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ARTICLE

Seed Priming with $MgCl_2$, $CaCl_2$, and $ZnCl_2$ as a Biofortification Based-Approach Induces Changes in Anise Seedlings Emergence

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ABSTRACT

Aromatic and medicinal plant species having small seeds have field emergence problems due to low nutrient supply. Therefore, *Pimpinella anisum* seeds were hydro and osmoprimed with 100 mM $MgCl_2$, $CaCl_2$, and $ZnCl_2$, for 2, 4, and 8 h each to compare their growth attributes during germination and seedling establishment stages. Non-treated seeds were used as control. Both hydro and osmo primed seeds were dried for 48 h before, they were sown in plastic trays in growth room conditions to see the impact of treatments on seedling emergence and growth. The maximum root length (12.90 cm), fresh weight (256.30 mg plant^{-1}), and mean emergence time (MET) were recorded from 8 h $MgCl_2$ primed seeds. Similarly, the maximum (97.50, and 98.00%) emergence percentage was observed using 8 h $MgCl_2$ primed seeds and nontreated seeds (control treatment). The evaluation of parameters like chlorophyll contents and electrical conductivity showed the 8 h $MgCl_2$ priming as the optimum treatment. The evaluation of parameters like chlorophyll contents and electrical conductivity showed the 8 h $MgCl_2$ priming as the optimum treatment. The result suggests $MgCl_2$ priming worked synergistically and improved seedling growth attributes under greenhouse conditions. The chlorophyll content ranged 25.94–35.69 SPAD unit. The highest chlorophyll content was obtained from the seedlings obtained from 4 h $CaCl_2$ treatment, which were statistically similar to the chlorophyll contents of the seedlings obtained after 8 h $MgCl_2$ treatment and nontreated seeds (control treatment). All other treatments showed inhibition in the chlorophyll contents and growth attributes of the seedlings. In conclusion, $MgCl_2$ osmopriming treatments were significantly promotive and better compared to hydro-priming and osmopriming treatments including control treatment in terms of anise seeds germination and emergence.

KEYWORDS

Pimpinella anisum L; seed treatment; hydropriming; osmopriming; electrolytes leakage

1 Introduction

Anise-*Pimpinella anisum* L. (Umbelliferae, Apiaceae) is an important flowering, medicinal and aromatic plant native to the Eastern Mediterranean and South Western Asian regions [1,2] that grows to around 40–50 cm with white or yellow flowers [3]. It has been cultivated in Egypt for the last 4,000 years; from where it spread to other parts of Europe and the Middle East [4]. The species belonging to the genus Pimpinella are represented by 27 taxa in the flora of Türkiye [5] and have multiple uses in ethnomedicinal systems. It is used singly or in combination with other herbs and taken as a carminative stomachic, stimulant, expectorant,

antispasmodic, and antiseptic [6]. It is also used as an aromatic flavoring agent in desserts and alcoholic beverages [7]. The plants grow and establish well on fertile, warm soils soon after spring [8].

Anise seeds have very small endosperm, and face difficulties during early stages of growth. Therefore seeds face multiple abiotic and biotic stresses; which may inhibit their germination, along with growth and development of seedlings under natural conditions [9,10]. Several strategies in soil fertilization are being employed by farmers to improve germination and emergence that vary depending on the farmers. Most of the farmers always tend to apply an easy and cheap way of crop production. Seed pre-sowing osmopriming treatments are widely used as an easy way to biofortify seeds with nutrient elements to stimulate their germination, emergence [11] and inhibit the effects of external stresses [12].

Hydroprimed and osmoprimed seeds of *Oryza sativa* L. [13,14], *Brassica juncea* L. [15], and *Lens culinaris* Medik. [16,17] showed improvement in germination and stand establishment. Although, anise is used as a multifunctional medicinal crop like *Nigella sativa* [18] and *Ocimum basilicum* [19] the studies on germination, seedling establishment and agronomic practices are rare. There is no study regarding the strategy of treating $MgCl_2$ (Magnesium chloride), $CaCl_2$ (Calcium chloride), and $ZnCl_2$ (Zinc chloride) to improve anise germination and protect them from stresses during germination. This study evaluated the metabolic stimuli induced changes in anise seeds due to $MgCl_2$, $CaCl_2$, and $ZnCl_2$ based osmopriming for obtaining uniform seed germination and stands in fields that are difficult to obtain under natural conditions [20]. These stored mineral nutrients like magnesium, calcium, and zinc have importance especially when seedlings are under conditions of limited nutrients. Enrichment of seeds via seed priming with these nutrients could promote germination and seedling growth.

Magnesium (Mg) is an important macronutrient element that is required to carry out several biochemical and physiological processes in plants [21] during photosynthesis by binding to chloroplasts, participating in the light harvest in PSI and PSII [22]. It is also involved in carbon fixation by chloroplasts [23]. Mg is a micronutrient element and an environment-friendly biostimulant. Mg deficiency reduces the rate of the biomass with a disruption of CO_2 fixation and generation of reactive oxygen species (ROS) ending up in cell damage [22]. It also acts as an enzyme cofactor activity with ATP [24] based on hydrolysis and synthesis [25]. It is an important constituent of chlorophyll molecules and the powerhouse behind photosynthesis.

Calcium (Ca) is one of the main components of cell structure and it takes a role in cell elongation, division, controlling nutrient absorption, and helping water absorption [26]. Furthermore, due to its role in cell structure, and signaling roles in plants; Ca^{2+} ions are important in charge, osmotic balance, and in seed germination. When the seeds are imbibed with the impact of rehydration, gibberellins cause Ca^{2+} flux into the cytosol and the expression of calmodulin (Ca^{2+} binding proteins); which are involved in signal transduction [27,28]. Alpha-amylase enzyme one of the Ca^{2+} metalloenzymes required at the beginning of germination [29,30], is the consequence of Ca^{2+} related post-imbibition metabolism.

Zinc (Zn) is crucial during seed germination and the early growth stage of plants until the root system gains the ability to nutrient uptake from the soil [31]. Zinc is a co-factor of various enzymes that take a role in the detoxification of ROS. Beyond that stage, the seeds containing low zinc show a delay in germination and poor seedling vigor [31,32].

Chlorides (Cl) prevent the accumulation of free amino acids and protect plants against diseases with easy management of intercellular transport of water [33]. It is understood that both Mg and Cl ions could improve quality and yields in cereals and other crops.

Therefore, the present study aimed to evaluate the impact of water, $MgCl_2$, $CaCl_2$, and $ZnCl_2$ priming on anise seeds germination and seedling growth under controlled conditions.

2 Materials and Methods

2.1 Seed Treatments (Priming) and Emergence Tests

The experimental material consisted of the anise seeds purchased from an anise farmer from Denizli Province in 2021.

Fifty seeds were used in each treatment with 4 replications. Nontreated seeds were used as control. Each of the aliquots of 50 seeds was immersed in 50 ml distilled water (hydropriming), 100 mM solutions of $MgCl_2$ (EC = 8.3 mS cm^{-1}), $CaCl_2$ (EC = 9.4 mS cm^{-1}), and $ZnCl_2$ (EC = 8.9 mS cm^{-1}) at 20°C for 2, 4, and 8 h under dark conditions. The osmoprimed seeds were rinsed and washed with distilled water to remove the traces of $MgCl_2$, $CaCl_2$ and $ZnCl_2$ on the seed surfaces. Thereafter, the surfaces of these seeds were dried and left at room temperature for 2 days (22 ± 1°C) to decrease moisture contents [34]. The seeds in each replicate were weighed before and after priming treatment to calculate their water uptake.

$$\text{Water uptake } (\%) = \frac{\text{Weight after priming (mg)} - \text{initial weight (mg)}}{\text{initial weight (mg)}} \times 100$$

Four replicates of 50 seeds (50 × 4 = 200 seeds) were sown at a depth of 2 cm in the plastic trays (30 cm × 21 cm × 9 cm) containing peat and placed in a growth chamber (Sanyo versatile Growth chamber, Japan) at 20 ± 1°C 45 μM photons m^{-2} s^{-1} light for 16 h. The peat used in the study had a pH of 6.5 and EC of 40 mS m^{-1}, the porosity of around 69% (v w^{-1}).

The number of emerged seedlings (unfolding cotyledons on the surface) was counted daily for up to 25 days, along with the emergence percentages of the respective seedlings. The plants were irrigated with 50 ml water 8 times during 26 days of the experiment. The mean emergence time (days) was calculated according to the formula given below ISTA [35]:

$$\text{MET} = \frac{\Sigma n \times t}{\Sigma n}$$

n = number of cotyledons on the turf surface at time t

t = days from planting

Σn = final number of the cotyledons on the turf surface

Chlorophyll contents were measured on the 25th day. Shoot length, root length, seedling fresh weight, and dry weight were measured for all seedlings from each replicate after the 26th day. Fresh weights of seedlings were measured soon after harvest to avoid weight loss [36]. The dry weight of the seedlings was measured after drying the samples in an oven at 70°C for 48 h [37].

2.2 Chlorophyll Content Measurement

Chlorophyll measurements were done with SPAD-502 Plus (Konica Minolta) using five leaves per seedling. Ten seedlings from each replicate were used for sampling [37].

2.3 Electrical Conductivity (EC) Test

The electrical conductivity (EC) of four replicates of 50 seeds each of soaked anise seeds with distilled water and different osmotic solutions for each treatment was measured using a WTW Cond 314i model conductivity meter [38]. The results were expressed in μS cm^{-1} to take account of variability in different treatments.

2.4 Experimental Design and Statistical Analysis

The experiment was arranged in a completely randomized design with four replicates. Water uptake and emergence percentage data were subjected to arcsine transformation before carrying out an analysis of variance with MSTAT-C statistical software (Michigan State University, version 2.10). The differences among the means were compared with Duncan's Multiple Range Test ($p < 0.01$ or $p < 0.05$).

3 Results

Seed water uptake varied among treatments ($p = 0.0000$). Seeds soaked in water for 8 h had the highest water uptake (96.00%) while the minimum water uptake (56.24%) was observed in 4 h $CaCl_2$ soaking (Table 1).

Table 1: Effects of different priming treatments on water uptake

Priming treatment	Duration (h)	Water uptake (%)
Hydropriming	2	64.79 ± 3.48^{cd}
$MgCl_2$	2	57.29 ± 0.67^{d}
$CaCl_2$	2	67.75 ± 0.37^{cd}
$ZnCl_2$	2	58.57 ± 0.58^{d}
Hydropriming	4	87.13 ± 1.43^{b}
$MgCl_2$	4	59.08 ± 5.33^{d}
$CaCl_2$	4	56.24 ± 3.06^{d}
$ZnCl_2$	4	62.32 ± 1.89^{cd}
Hydropriming	8	96.00 ± 9.20^{a}
$MgCl_2$	8	72.85 ± 8.14^{bcd}
$CaCl_2$	8	78.03 ± 5.29^{bc}
$ZnCl_2$	8	68.98 ± 0.94^{cd}

Note: All values shown with different letters in single columns are statistically different using Duncan's Multiple Range Test ($p < 0.01$), ±: Standard Deviation.

Mean emergence time represented a statistical difference over seed treatments ($p < 0.0000$). A comparison among seed priming treatments showed statistical differences among the non-treated, hydroprimed, and osmoprimed seeds. Non-primed seeds took 11.20 days to emerge. 8 h $MgCl_2$ priming also shortened the mean emergence time over control, which was statistically different from non-treated control treatment and were statistically different among themselves showing a range of 9.73 to 17.09 days to emerge. The minimum and the maximum time to emergence were noted in 8 and 2 h $MgCl_2$ priming. $CaCl_2$ and $ZnCl_2$ priming did not show any superiority over control or 8 h $MgCl_2$ priming and the emergence time was slower (Table 2).

Table 2: Effects of priming treatments on days to emergence and emergence percentages of anise

Treatment	Duration (h)	Mean emergence time (day)	Emergence percentages (%)
Control	0	11.20 ± 0.14^{e}	98.00 ± 1.63^{a}
Hydropriming	2	15.70 ± 0.49^{b}	56.00 ± 4.89^{cde}
$MgCl_2$	2	17.09 ± 0.69^{a}	65.00 ± 3.46^{bcd}
$CaCl_2$	2	14.84 ± 0.38^{c}	66.50 ± 3.00^{bc}

(Continued)

Table 2 (continued)

Treatment	Duration (h)	Mean emergence time (day)	Emergence percentages (%)
$ZnCl_2$	2	14.91 ± 0.42^{c}	67.50 ± 2.52^{bc}
Hydropriming	4	15.80 ± 0.53^{b}	56.50 ± 1.91^{cde}
$MgCl_2$	4	15.77 ± 0.09^{b}	63.50 ± 1.00^{bcd}
$CaCl_2$	4	14.90 ± 0.39^{c}	54.00 ± 2.82^{e}
$ZnCl_2$	4	13.56 ± 0.33^{d}	54.50 ± 3.42^{de}
Hydropriming	8	10.53 ± 0.39^{e}	48.50 ± 3.00^{e}
$MgCl_2$	8	9.73 ± 0.26^{f}	97.50 ± 1.00^{a}
$CaCl_2$	8	16.01 ± 0.50^{b}	68.50 ± 5.74^{b}
$ZnCl_2$	8	15.77 ± 0.54^{b}	58.00 ± 6.73^{bcde}

Note: All values shown with different letters in single columns are statistically different using Duncan's Multiple Range Test ($p < 0.01$); ±: Standard Deviation.

Impact of seed treatment on the emergence percentage showed statistically significant differences ($p < 0.0000$). The control and $MgCl_2$ treatments showed maximum and statistically similar values of 98.00% and 97.50%, respectively. The hydroprimed seeds were negatively affected in terms of the emergence percentage compared to the non-treated seeds (control treatment). The minimum emergence percentage of 48.50% was noted on 8 h of hydroprimed seeds. Emergence percentages of $CaCl_2$ primed seeds varied between 54.00% to 68.50% and $ZnCl_2$ primed seeds varied between 54.50% to 67.50% (Table 2).

The root length of anise seedlings showed significant differences ($p < 0.0000$) among non-treated, hydroprimed, and osmoprimed seeds (Table 3). A comparison of the mean root length values showed that the maximum (12.90 cm) root length was noted from 8 h of $MgCl_2$ primed seeds. Minimum root length was noted (5.43 cm) on 8 h $ZnCl_2$ primed seeds. Hydroprimed and $CaCl_2$ primed seeds had the longest roots in 8 and 2 h duration in the same order.

Table 3: Effects of different priming treatments on root & shoot lengths, and seedlings' fresh and dry weights

Treatment	Duration (h)	Root length (cm)	Shoot length (cm)	Seedling fresh weight (mg plant^{-1})	Seedling dry weight (mg plant^{-1})
Control	0	$11,33 \pm 0.62^{b}$	10.77 ± 0.430^{a}	254.00 ± 3.46^{a}	26.25 ± 2.63^{a}
Hydropriming	2	$6,32 \pm 0.37^{gh}$	9.59 ± 0.51^{abc}	$237,50 \pm 12.58^{a}$	21.00 ± 3.46^{ab}
$MgCl_2$	2	$6,45 \pm 0.68^{fgh}$	9.12 ± 1.02^{bcd}	132.30 ± 16.21^{cd}	13.50 ± 2.64^{cde}
$CaCl_2$	2	$9,60 \pm 1.24^{c}$	$9,62 \pm 0.58^{abc}$	150.50 ± 7.14^{cd}	15.00 ± 0.82^{cd}
$ZnCl_2$	2	$8,70 \pm 0.79^{cd}$	$10,43 \pm 0.48^{ab}$	253.30 ± 31.30^{a}	15.50 ± 1.73^{bcd}
Hydropriming	4	$6,60 \pm 0.66^{efgh}$	$7,30 \pm 0.66^{e}$	122.50 ± 6.56^{d}	13.25 ± 1.50^{cde}
$MgCl_2$	4	$7,23 \pm 0.90^{defg}$	10.18 ± 1.05^{ab}	170.30 ± 12.31^{bc}	16.75 ± 5.56^{bc}
$CaCl_2$	4	$8,52 \pm 0.81^{cd}$	$8,88 \pm 0.45^{cd}$	195.80 ± 30.65^{b}	12.75 ± 1.50^{cde}
$ZnCl_2$	4	$8,00 \pm 0.98^{def}$	$7,98 \pm 0.79^{de}$	132.80 ± 15.43^{cd}	10.00 ± 0.00^{de}

(Continued)

Table 3 (continued)

Treatment	Duration (h)	Root length (cm)	Shoot length (cm)	Seedling fresh weight (mg plant^{-1})	Seedling dry weight (mg plant^{-1})
Hydropriming	8	8,11 ± 0.90^{cde}	6.92 ± 0.60^{e}	190.30 ± 33.14^{b}	24.00 ± 5.89^{a}
$MgCl_2$	8	12,90 ± 1.09^{a}	10.35 ± 0.24^{ab}	256.30 ± 38.22^{a}	23.00 ± 1.83^{a}
$CaCl_2$	8	7,10 ± 0.59^{defg}	7,36 ± 0.05^{e}	148.50 ± 15.52^{cd}	15.50 ± 2.52^{bcd}
$ZnCl_2$	8	5,43 ± 0.57^{h}	7,35 ± 0.66^{e}	112.30 ± 14.57^{d}	8.25 ± 0.96^{e}

Note: All values shown with different letters in single columns are statistically different using Duncan's Multiple Range Test ($p < 0.01$); ±: Standard Deviation.

Analysis of variance showed significant differences ($p < 0.0000$) among treatments for shoot length. The maximum shoot length value of 10.77 cm was observed in the control (non-treated) treatment. This value was statistically similar to the 2 h hydroprimed seeds with values of 9.59 cm, 4 and 8 h $MgCl_2$ primed seeds with values of 10.18, 10.35 cm and 2 h $CaCl_2$ and $ZnCl_2$ primed seeds with values of 9.62 and 10.43 cm. The minimum shoot length value of 6.92 cm was noted from 8 h hydroprimed seeds. It seemed that the negative effects of hydropriming on growth parameters were due to over-imbibition by anise seeds during hydropriming (Table 3).

Seedling fresh weight was significantly ($p < 0.0000$) affected by seed treatments (Table 3). The maximum seedling fresh weight value of 256.30 mg plant^{-1} was noted in 8 h $MgCl_2$ treated seeds, which showed statistical similarity with non-treated seeds (254.00 mg plant^{-1}), 2 h hydroprimed seeds (237.50 mg plant^{-1}), and 2 h $ZnCl_2$ treatment (253.30 mg plant^{-1}). The lowest seedling fresh weights were obtained from 8 h $ZnCl_2$ primed seeds with a value of 112.30 mg plant^{-1}.

The seedling dry weight values of anise were significantly affected ($p < 0.0000$) by seed treatments. While the maximum seedling dry weight value was observed in non-treated seeds (26.25 mg plant^{-1}), statistical similarity with 8 h hydropriming (24.00 mg plant^{-1}) and 8 h $MgCl_2$ (23.00 mg plant^{-1}) treatment were also observed (Table 3).

Results about chlorophyll contents measurements (Table 4) clearly reflected the sharp impact ($p < 0.0000$) of seed treatments on chlorophyll contents. The maximum chlorophyll contents in terms of SPAD unit were observed in 4 h $CaCl_2$ priming with 35.69 SPAD units and statistically similar values were noted in control (34.51 SPAD units) and 8 h $MgCl_2$ priming (35.18 SPAD units). The osmoprimed seeds improved the chlorophyll contents depending on the duration of treatment.

Table 4: Effects of different priming treatments on chlorophyll contents of anise seedlings and EC of seed leakage

Priming	Duration (h)	Chlorophyll contents (SPAD unit)	EC (μS cm^{-1})
Nontreated seeds	0	34.51 ± 0.47^{a}	0,050 ± 0.008^{i}
Hydropriming	2	26.96 ± 0.39^{fg}	402,1 ± 0.89^{c}
$MgCl_2$	2	25.94 ± 0.54^{g}	9.99 ± 0.71^{h}
$CaCl_2$	2	27.34 ± 0.91^{efg}	10.17 ± 0.01^{h}
$ZnCl_2$	2	28.10 ± 0.62^{def}	95.20 ± 0.08^{e}
Hydropriming	4	29.97 ± 0.54^{c}	533.7 ± 0.82^{b}

(Continued)

Table 4 (continued)

Priming	Duration (h)	Chlorophyll contents (SPAD unit)	EC (μS cm^{-1})
$MgCl_2$	4	29.67 ± 0.42^{cd}	9.71 ± 0.06^{h}
$CaCl_2$	4	35.84 ± 1.05^{a}	98.51 ± 1.23^{d}
$ZnCl_2$	4	31.89 ± 0.46^{b}	93.80 ± 0.08^{f}
Hydropriming	8	29.18 ± 1.04^{cd}	671.7 ± 0.58^{a}
$MgCl_2$	8	35.18 ± 2.04^{a}	9.73 ± 0.01^{h}
$CaCl_2$	8	30.26 ± 0.34^{c}	98.53 ± 0.48^{d}
$ZnCl_2$	8	28.75 ± 0.41^{cde}	92.40 ± 0.08^{g}

Note: All values shown with different letters in single columns are statistically different using Duncan's Multiple Range Test ($p < 0.01$), ±: Standard Deviation.

The electrical conductivity test showed significantly dissimilar variations ($p < 0.0000$). The minimum values were observed in $MgCl_2$ priming in all treatment durations between 9.71–9.99 μS cm^{-1} (Table 4). The maximum EC value was observed in 8 h hydropriming with 671.7 μS cm^{-1}.

4 Discussion

During priming treatments, the seeds were partially hydrated with water and different osmotic agents to a point (2, 4, and 8 h). Water uptake showed diversity among treatments. The results revealed that among priming treatments hydropriming had the highest water uptake in all durations. This could be attributed to a low EC value of distilled water compared to other treatments, leading to uncontrolled water uptake.

$MgCl_2$ priming treatments showed an increase in root length, fresh and dry weight along with chlorophyll contents with each increase in priming treatment duration of 2 to 8 h. $CaCl_2$ priming treatment showed maximum improvement after 4 h of priming treatment. These two treatments were at par with the values obtained from nonprimed seeds used as control treatment. $ZnCl_2$ priming treatments were noneffective at any duration of treatment and were inferior compared to the control treatment. The maximum values for these parameters were noted on 2 h treatment duration followed by obtaining decreased values for each parameter on 4 and 8 h priming duration treatments. The results indicated variable inhibition in these parameters for the rest of the primings for 2, 4, and 8 h excluding 8 h $MgCl_2$ treatment.

Seedling dry weight in control had the maximum value among all treatments but no statistical difference was observed with 8 h $MgCl_2$ priming. Priming duration in each treatment showed dissimilar results. This showed that the seeds could end up with either of the

a) replacement of seed nutrient matters in the cytoplasm with water moving from outside.
b) accumulation of the undesired amount of water into the seed cells.
c) bursting of cells, which are not desired beyond maintenance of osmotic balance between the internal and external forces.

An imbalance in the solid loss/water gain ratio is not desired and ends up in embryo damage with negative implications on the growth of seedlings' roots and shoots depending on the environmental factors and genetic potential of the seeds [39,40].

Mg has an important role in the development and formation of sink organs [41,42] and the extra Mg given through osmopriming helped seed and seedling attributes to improve them in terms of fresh seedling weight per plant over other treatments. The nutrient matters are lost and washed away through

osmosis with each increase in the duration of hydropriming, which influence the growth and development of roots from the seeds [42]. $MgCl_2$ osmopriming maintains the genetic fidelity of the seeds, which should be carried out very carefully. Taking water up to a critical point is essential for germination and improving seed vigor, with better root growth and uniform crop establishment, and larger canopy [43,27]. This helps in improved competition with the surrounding plants and higher yields [44].

Zinc is important during germination against abiotic stress and for seedling vigor. However, $ZnCl_2$ priming induced inhibition, toxicity, and dormancy during germination and growth compared to the control or other priming treatments. It seemed the amount and duration of $ZnCl_2$ (100 mM) treatments used in the current experiment were toxic for anise. These findings were corroborated by Stanković et al. [45] who had similar findings using different levels of $ZnCl_2$ to germinate wheat seeds germination observing limited seedling growth.

Hydropriming during 2, 4 and 8 h led to uncontrolled water uptake of anise seeds and less emergence percentage than other treatments. Imbibition damage was also evident due to minimum germination in 8 h hydropriming. Imbibition damage due to uncontrolled rapid water uptake causes cell death and high solute leakage which was observed as the highest in hydropriming compared to other treatments. Considering these results 8 h of priming with water reduced the emergence percentage for anise seeds. The disadvantages of hydropriming were mentioned by Lutts et al. [46] and it is also recommended to define accurate treatment duration, temperature, and water volume for every species. On the contrary osmopriming with 8 h $MgCl_2$ created a positive impact on emergence percentage due to slow water uptake related to low water potential. Positive effects of $MgCl_2$ seed priming on germination percentage have been noted in rice by Brooks et al. [47].

A decrease in chlorophyll content leads to low photosynthesis which has a negative impact on plant growth [37]. Magnesium is necessary for the synthesis of chlorophyll, and essential for photosynthesis [14] and its deficiency causes a reduction in chlorophyll concentrations in leaves [42]. Additionally, Wang et al. [48] have already mentioned the protective role of Ca^{2+} on photosynthetic electron transport. The results show similarity with the observation showing 8 h $MgCl_2$ and 4 h $CaCl_2$ priming of anise seeds led to an increase in chlorophyll synthesis compared to hydroprimed seeds in this research.

Electrolyte leakage has been reported as the determiner of seed viability and vigor in several species. The seeds having less vigor leak more electrolytes during imbibition [49,50]. The hydroprimed anise seeds had less emergence percentage and had the maximum EC values which seemed as an indicator of hydropriming with a negative impact on anise seeds and germination. However, priming with $MgCl_2$ gave the minimum EC values among priming treatments, which was the evidence of repair mechanism of the membrane, especially for 8 h $MgCl_2$ priming.

Sustaining favorable water under field conditions is critical for the germination process. Priming with different osmotic agents and water generated moderate abiotic stress (osmotic stress, saline, and drought stress) during soaking, and accumulation of osmotically active solutes like proline, which is reported in a dissimilar number of species during seed priming [51–53,46,42].

5 Conclusion

Any duration of hydropriming treatments is not recommended for anise. It seemed all treatment durations in hydropriming had inhibitory effects due to imbibition-based damages. This trend was also indicative in all osmoprimed seeds, excluding 8 h $MgCl_2$ priming treated seeds. Although 8 h $MgCl_2$ priming treatment and non-treated control treatment were statistically similar the former showed a numerical improvement over the other. This showed that biofortification of $MgCl_2$ improved anise seed germination and emergence.

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