms, but to enhance germination and growth, seed priming has usually described as pre-sowing treatment in water or chemical solutions. Seed priming different chemical solutions, especially Ammonium Molybdate, can lead to improved germination and growth in many crops such as Pea (Swami *et al*., 2021); wheat (Lazim and Ramadhan, 2019); cowpea (Arun *et al*., 2020); bean (Majda *et al*., 2019); and chickpea (Rahman *et al*., 2014). Recently the interest in the uses of the physical seed priming materials (ultrasonic, gamma, laser, ultraviolet irradiation, magnetic field, microwave, and hydro-priming) on germination, growth, and yield of various crop plants has increased. For example, magnetic field treatment of seeds, as published by Lazim (2021); Bukhari *et al*., (2021); Lazim and Ramadhan (2020b); and Lazim and Nasur, (2017), stimulates the seed germination and growth of barley, sunflower, wheat, and sorghum, respectively. On the other hand, Lazim and Ramadhan, (2020a) show that the effects of Microwave radiation on

the germination and growth of barley seeds gave the best germination results. In addition, Semenov *et al*., (2020); and Sadeghianfar *et al*., (2019) show that UV irradiation positively affects the biometric parameters of wheat and cowpea seedlings, respectively. Moreover, the treatment of soybean seeds with irradiation of laser light showed positive effects in some of the characteristics biostimulation process (Sarreta and de Castro Neto, 2021). The process of passing water through or exposing seeds to a magnetic field is known as a magnetic treatment. Furthermore, the water has treated by the magnetic field or passes through a magnetic device called magnetized water (Alattar *et al*., 2021). Magnetizing water (MT) and irrigation of seeds are considered one technique of magnetic field application. Also, it had shown increased absorption and assimilation of nutrients resulting increase in plant growth (Massah *et al*., 2019). Therefore, the application of magnetic water could be one of the most promising methods for plant growth stimulation, also considered environmentally friendly physical presowing seed germination (Abd Ellateef and Mutwali, 2020; Aghamir *et al*., 2015). In addition, the magnetized water has been shown to improve plant germination and growth in several field crops, such as sorghum (Teressa *et al*., 2021); sunflower (Afzal *et al*., 2021); barley (El-Zanaty *et al*., 2021; Hozayn *et al*., 2020); wheat (Massah *et al*., 2019); and maize seeds (Alattar *et al*., 2021; Aghamir *et al*., 2015).

There has a lack of knowledge about the type effects of irrigation magnetized water and different seed priming treatments on seed germination and seedling growth parameters of sorghum. Moreover, there hasn't been enough research found in this respect. Therefore, this research aimed to study the type effects of irrigation (magnetized water and tap water) and seed priming treatments (soaking in distilled water, ammonium molybdate, and magnetized water) on some of the seed germination and seedling growth parameters of sorghum.

II. Materials and methods

Experimental Design and Treatments:

Healthy seeds of sorghum (*Sorghum bicolor L. Moench*) selected for uniform size and shape, were used in this experiment. Pot experiments were conducted in the laboratory physics at the department of agricultural machinery and equipment, Agriculture College, the University of Basrah, to study the response of growth germination of a sorghum plant for priming and watering treatment. The replicated pot experiment was conducted as a factorial experiment, using a completely randomized design with three replicates for each treatment, involved four priming seeds techniques and three types of irrigation water. Before the treatments, seeds have divided into three groups. For each group, the seeds were first subjected to four priming treatments with three replications of each as shown below:

1. Unprimed seeds (dry seeds) were taken as the control treatment (UP).
2. Seeds were primed with distilled water (hydro-priming) for 12 h (DW).
3. Seeds were primed in 500 ppm solution of ammonium molybdate for 12 h (Mo).
4. Seeds were soaking primed with magnetized water for 12 h (MW).

At the end of the priming process, the seeds were placed in open air to dry. For each group of the four treatments, 30 seeds were planted in plastic pots (20 cm in diameter and 15 cm in depth) in 2 cm depth of soil (each pot ten seeds). Each pot group treatment was watered daily separately with about 200 ml/pot from the time of planting until the end of the germination period by three types of water as shown below:

1. The first group was watered with normal tap water as a control (ITW).
2. The second group was watered with distilled water (IDW).
3. The third group was watered with magnetized distill water (IMW).

There were 36 treatments and each was replicated three times. The magnetic water device has been used to magnetize distilled water for 6 hours, which then has used to soaked seeds for 12 h. The magnetic device had a magnetic field strength of nearly 120 mT. The magnetic strength field has determined by a Gaussmeter produced by Nv. Ltd, India, unit type Nv621. The magnetized distill water used in this experiment has prepared using a static magnetic field and then used for irrigation pots in group 3.The magnetization time was 6 hours. During the growth of the plants, the irrigation of all three groups has continued at adequate water amounts for all types of water used, with a consistent watering period maintained similar to all tested seedlings.

Data Collection:

After two days, the plants have become to grow over the soil level. Then the counting of germinated seeds has started at a specific hour. The measurements and observations were recorded daily for ten days consecutively. The experiment has terminated until no more seeds germinated on day 10.

Measurements of seed germination and seedling growth:

Every day, the total number of seeds that germinated has counted. After that, on the tenth day after seeding, the final germination percentage was calculated using the following formula:

$FGB\% = \frac{N_g}{N_t} \times 100$, where N_g is the number of germinated seeds 10 Days after sowing, and N_t is the total number of seeds planted.

The time to reach 50% of the final germination percentage (T_{50}) was calculated according to the formula of Coolbear *et al*., (1984) modified by Farooq *et al*., (2005), and Tounekti *et al*., (2020) as follows:

$T_{50} = t_i + \frac{\left[(\frac{N}{2} - n_i)(t_j - t_i)\right]}{n_j - n_i}$ (day) ,where N is the final number of germination and ni , nj are the cumulative number of seeds germinated by adjacent counts at times when ni <N/2<nj

Mean Germination Time (MGT) was calculated according to the following formula of Ellis and Roberts, (1981):

$MGT = \frac{\sum nD}{\sum n}(\text{Day})$, where n is the number of seeds, which were germinated corresponds to the day D observation (not the accumulated number), and D is the number of days counted from the beginning of germination.

Germination Rate Index (GRI) was calculated based on Al-Mudaris, (1998) using the following equation:

GRI= G_1/D_1+ G_2/D_2+ … + G_n/D_n (%/day), where G_1, G_2, G_3….G_n is the number of seeds newly germinated on 1, 2, 3… n^{th} day, respectively, and D_1, D_2, D_3,…D_n count on 1, 2, 3,… n^{th} day respectively.

Mean seedling length (cm): Ten days after sowing, five normal seedlings from each treatment have randomly selected to measure shoot (SL) and root length (RL) using a graduated scale in cm. Then the mean seedling length (SL+RL) were calculated.

Seedling Vigor Index (SVI) was calculated by using the below formula as suggested by Abdul-Baki *et al.,* (1973):

SVI= Germination % × Seedling Length (SL+RL)

Leaf area was calculated using the following formula of Amanullah et al., (2007):

Leaf area = leaf length × leaf width × factor (0.75)

Statical Analysis:

Statistical analysis of recorded data of all parameters was performed in triplicates using the variance analysis (ANOVA). All treatments were subjected to the test of Least Significance Difference (L.S.D) to compare the variation between the treatment means and their interaction at the 5 % probability level.

III. Results And Discussion

The results of the independent effect of both priming and irrigated water treatments on seed germination characters such as FGP, MGT, GRI, and T_{50} have presented in Tables (1 and 2). The data presented in the Table 1 show that FGP was significantly increased by seed priming with MW and Mo with 20.28 and 15.93 %, respectively, compared to dry seeds (control). While the data in the same table show that MGT has significantly decreased with all priming seeds, as compared to control. The time required for each treatment to germination was, in general, less than the corresponding control values. Thus the rate of germination of priming seeds has higher than that of dry seeds (control). Table 1 demonstrates, on the other hand, that priming treatment had no significant effect on GRI and T50. However, MW is priming improves the GRI and T_{50} to 3.38 %day and 2.58 days, respectively.

My findings on seed priming with MW are consistent with those of Elfadil and Abdallah (2013), who found that pretreatment of seeds with magnetized water improves the growth and development of Sorghum plants. Similar results had found by Kumar and Krishnaraj (2018), who showed that high germination from magnetized seed treated of cowpea, soybean, corn compared to seeds treated with those normal tap water. In agreement with these results, Afzal *et al*., (2021); Cheikh *et al*., (2018); and Noor *et al*., (2016) revealed that magnetic treatment of sunflower, cucumber, and Pea seeds, respectively, can accelerate germination compared to untreated samples. The water is either treated by a magnetic field or passes through a magnetized water device called magnetized water. Some biophysical, physicochemical, chemical and physical properties of water are changed when it's magnetized, which may be lead to changes in plant characteristics, growth, and production (Alattar *et al*., 2021). Magnetizing treated water (MTW) is considered one technique of

magnetic field application. Also, MTW has been shown as an environmentally friendly method of seed germination presowing. Therefore, MTW has been considered one of the most promising methods for plant growth stimulation (Aghamir *et al*., 2015). Seeds treated with magnetized water might have enhanced enzymatic and photosynthetic activities and increased the absorption and assimilation of nutrients, which results in increased plant growth (Cheikh *et al*., 2018).

On the other hand, it is well-known that seed priming with different chemicals, especially Ammonium Molybdate, has been shown to enhance the germination and growth of many crops (Arun *et al*., 2017; Rahman *et al*., 2014; Kumar and Krishnaraj, 2018). My results in Mo seed priming agree with the findings of Lazim and Ramadhan (2019) on Wheat; Arun *et al*., (2016) on cowpea; and Baker *et al*., (1976) on corn. Seed priming is a simple, cost effective pre-sowing technique that helps seeds absorb water and activate early germination activities while preventing radicle protrusion followed by drying (Satani *et al*., 2021). Seed priming can take various types, but here only hydropriming, Ammonium Molybdate priming, and magnetized water (physical priming) have been done to enhance germination and growth. Seed priming is a seed improvement technique that improves germination, seedling growth, and seedling establishment rate and uniformity (Khan *et al*., 2009). The increase in the germination of priming seeds with ammonium molybdate might be due to different biochemical and physiological changes in priming seeds, causing the enhanced synthesis of α-amylase, peroxidase, and dehydrogenase activity. The synthesis of enzymes might have an earlier initiation of metabolic processes leads to enhanced germination events in seed priming (Arun *et al*., 2017).

Table 1. **Effect of different priming treatments on seed germination parameters of sorghum**

Priming treatments	Seeds germination parameters			
	FGP %	MG Tday	GRI %/day	T_{50} day
Unprimed (UP)	76.67^{b}	3.54^{a}	2.47	3.20
DW	85.56^{ab}	3.19^{b}	3.01	2.85
Mo	88.89^{a}	3.21^{b}	3.12	2.59
MW	92.22^{a}	3.07^{b}	3.38	2.58
$L.S.D_{0.05}$	9.44	0.3063	N.S	N.S

DW= Hydro-priming, Mo= Ammonium Molybdate priming, magnetized water priming. Different superscript letters across each column are mean significantly different at P< 0.05 level; N.S=Non-significance.

Regarding seed germination parameters, results clear that a positive effect of type irrigated water treatments on FGB and MGTcompared with untreated treatment (Table 2). Concerning FGB (Table 2), the analysis showed a significant increase in treatments, irrigation with magnetized water by 17.03%, and irrigation distill water by 11.70%, compared to irrigation with tap water (control). While concerning MGT, showed a significant decrease in treatments, irrigation magnetized water and irrigation with distilled water by 13.68% compared to irrigation with tap water (control). The time required for each treatment to germination was, in general, less than the corresponding control values. As a result, the rate of treated seeds germinated more rapidly than untreated seeds. On the other hand, irrigated water treatments on GRI and T_{50} have found no significant effects. However, there found an enhancement in the GRI of 3.35 %day and T50 of 2.55 days with irrigated magnetized water.

The positive effect of magnetized water for irrigation on seed germination agrees with those shown in several field crops by many recent types of research. For example, Massah *et al*., (2019) noticed that magnetized water increased the germination and growth characteristic of wheat seeds. Also, the physiological factors of bean seeds, such as germination percentage and index, were shown to be significantly increased by irrigation magnetized water, according to Aghamir *et al*., (2016). On the other hand, these results also coincided with those reported by Aghamir *et al*., (2015) for corn seeds irrigated with magnetic water. Saeed and Mutwali (2019) reported that the activation of enzymes and hormones involved in the germination process and nutrient mobilization and seed absorption of magnetized water before sowing might be due to irrigation of cowpea seeds with magnetically treated water.

As a result, there is probably an increase in nutrient mobilization and transportation to the embryonic axis and a resultant increase in emergence speed and germination rate.

Table 2. Effect of different irrigation treatments on seed germination parameters of sorghum

Irrigation treatments	Seeds germination parameters			
	FGP %	MGT day	GRI %/day	T_{50} day
ITW (Control)	78.33^{b}	3.58^{a}	2.49	2.94
IDW	87.50^{a}	3.09^{b}	3.14	2.94

IMW	91.67[a]	3.09[b]	3.35	2.55
$L.S.D_{0.05}$	8.17	0.2652	N.S	N.S

DW= Hydro-priming, Mo= Ammonium Molybdate priming, magnetized water priming. Different superscript letters across each column are mean significantly different at P< 0.05 level; N.S=Non-significance

Also, the results of combined treatment with priming seeds and irrigation water have shown in table (3). According to obtained results in Table 3, no significant combination treatments have been recorded on all seed germination characters, except for the FGB. The IMW×MW and IMW × Mo treatment combination had a higher FGB percentage of 100% and 93.33%, respectively. On the other hand, in the same Table, the IMW × MW treatment combination had no significant effect recording on the other germination characters. However, there found an enhancement in the MGT (2.7 days), GRI (3.94%/day), and T_{50} (2.22 days).

Table 3: Interaction effect of different priming and irrigation treatments on seed studied germination parameters of sorghum.

Treatments	Seeds germination parameters			
	FGP %	MGT day	GRI %/day	T_{50} day
ITW×UP	66.66	3.90	2.01	3.33
ITW ×DW	80	3.35	2.65	2.96
ITW ×Mo	83.33	3.56	2.51	2.61
ITW ×MW	83.33	3.49	2.80	2.87
IDW × UP	80	3.35	2.60	3.69
IDW × DW	86.66	3.06	3.12	2.88
IDW × Mo	90	2.91	3.41	2.52
IDW × MW	93.32	3.01	3.43	2.66
IMW × UP	83.33	3.37	2.80	2.59
IMW × DW	90	3.16	3.28	2.72
IMW × Mo	93.33	3.14	3.44	2.66
IMW × MW	100	2.7	3.94	2.22
$L.S.D_{0.05}$	16.35	N.S	N.S	N.S

DW= Hydro-priming, Mo= Ammonium Molybdate priming, magnetized water priming, ITW= irrigation; N.S=Non-significance normal tap water, IDW=irrigation distill water, IMW=irrigation magnetic water; N.S=Non-significance

Regarding seedling growth characters, Table 4 shows a significant positive effect between priming treatments and unprimed seed treatment (control) in leaf area (LA), shoot length (SL), root length (RL), and seedling vigor index (SVI). The highest values of LA (10.35 cm2), SL (25.11 cm), RL (14.14 cm), and SVI (3760) have recorded at the soaking primed with magnetized water compared with unprimed seeds, with an increase of 87.65, 37.43, 41.96, and 70.67 %, respectively (Table 4). These results agree with those shown by Kumar and Krishnaraj (2018), who report that soaking primed with magnetized water cowpea, soybean, and corn seeds results in a significant increase in shoot and root length, and leaf sizes, compared with unprimed treatment.

Seed priming regulates various molecular, biochemical, and physiological activities in seedlings, including cell division, elongation, and stress response protein activation. As a result, the seed priming technique has played an essential role in plant growth and development (Anwar *et al*., 2020). Afza *et al*., (2021) found that the positive effects prim of magnetized water on the growth of root and leaf of Sunflower is due to an increased rate of cell division in the root tips and earlier start of emergence since they appear to induce an improved capacity for nutrients and water uptake. Similarly, the best results were found in Mo seed priming compared with unprimed seeds, with an increase of 80, 33, 32.83, and 53.10 % in LA, SL, RL, and SVI, respectively (Table 4). These results agree with those shown by Arun *et al*., (2020) and Singh *et al*., (2014), who reported that Mo seed priming of cowpea and chickpea respectively, results in a significant increase in growth parameters compared with unprimed treatment (Table 4).

Table 4. Effect of different priming treatments on seedling growth parameters of sorghum

Priming treatments	seedling growth parameters			
	Leaf area (cm^2)	SL (cm)	RL (cm)	SVI
Unprimed (UP)	5.51[c]	18.27[c]	9.96[d]	2203[d]
DW	8.27[b]	22.35[b]	11.94[c]	2840[c]
Mo	9.95[a]	24.43[a]	13.23[b]	3373[b]

MW	10.35^{a}	25.11^{a}	14.14^{a}	3760^{a}
$L.S.D_{0.05}$	0.846	1.334	0.641	359.4

DW= Hydro-priming, Mo= Ammonium Molybdate priming, magnetized water priming. Different superscript letters across each column are mean significantly different at P< 0.05 level

Similarly, all results in Table 5 showed a significant difference between all irrigation treatments in seedling growth parameters compared to irrigation with tap water (control). As shown in Table (5), the irrigation with magnetized water increased LA (86%), SL (42.76%), RL (46.83%), and SVI (63.91%) as compared to irrigation with tap water.

Also, the data in the same Table show that irrigation with distill water significantly increased the seedling growth parameters LA, SL, RL, and SVI, by 79.74, 36.26, 36.86, and 54.83 %, respectively, compared to irrigation with tap water. The obtained results are consistent with those reported by Sadeghipour and Aghaei (2013), who found that irrigation with magnetized water increased the leaf area and root growth of cowpea plants significantly. Moreover, in this connection, Alattar *et al.*, 2021 showed that the corn plants watered with magnetized water had higher shoot lengths than those watering normal tap water.

Similar results had obtained by Abd Ellateef and Mutwali, 2020 who reported that irrigation of broad bean with magnetized water-induced significant increases in plant height, number of leaves, and leaf area compared with non-magnetized water.

The increase in treatments irrigated with magnetized water may be attributed to the increases in the ability of water absorption and nutrients by the seeds and consequently lead to an increase in seed germination and plant growth (Abd Ellateef and Mutwali, 2020).

Table 5. Effect of different irrigation treatments on seedling growth parameters of sorghum

Irrigation treatments	Seeds germination parameters			
	Leaf area (cm^2)	SL (cm)	RL (cm)	SVI
ITW (Control)	5.49^{b}	17.84^{c}	9.63^{c}	2181^{b}
IDW	9.86^{a}	24.31^{b}	13.18^{b}	3377^{a}
IMW	10.22^{a}	25.47^{a}	14.14^{a}	3575^{a}
$L.S.D_{0.05}$	0.732	1.155	0.555	311.3

ITW= irrigation normal tap water, IDW=irrigation distill water, IMW=irrigation magnetic water. Different superscript letters across each column are mean significantly different at P< 0.05 level

On the other hand, the results of combination treatment with priming seeds and irrigation water have shown in table (6). The seedling growth parameters exhibited significant responses to treatment combinations. As shown in Table (6), the IMW×MW treatment combination had a higher LA (13.26 cm2), SL (28.80 cm), RL (17.03 cm), and SVI (4884). Also, in the same Table the IMW × Mo treatment combination had a higher LA (12.96 cm2), SL (28.27 cm), RL (15.17 cm), and SVI (4048).

According to our knowledge, there are no other studies on combination treatments of seed priming with magnetized water or ammonium molybdate and with irrigation magnetized water concerning their seed germination and seedling growth of presowing sorghum. Therefore, it is not possible to compare our results with another study.

Table 6: Interaction effect of different priming and irrigation treatments on studied seedling growth parameters

Treatments	Seeds germination parameters			
	Leaf area (cm^2)	SL (cm)	RL (cm)	SVI
ITW×UP	3.69^{f}	14.07^{f}	7.5^{h}	1445
ITW ×DW	5.82^{e}	18.15^{e}	9.4^{g}	2212
ITW ×Mo	5.54^{e}	18.13^{e}	10.07^{fg}	2353
ITW ×MW	6.91^{e}	20.99^{d}	11.53^{e}	2713
IDW × UP	6.39^{e}	19.83^{de}	10.9^{ef}	2471
IDW × DW	9.82^{cd}	24.99^{bc}	13.5^{cd}	3333
IDW × Mo	12.36^{a}	26.91^{ab}	14.46^{bc}	3718
IDW × MW	10.86^{bc}	25.53^{bc}	13.87^{cd}	3682
IMW × UP	6.46^{e}	20.91^{d}	11.47^{e}	2693
IMW × DW	9.18^{d}	23.90^{c}	12.93^{d}	2985
IMW × Mo	12.96^{ab}	28.27^{a}	15.17^{b}	4048
IMW × MW	13.26^{a}	28.80^{a}	17.03^{a}	4884
$L.S.D_{0.05}$	1.465	2.310	1.111	622.6

DW= Hydro-priming, Mo= Ammonium Molybdate priming, magnetized water priming, ITW= irrigation normal tap water, IDW=irrigation distill water, IMW=irrigation magnetic water. Different superscript letters across each column are mean significantly different at P< 0.05 level

IV. Conclusion

As conclusions, this study shows that MW and Mo priming seeds and the irrigation magnetized water significantly affected the sorghum germination and growth development parameters, including FGB, MGT, LA, SL, RL, and SVI. Moreover, FGB, LA, SL, and RL exhibited significant responses to treatment combinations of MW and Mo priming seeds and IMW irrigation water treatments. So as a simple cost and safe method, seed priming with magnetically treated water and Ammonium Molybdate and irrigation with magnetized water can be used to improve sorghum germination and growth.

Conflict of interest: Authors declare that there is no conflict of interests.

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