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Original article

Zn application through seed priming improves productivity and grain nutritional quality of silage corn

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ABSTRACT

The micronutrient application in agriculture takes place through soil application, foliar spraying or added as seed treatments. The latter method, the nutri-priming, is an appealing option due to the easiness in handling it, environment-friendly, cost effectiveness and efficient against multiple environmental stressors. To assess the feasibility of Zn-priming technique on seeds germination, two experiments were conducted and assessed the efficiency on the growth rate, yield and biofortification on the forage maize (*Zea mays* L.). The first laboratory experiment assessed the effect of Zn-priming for three-time exposures (i.e., 8, 16 and 24 h) on germination parameters. The second experiment was done in a greenhouse, by using the 10 seeds obtained from 24 h priming. Five seed pretreatments were studied (0, 0.1, 0.5, 1 and 11 2 % of zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$)) compared to the recommended dose (5 ppm of Zn at 5–9 leaf stage) provided by soil application. The obtained results revealed that all seed priming, including hydro-priming, improve seed germination performance. Zn-priming increased the grain yield and helped to enrich the seeds in this element, especially seedlings treated with 0.5 % Zn sulphate for 24 h leading to an increase in yield by 47 % and in Zn content by 15 %. The comparison of the results from both techniques showed that Zn-priming could be was very effective than the traditional direct application in soil.

1. Introduction

The physical, mental and psycho-emotional development and homeostatis for children and adults alike is an essential condition for life (SNN, 2011). Each element has a specific role in the human body, and any nutritional imbalance leads to a health imbalance. Plants and animals are the sources of these elements, while vegetables and fruits remain the main source of microelements. Micronutrient deficiency can induce malnutrition caused by insufficient uptake rate of essential vitamins and minerals and is called the "invisible hunger" or "hidden hunger". The "hidden hunger" comprises the most worrying human health issue in most developing countries (Aguenaou, 2007). The deficiencies in these micronutrients assimilation by human body can induce various illnesses such as blindness, immunodeficiency, mental issues and in the end, death (Aguenaou, 2007). Around two billion people worldwide were suffering from this hidden hunger, fact that will be perceived too late (Ludwig and Slamet-Loedin, 2019), the main culprit is Zn deficiency (Hefferon, 2019).

The biofortification of staple crops with micronutrients has the main objective in increase the concentration of micronutrients in the edible parts of seeds. It aims remedying to nutritional deficiencies. This is a strategy adopted to overcome human malnutrition. Two main methods are used for biofortification: genetic selection techniques (Ludwig and Slamet-Loedin, 2019) and agronomic fortification (Nissar et al. 2019) such as the application of fertilizers called fertifortification by prasad (2009).

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Within crops, Zn is an important microelement in various biochemical processes (Veena and Puthur, 2021), such as growth and reproduction (Chasapis et al., 2011). Also, the Zn is effective in antiviral immunity, being currently in use as supplement for COVID-19 treatment (Rahman & Idid, 2020). However, nearly half of the world's population suffers from Zn deficiency (WHO, 2002), mainly in rural communities (Stein, 2010) and apparently the deficiency of this element in crops and humans is strongly interconnected (Younas et al., 2022).

The suitability of different mineral micronutrients for biofortification is examined by previous studies (Haider et al., 2020; Dhaliwal et al., 2021). In general, Zn is a suitable candidate for agronomic biofortification. This approach has been shown to be effective in cereals (Cakmak, 2008; Meena and Fathima, 2017; Hassan et al., 2018; Rehman et al., 2018) especially corn (Ladumor et al., 2019). However, the efficiency of Zn assimilation in crops is strongly dependent to its application method and the type form of fertilizer applied (Cakmak, 2008). The application of Zn is usually done by soil supply, foliar application or the treatment of seeds (Farooq et al., 2012, 2018). Previous studies (Cakmak, 2008; Chattha et al., 2017; Hidoto et al., 2017) came to the conclusion that direct foliar spraying was more efficient for increasing the crop yield, as well as for seeds biofortification and enrichment. The priming with Zn was also showed to be an efficient method in increasing its content in seeds (Harris et al., 2007; Imran et al., 2013; Imran et al., 2017).

The current study assessed the effects of priming (i.e. Hydropriming and Zn-priming) on the rate of germination and growth, as well as on the yield and grain Zn content in forage maize (*Zea mayz* L.). Thus, the current study was conducted as two different experiment. The first experiment assessed the effect of tree time exposure (i.e., 8, 16 and 24 h) of Zn-priming on the germination rate of maize seeds. After choosing the best soaking time, a second experiment was carried in a greenhouse, to assess the efficiency of this technique on biofortification and yield. Additionally, a comparison was taken place between nutri-priming and soil application of recommended dose.

2. Materials and methods

2.1. The priming with nutrients

Seeds of *Zea mays* L. (var. Rulexx, obtained by RAGT SEMENCES / AGRIN MAROC and registered in the national catalog in 2007) were used. Five treatments were defined (0, 0.1, 0.5, 1 and 2 % zinc sulfate heptahydrate ($ZnSO_4$, $7H_2O$)) with three priming durations (8, 16 and 24 h). After selecting healthy seeds having the same shape and size, we measured 95 ± 2 g, approximately 250 seeds, for each treatment. Seeds were treated following the standard procedure as detailed in Choukri et al. 2019. The weight evolution and volume of the solution absorbed before and after priming were explored to calculate the hydration capacity (HC) and hydration index (HI) as mentioned in Table 1.

To define the optimal time required for priming the maize seeds, treated and not treated, their germination was carried following the standard procedure of Choukri et al. 2019. When the germination test was over, we have also calculated the speed of germination (hereafter SG) and the final germination percentage (hereafter FGP) (Table 1).

2.2. Zn concentration of primed seeds and seeds harvested

In order to characterize seed enrichment (primed seeds by Zn-priming and seeds harvested by biofortification), Zn contents were determined in primed and unprimed seeds according to the prescribed protocol (Choukri et al., 2019) used for Molybdenum analysis in bean seeds. The reading of the results was carried out by an atomic absorption spectrophotometer. Then, seed Zn content was calculated using formula mentioned in Table 1.

2.3. Experimental design

A shelter experiment was carried out in pots at the National School of Agriculture of Meknes (Meknes-Fes Region, Morocco). The physico-chemical soil parameters (33°44′59″ N latitude, 5°34′29″W longitude, Morocco) were determined in laboratory. The soil used in the current experiment had the following physic-chemical characteristics: sandy texture (87 %), pH 8.3, electrical conductivity 0.05(dS m^{-1}), organic matter content 0.58 %, mineral nitrogen 10.5 mg kg^{-1}, Olsen P_2O_5 26,34 mg kg^{-1}, Extractable[a] K_2O 115,20 mg kg^{-1}, Extractable[b] Zn of 12,86 mg kg^{-1}, Extractable[b] Mo of 0,83 mg kg^1. (Extractants: a. Ammonium Acetate; b. Diethylene Triamine Penta-Acetic acid (DTPA)).

Depending on germination parameters (speed and final germination percentage), primed seeds for 24 h were chosen for cultivation. Four seeds were sown in each pot (30 cm × 27 cm) and one healthy plant was retained after thinning. The pots were irrigated with tap water up to 70 % of the field capacity, three times per week and twice a day in increased temperature conditions. The experiment was carried between March 26 to July 10, 2018. It was marked by very frequent daily temperature drops. A randomized block factorial design was used with seven treatments of nutrient priming, each in five replicates. Treatments studied are presented in Table 2. Fig. 1 illustrate the experimental setup.

During the growing season, the soil was fertilized with 260 kg ha^{-1} of N as ammonium nitrate, 110 of P_2O_5 as triple super phosphate, 180 kg ha^{-1} of K_2O as sulphate of potash. Symptoms of manganese deficiency appeared later (10–11 leaf stage just before male flowering). Thus, two foliar applications of 1 kg ha^{-1} of Mn as manganese sulphate monohydrate were sufficient to rectify the crop requirements. In addition, no Zn deficiency symptoms were observed in all treatments.

During the experiment, the height of plants, the stem diameter, number of leaves and their area, relative growth rate and chlorophyll content were measured (Table 1). The relative chlorophyll content index (RCCI), on a scale ranging from 0 to 999, was measured with a chlorophyll meter (CCM-200, Opti Sciences, USA). Two RCCI measurements per plant were carried out in three stages (before the appearance of female flowers, pollination stage and at the 90th day after emergence). The average of the two measurements was used to calculate the chlorophyll content (Chl) in g/m^{-2}(−|−) according to equation cited in Table 1.

At harvest, growth and other yield components, such as the length of roots fresh and dry mass, number of ears and rows per plant, number of kernels per row and the mass of 1000 randomly selected kernel masses were measured. The data collected from each experimental unit was analyzed by SPSS software (version 20). Data were first tested for normality and homogeneity of variance, followed by one-way ANOVA and subsequently the Student-Newman-Keuls (SNK) multiple comparison tests, for multiple comparisons among treatments.

3. Results

3.1. Hydration, germination and Zn content

Table 3 summarizes the data for the hydration indices (HC and HI), germination indices (SG and FGP) and Zn contents.

Soaking for 8 h allowed the seeds to absorb 0.09 to 0.11 g $seed^{-1}$ of Zn sulfate solution. After 8 h, this HC increased slightly and

Table 1
The formulas used to measure the parameters tracked.

Parameter	Formula	Formula details	Reference
Hydration capacity (g of solution seed^{-1})	$HC = \frac{FW-IW}{N}$	FW: final weight of primed seeds IW: initial weight of seeds N: number of seeds	Choukri et al. 2019
Hydration index (g of solution g^{-1} of seed)	$HI = \frac{HC}{\text{Weight of one seed}}$		Choukri et al. 2019
Speed of germination	$SG = \sum(\frac{Ni}{Di})$	Ni: number of seeds germinated on the ith day Di: number of days to count the nth	Maguire, 1962
Final germination percentage (%)	$FGP = \frac{Nt*100}{N}$	Nt: total number of germinated seeds in each treatment N: number of seeds used in the bioassay	Belcher and Miller, 1974
Seed Zn contents $\left(\mu g\, seed^{-1}\right)$	$\text{Zn contents} = \text{seed dry biomass (g)} * \text{Zn concentration } (\mu g\, g^{-1})$		Imran et al., 2017
Total leaf area	$TLA = \Sigma\,(\text{large leaf length} \times \text{leaf greatest width} \times 0.75)$		(Mokhtarpour et al., 2010).
Relative growth rate (cm cm-1 day-1)	$RGR = \frac{\text{Ln xt2-Ln xt1}}{\text{t2-t1}}$	x_{t1} is plant height measured at time t_1 x_{t2} is plant height measured at time t_2 t_2-t_1 is the number of days between x_{t1} and x_{t2}	James and Richards, 2006
chlorophyll content (Chl)	$Chl = 0.0214 \times (RCCI) + 0.0424(g/m^{-2}(-\vert-))$	The relative chlorophyll content index (RCCI)	(Bagard et al., 2008):

Table 2
Treatments steadied in pot experiment.

Treatment	Dose	Application method
Z0	0 % ($Zn\ SO_4{\cdot}7H_2O$)	Hydro-priming
Z1, Z2, Z3 and Z4	0.1, 0.5, 1 and 2 % ($Zn\ SO_4{\cdot}7H_2O$)	Nutri-priming
NC (Negative control)	0 % ($ZnSO_4{\cdot}7H_2O$)	Unprimed seeds
PC (Positive control)	5 ppm of Zn as $Zn\ SO_4{\cdot}7H_2O$	Soil application at the 5–6 leaf stage (Drissi et al., 2015)

remains almost stable after 16 h. As showed in Fig. 2, the imbibition phase coincided with the first 16 h of priming. A faster rate of water absorption for the first eight hours, with an imbibition speed of 1.35 mg h^{-1} of solution, was observed. This speed decreased during the second eight hours to 0.36 mg h^{-1} of solution, then it became null between 16 and 24 h. The priming process comprised the soaking the seeds in water or osmotic solution for an estimated period of time period to complete the imbibition and activation phase of the germination process. The first imbibition phase was characterized by the rapid absorption of water that coincided with the first 16 h of priming. Thus, the activation phase seems to begin after 16 h of priming.

The untreated seeds had a final germination percentage of 76.7 %, which increased significantly in all treatments (for all priming durations), with the exception of Z4 treatment. All seeds germinated in Z1 and Z2 treatments, respectively, for 8 and 16 h. Nevertheless, no significant difference was detected between Z0, Z1, Z2 and Z3 treatments. In addition, hydro-priming and Zn-priming accelerated the seeds' germination. The faster germination speed (>18) was recorded in primed seeds into 0, 0.1 and 0.5 % Zn sulphate for 24 h. Moreover, the concentration of 2 % did not affect the germination speed, which remained similar to control (7.9), whatever the time of exposure.

Compared to the initial content, the Zn-priming significantly increased the content of this oligonutrient in seeds. Furthermore, the results revealed its accumulation directly with the dosages of Zn sulfate and priming duration. In fact, the highest seed Zn value was observed in Z4 treatment after 24 h of priming (906.6 µg seed^{-1}), which is an enrichment 77-fold higher than unprimed seeds (11.7 µg seed^{-1}). Additionally, hydro-primed seeds (Z0) showed similar values to those recorded in untreated seeds.

The germination kinetics is represented in Fig. 3. Over the first day, the germination rate increased with increasing priming duration. For 0, 0.1 and 0.5 % Zn sulfate treatments, the germination rate of primed seeds for 8 (Fig. 3a) and 24 (Fig. 3c) hours exceeded respectively 50 and 80 %, while the unprimed seeds reached 10.8 % of germinated seeds. Whatever may be the priming duration, the percentage of germination exceeded 90 % from the 6th day with the exception of the Z4 treatment. On the contrary, primed seeds in 2 % Zn sulfate presented similar values than those observed from unprimed seeds, which not exceed 76.7 %.

3.2. Growth parameters

Time course of stem height growth (Fig. 4a) was not affected by Zn nutrition, where no significant difference was recorded between treatments during the whole crop cycle. Height growth stopped on the 80th day after emergence. At harvest, the height growth was noted between 209.1 (NC) and 215.3 cm (Z4) (Table 4). Except for the time period between 60 and 70 days after emergence, the ANOVA showed no significant effects of treatments on RGR. The RGR values declined directly with crop growth. The highest values of RGR were observed between 10 and 20 days after emergence with values ranging from 0.085 to 0.108 cm cm^{-1} d^{-1}. Between 60 and 70 days after emergence, a clear growth peak were detected with a highest average value in the PC (0.038 cm cm^{-1} d^{-1}), followed by Z1 and Z3 treatments without showing any significant difference. However, a statistically lower RGR were noted in NC with an average of 0.031 cm cm^{-1} d^{-1}.

Regarding the leaf number, from the 50th day after emergence, a significant difference between treatments has been noted. The high number of leaves, ranged from 14 to 15, was recorded in all plants that received zinc fertilization as well as plants that had undergone hydro-priming. In contrast, the lowest mean value (13 leaves) was recorded in the NC. For stem diameter, the two methods of Zn application did not affect this parameter. At harvest, it varies between 24.4 and 26.4 mm respectively for NC and Z2. For leaf area, no significant difference was detected between treatments. However, plants grown without Zn application recorded the smallest total leaf area (0.52 m^2). The maximum average of leaf

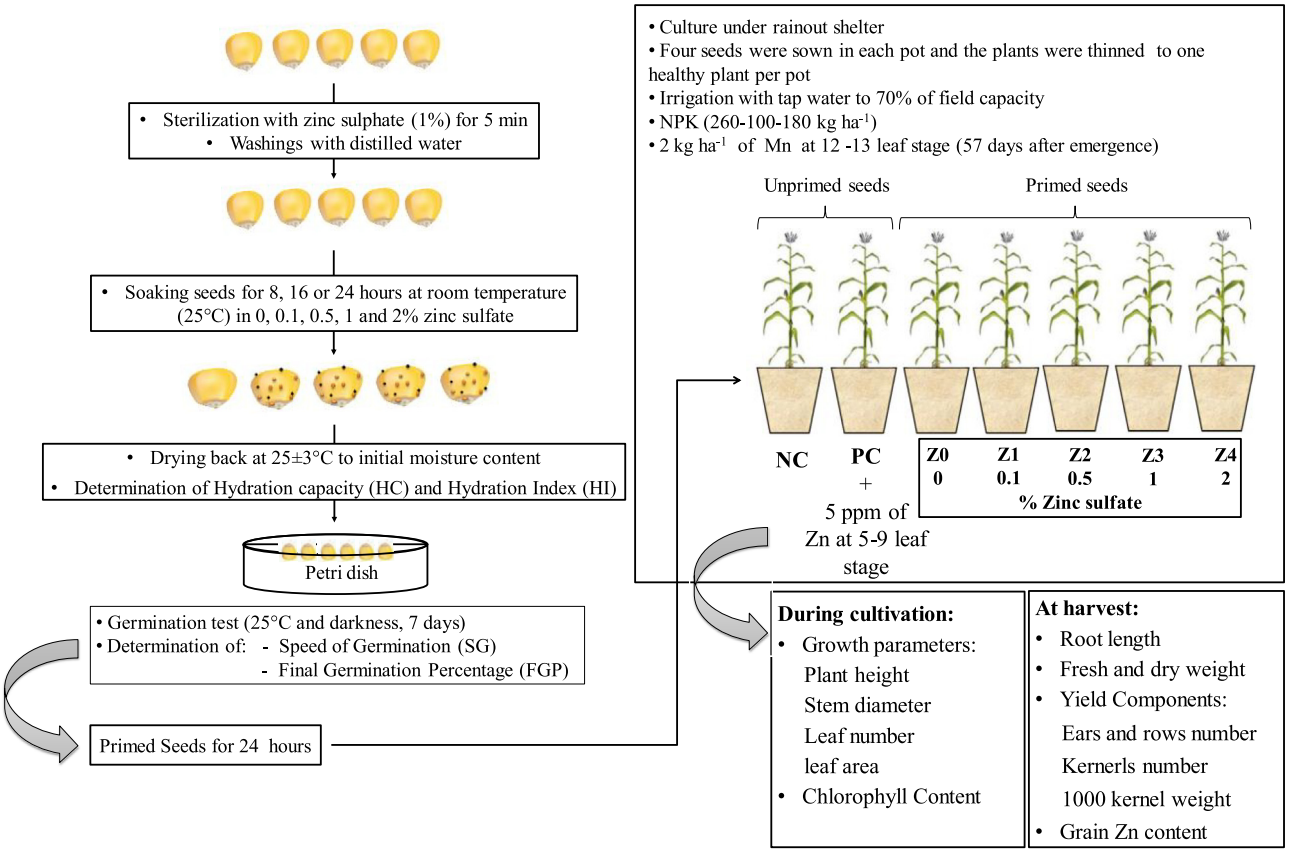

Fig. 1. Schematic illustrating the experimental set up.

Table 3
Hydration indices, germination indices and Zn content of maize seeds (*Zea mays*) control and pretreated (Z0: 0. Z1: 0.1. Z2: 0.5 Z3: 1 and Z4: 2 % Zn sulfate) with three priming durations (8. 16 and 28 h). The data represent Mean ± SD (n = 3). The different letters indicate significant differences according to the SNK test at $p < 0.05$.

Parameters	Duration (h)	Control	Z0	Z1	Z2	Z3	Z4
Hydration capacity	8		0.11	0.10	0.09	0.10	0.10
(g of solution seed^{-1})	16		0.14	0.13	0.14	0.13	0.12
	24		0.13	0.14	0.13	0.13	0.13
Hydration index	8		0.28	0.27	0.24	0.26	0.26
(g of solution g^{-1} of seed)	16		0.36	0.35	0.36	0.34	0.32
	24		0.35	0.36	0.35	0.35	0.34
Final germination	8	76.7 ± 6.1^{b}	98.3 ± 2.2^{a}	100.0 ± 0.0^{a}	99.2 ± 4.4^{a}	98.3 ± 2.2^{a}	73.3 ± 5.6^{b}
(%)	16		96.7 ± 2.2^{a}	98.3 ± 2.2^{a}	100.0 ± 0.0^{a}	95.0 ± 3.3^{a}	66.7 ± 2.2^{b}
	24		98.3 ± 2.2^{a}	96.7 ± 2.2^{a}	90.0 ± 0.0^{a}	91.7 ± 2.2^{a}	68.3 ± 5.6^{b}
Speed germination	8	7.9 ± 0.5^{f}	16.0 ± 1.3^{abcd}	17.0 ± 0.9^{abcd}	15.4 ± 0.8^{bcd}	12.7 ± 0.7^{e}	7.9 ± 1.2^{f}
	16		17.4 ± 0.2^{abc}	17.8 ± 0.5^{abcd}	17.3 ± 0.7^{abcd}	14.4 ± 1.1^{de}	7.5 ± 0.8^{f}
	24		18.8 ± 0.2^{a}	18.4 ± 0.6^{ab}	18.1 ± 0.1^{ab}	14.8 ± 0.6^{cde}	7.1 ± 1.2^{f}
Zn content	8	11.7 ± 0.5^{f}	14.6 ± 0.8^{f}	297.9 ± 25.3^{e}	530.2 ± 12.3^{d}	573.1 ± 27.6^{d}	768.6 ± 72.8^{b}
(μg seed^{-1})	16		20.2 ± 1.0^{f}	517.9 ± 18.1^{d}	627.9 ± 22.5 ^{cd}	630.2 ± 42.9^{bc}	680.8 ± 58.3^{b}
	24		14.8 ± 1.0^{f}	558.5 ± 56.5^{d}	667.8 ± 19.7 ^{cd}	773.9 ± 72.8^{b}	906.6 ± 77.4^{a}

area was reached under the Z2 treatment, with a 21 % increase over the NC. Likewise, no significant difference was observed between all treatments for total fresh biomass, total dry biomass as well as for root length.

3.3. Chlorophyll content

Fig. 5 shows the variation of the chlorophyll content expressed in g/m^{-2}(−|−) as a function of the different treatments studied in 3 different stages (before the appearance of female flowers, pollination stage and ripening). Statistical analysis showed no effect of zinc fertilization on the chlorophyll content whatever the stage.

3.4. Yield components and seed Zn enrichment (Biofortification)

Zn fertilization did not influence the number of ears per plant, of rows per ear and of kernels per row (Table 4). In contrast to the yield components mentioned above, it has been found that Zn-priming in 0.5 % Zn sulfate significantly increased the1000-grain weight. The average value (215.1 g) was 27 and 24 % greater than NC and PC, respectively. However, no significant effect of all treatments was remarked. Similarly, grain yield showed significant positive response to Zn-priming in 0.5 % Zn sulphate with an average increase of 47 % compared to the NC.

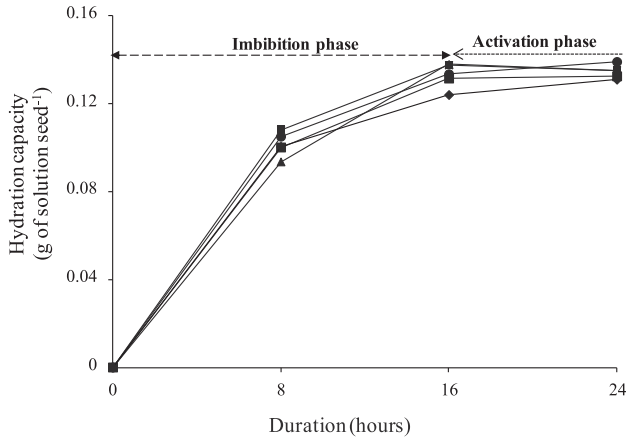

Fig. 2. Variation of hydration capacity of primed (Z0: 0 ×, Z1: 0.1 •, Z2: 0.5 ▲, Z3: 1 ■and Z4: 2 % ◆of zinc sulfate) maize seeds (*Zea mays*) with three priming durations (a: 8 h, b: 16 and c: 24 h).

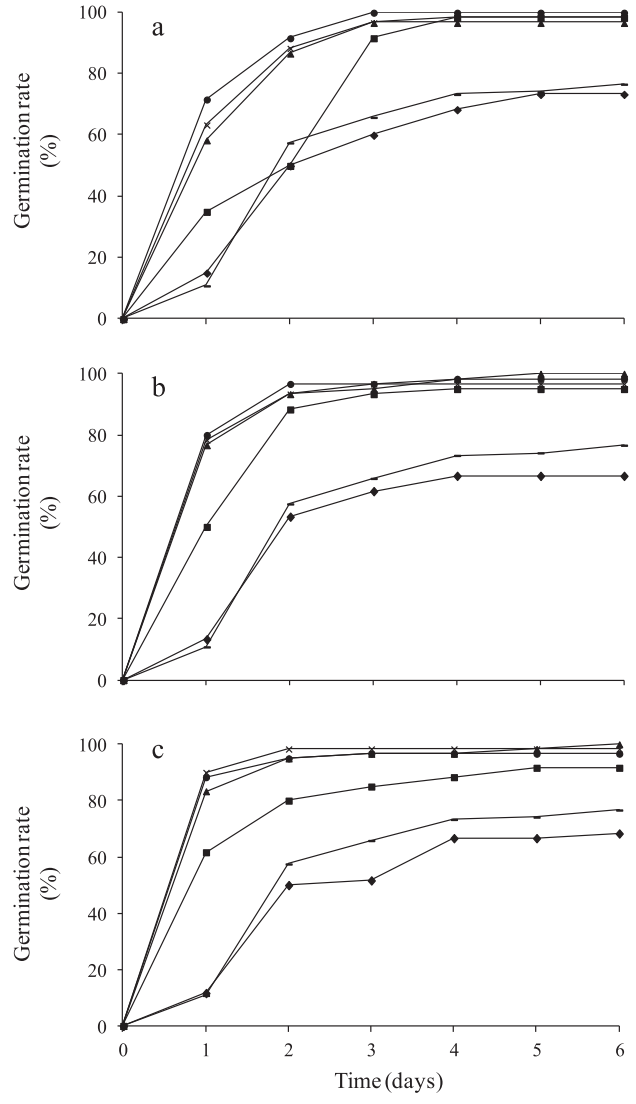

Fig. 3. Germination kinetics of unprimed (control: -) and primed (Z0: 0 ×, Z1: 0.1 •, Z2: 0.5 ▲, Z3: 1 ■and Z4: 2 % ◆of zinc sulfate) maize seeds (*Zea mays*) with three priming durations (a: 8 h, b: 16 and c: 24 h).

Generally, Zn fertilization influenced positively the grain Zn content (Fig. 6). Plants with Zn supply produced grains with high Zn content as compared to both NC and Z0 treatment. However, only Z1 and Z2 treatments significantly increased grain Zn content respectively by 11 and 15 % compared to the NC.

4. Discussion

4.1. Germination parameters

The priming process comprises a physiological method that in the end improves the seeds' performance, leading to faster and better synchronized germination because of the activation of many metabolic processes implicated in the early stages of germination (Nawaz et al. 2013; Ibrahim, 2019). In this sense, the germination test revealed that all treatments increased the two germination indices calculated (Final germination rate and germination speed), with the exception of the Z4 treatment (2 % $ZnSO_4$) whose rate remained similar to the control one. Nevertheless, the soaked seeds for 24 h in water or in 0.1 % $ZnSO_4$ solution had the highest values without a notable significant difference. Therefore, the increase in germination indices is due to the biochemical advantages of hydro-priming and not to the nutritional effect of Zn. The acceleration of the germination rate of treated seeds for 24 h could be justified by entry into the activation phase. During this phase, most of the biochemical processes of germination are accomplished. For example, (Gallardo et al., 2001) and (Job et al., 1997) found that hydro-priming increased the polypeptide fractions, which was interpreted as a degradation of stored proteins, in Arabidopsis and sugar beet seeds, respectively. The acceleration of germination was also caused by the mobilization of reserved accumulated in seeds during priming, hydrolysis of germination inhibitors and hydrolysis of ABA (Imran et al., 2015).

Our results are consistent previous studies, such as the one of Rehman et al. (2015), who reported that Zn-priming treatments (i.e., 0.01, 0.05, 0.1, 0.5 and 1 M $ZnSO_4$), including hydro-priming, improved the germination rate of seeds. However, the priming of seeds with 0.5 M $ZnSO_4$ for 12 h proved to be more effective than other treatments. Similarly, Imran et al. (2015) reported that hydro-priming of maize seeds for 24 and 36 h increase the speed of their germination. Accelerated and high germination, following priming in water or Zn solutions, have been reported in the same way in maize (Harris et al., 2007), Common bean (Choukri et al., 2019) and rice (Salleh et al., 2020).

The toxic effect of the highest concentrations on germination could be attributed to the inhibitory effect that the Zn has on cell division and associated cellular anomalies. In this sense, Reis et al. (2018) stated that doses of micronutrients (Zn and Fe) exceeding 4 mg l^{-1} of the priming solution (8 h) negatively affect germination. Thus, they pointed out that these concentrations significantly reduced the rate of cell division, but increased the number of abnormalities. Also, Rehman et al. (2015) reported that increasing concentrations of Zn in solution higher than 0.5 M ($ZnSO_4$, 12 h) inhibited the growth of seedling. Higher concentrations of Zn may restrict the growth of roots because of its toxicity and inhibitory effect on cell division (Prasad et al., 1999; Natasha et al., 2022). Zn toxicity slows the normal development of root and leaves due the substantial decrease in NADPH production by plant chloroplasts (Mousavi, 2011).

Seed analyzes before sowing revealed that Zn-priming increased their Zn content. Our results were similar with previous experiments carried on barley (Ajouri et al., 2004) and maize (Harris et al., 2007, Imran et al., 2013; Imran et al., 2017). For example, Johanson et al. (2005) reported that priming of chickpea seeds in solutions of 4 mM Zn resulted in an increase in its content

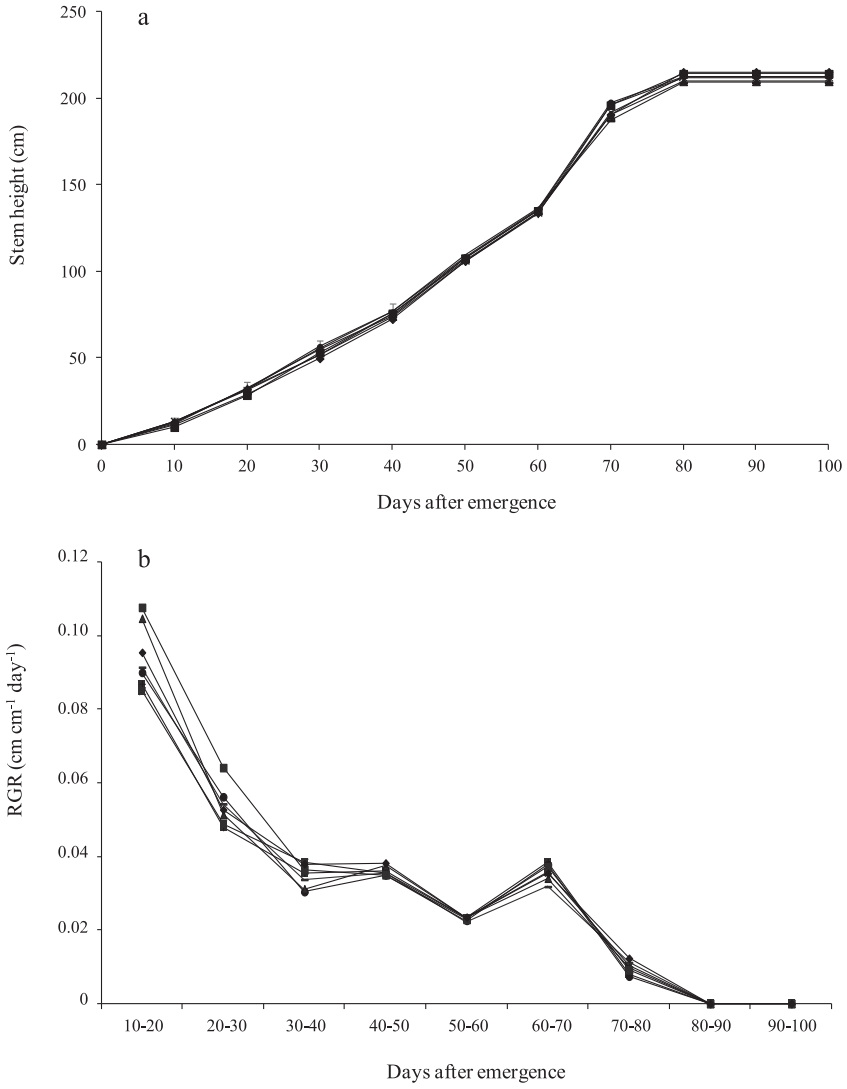

Fig. 4. Changes in the stem height (a) and relative growth rate (RGR) (b) of maize plants (Zea mays) from unprimed (NC -: without Zn fertilization, PC +: with recommended dose of Zn provided by soil application) and primed seeds (Z0: 0 ×, Z1: 0.1 •, Z2: 0.5▲, Z3: 1 ■and Z4: 2% ◆of zinc sulfate). Each symbol represents the mean of 5 replicates.

Table 4
Growth and yield parameters of maize plants (*Zea mays*) from untreated seeds (NC: without Zn fertilization. PC: Soil application of the recommended Zn dose) and pretreated (Z0: 0. Z1: 0.1. Z2: 0.5. Z3: 1 and Z4: 2 % Zn sulfate). The data represent Mean ± SD (n = 5). The different letters indicate significant differences according to the SNK test at $p < 0.05$.

Parameters (Unit)	NC	PC	Z0	Z1	Z2	Z3	Z4
Stem height (cm)	209.1 ± 5.7^{a}	211.8 ± 8.3^{a}	212.3 ± 14.8^{a}	212.8 ± 5.3^{a}	209.8 ± 7.8^{a}	214.2 ± 9.4^{a}	215.3 ± 6.2^{a}
Leaf number	13.0 ± 0.0^{b}	14.0 ± 0.4^{a}	14.8 ± 0.3^{a}	14.6 ± 0.6^{a}	15 ± 0.0^{a}	15.0 ± 0.4^{a}	14.2 ± 0.6^{a}
Stem diameter (mm)	24.4 ± 1.0^{a}	25.2 ± 0.5^{a}	26.00 ± 0.7^{a}	25.4 ± 0.1.2^{a}	26.4 ± 0.5^{a}	25.0 ± 0.4^{a}	24.8 ± 0.7^{a}
Total leaf area (m^2)	0.52 ± 0.04^{a}	0.55 ± 0.05^{a}	0.62 ± 0.03^{a}	0.60 ± 0.08^{a}	0.63 ± 0.06^{a}	0.60 ± 0.02^{a}	0.57 ± 0.04^{a}
Root length (cm)	85.7 ± 8.2^{a}	84.3 ± 3.7^{a}	86.7 ± 1.2^{a}	82.2 ± 2.2^{a}	87.8 ± 2.5^{a}	92.2 ± 1.6^{a}	81.8 ± 4.1^{a}
Total fresh biomass (g)	469.4 ± 34.1^{a}	445.2 ± 55.9^{a}	512.6 ± 44.7^{a}	455.6 ± 50.8^{a}	494.8 ± 67.4^{a}	454.8 ± 26.2^{a}	488.2 ± 44.9^{a}
Total dry biomass (g)	116.8 ± 6.7^{a}	108.3 ± 4.8^{a}	137.2 ± 8.4^{a}	112.5 ± 8.3^{a}	123.9 ± 5.9^{a}	120.2 ± 8.2^{a}	124.0 ± 11.8^{a}
Number of rows (ear^{-1})	14.0 ± 0.8^{a}	15.6 ± 0.7^{a}	14.5 ± 0.7^{a}	16.0 ± 0.8^{a}	15.0 ± 1.0^{a}	14.8 ± 0.9^{a}	15.5 ± 1.5^{a}
Number of kernels (row^{-1})	17.1 ± 1.2^{a}	18.2 ± 2.4^{a}	16.6 ± 0.9^{a}	16.9 ± 4.0^{a}	18.6 ± 1.8^{a}	19.6 ± 1.9^{a}	16.7 ± 0.8^{a}
1000-grain weight (g)	168.8 ± 4.5^{b}	174.2 ± 6.3^{b}	182.4 ± 4.6^{b}	187.3 ± 12.4^{b}	215.1 ± 27.1^{a}	183.1 ± 8.6^{b}	180.2 ± 5.4^{b}
Grain yield (g $plant^{-1}$)	40.3 ± 3.8^{b}	49.3 ± 4.8^{ab}	44.1 ± 5.3^{ab}	49.9 ± 9.0^{ab}	59.4 ± 6.2^{a}	52.9 ± 3.4^{ab}	46.3 ± 3.4^{ab}

from 40 to 60 (control) to 500–800 ppm (Zn-priming). Similarly, Imran et al. (2017) demonstrated that seeds priming in a solution of 4 mM $ZnSO_4{\cdot}7H_2O$ for 24 h resulted in a 7-fold increase in maize seed reserves in Zn compared to untreated seeds (43.2 ppm).

The weak influence the priming solution concentration on the hydration capacity of the seeds is related to the low osmotic potential of these solutions. The hydration capacity of seeds soaked in water is similar to that of seeds soaked in Zn sulfate solutions. According to the kinetics of hydration, it seems that the imbibition phase agrees with the first 16 h, allowing the seeds treated for 24 h to begin the activation phase without crossing it. However, the largest amount of accumulated Zn was absorbed during the first 16 h.

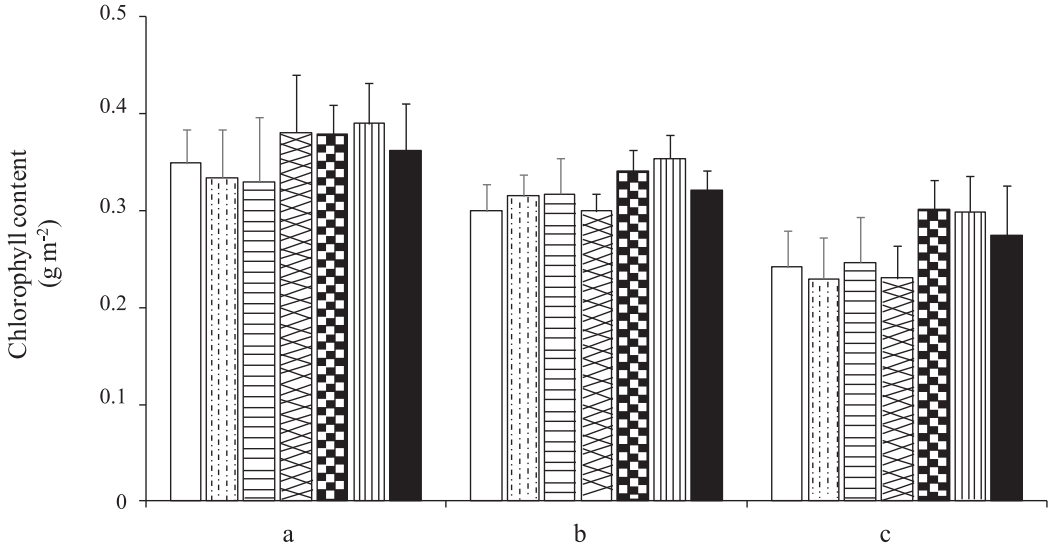

Fig. 5. Leaf chlorophyll contents in three stages (a: before the appearance of female flowers, b: pollination stage and c: the 90th day after emergence) reported as $g/m^{-2}(-|-)$ of maize plants derived from unprimed (NC: without Zn fertilization, PC: soil application of the recommended Zn dose) and primed seeds (Z0: 0, Z1: 0.1, Z2: 0.5, Z3: 0.1 and Z4: 0.2 %zinc sulfate). Data are presented as the means of 5 replicates; the vertical bars indicate standard deviations. Different letters indicate significant differences according to the SNK test at $p < 0.05$.

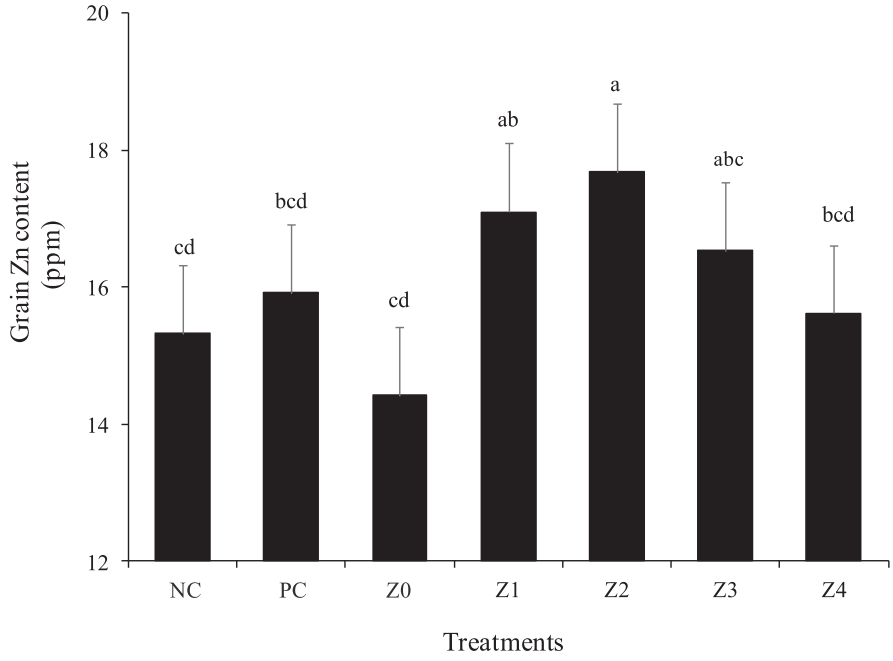

Fig. 6. Zn content (mg kg^{-1} of dry biomass) of produced grain by plants derived from unprimed (NC: without Zn fertilization, PC: soil application of the recommended Zn dose) and primed seeds (Z0: 0, Z1: 0.1, Z2: 0.5, Z3: 0.1 and Z4: 0.2 % zinc sulfate). Data are presented as the means of 5 replicates; the vertical bars indicate standard deviations. Different letters indicate significant differences according to the SNK test at $p < 0.05$.

It represents 90 % of the Zn content of primed seeds for 24 h. In addition to hydration and Zn accumulation, the germination speed is higher after 24 h soak, which justify the choice for the second part of the study.

4.2. Growth parameters

The absence of Zn fertilization effect on growth parameters could be explained by the Zn richness of the used soil (12.8 ppm), which is superior than the optimal content (0.8 mg kg^{-1} EDTPA) for maize growth (Lindsay and Norvell, 1978). In the Moroccan context, Drissi et al. (2015) reported that 5 mg kg^{-1} of Zn (EDTPA) is optimal for successful maize production on sandy soils. Therefore, all plants had enough Zn for normal growth. So, this initial soil richness did not allow to visualize the positive effects of Zn-application (Soil application and Zn-priming), which is a very promising alternative to satisfy crop requirement in micronutrients especially for crops growing on soil deficient in this element (Marschner and Marschner, 2012).

The positive effect of fertilization with zinc on RGR was recorded between 60 and 70 days after emergence. Similarly, a slight increase in RGR under Zn-priming has been reported by Afzal et al. (2013) in maize. This positive effect could be related to the role that zinc plays in the biosynthesis of tryptophan (Hera et al., 2018), which is a precursor for auxin, promoting cell elongation (Memari Tabrizi et al., 2011).

4.3. Grain yield and seed quality (Zn content)

This study showed that primed seed in 0.5 % Zn enhanced grain yield by 47 and 21 % compared respectively to plants without (NC) and with Zn fertilization by soil application of recommended dose (PC). Improved crop yield and 1000-grain mass was attributes to i) the role of this element in the carbohydrate metabolism (Marschner, 1995) and ii) Zn is part of the carbonic anhydrase enzyme catalysing the conversion of CO_2 to HCO_3^-. It is also a constituent of Ribulose 1,5-bisphosphate carboxylase (RuBPC) that catalyzes the first stage of carbon dioxide fixation (Burnell, 1990). The increase in 1000-grain weight with the Zn application to seeds may be due to the increased bioavailability of this element, and its direct translocation to young seedlings. This adequate intake of Zn probably increased nitrogen uptake during the development of grains and increase of crop yield (Siddiqui et al., 2009). In fact, Grzebisz et al. (2008) showed that early application of zinc positively affected the nitrogen absorption during reproductive phase. In contrast, the soil uptake was less effective, potentially due to the low mobility of this element and its rapid adsorption by soil particles (Alloway, 2008). In addition, Holloway et al. (2010) have explained the low efficiency of soil Zn application by both root and Zn distributions along soil profile. Drissi et al. (2016) reported that Zn retained on the top ten centimeters in sandy soil, even after a high leaching with large amounts of drained water (497 mm).

This positive effect on grain yield supported the results of Afzal et al. (2013) who demonstrated an increase of 34,3% in maize crop index after seed priming in 1.5 % $ZnSO_4$. Similarly, Zn-priming significantly increased the mass of maize grains exposed to heat stress during early growth (Imran et al., 2013). Harris et al. (2007) recorded increases in yield between 24 and 44 % after seed priming in 1 % $ZnSO_4$ for 16 h. In the present study, 31 and 47 % in yield improvement were obtained with primed seeds in 1 and 0.5 % $ZnSO_4$ for 24 h respectively. Many articles have reported the beneficial effects on yield in various crops, in chickpea (Harris et al., 2008), common beans (Kaya et al., 2007, Choukri et al., 2019), and wheat (Reis et al., 2018). In contrast, Chattha et al. (2017) observed only a slight increase in yield (9 %) in wheat plants from seeds soaked in 0.3 % $ZnSO_4$ compared to the soil fertilization of 50 kg ha^{-1} $ZnSO_4$ (28 %).

To reduce Zn malnutrition in human being, agronomic biofortification is expected to improve the concentration of this element in grains from 35 to 45 mg kg^{-1} (Cakmak, 2008). For this research study, seed Zn levels were low in all treatments, which may be explained by the low translocation to maize grain caused by the low temperatures during the crop growing cycle. Accordingly, Moraghan and Mascagni (1991) showed that, at low temperatures, Zn absorption and translocation from the root to the aerial part are generally decreased, leading to a lower concentration in shoots compared to roots. Also, it could be due to the phosphorus antagonistic effect on the Zn absorption, which worsens under low temperatures (Martin et al., 1965). Nevertheless, Zn-priming significantly increased seed Zn content compared to both controls (NC and PC). The highest level was found in maize grain from primed seeds in 0.5 % $ZnSO_4$. Zn concentration improvement in grain following Zn-priming could be due to the highest Zn availability, maintained by a larger pool of Zn in seed tissues during early growth stages. This has been demonstrated in maize (Imran et al., 2015) and rice (Prom-u-thai et al., 2012). Also, Ullah et al. (2019) reported that Zn-priming in chickpea increase seedling Zn concentration. Contrary, Zn uptake from the soil is generally reduced during the reproductive stages, which significantly reduces Zn accumulation in seeds (Chattha et al., 2017). Additionally, the fertilization of soil proved to be less effective for different factors like poor mobility (Alloway, 2008). Similarly, to our results, other studies on mungbean (Haider et al., 2020), wheat and chickpea (Harris et al. 2008) showed that Zn-priming increases the seed Zn contents.

5. Conclusions

The present study shows that the increase in germination indices is due to the biochemical advantages of hydro-priming and not to the nutritional effect of Zn. Thus, hydropriming alone can offer a realistic perspective to mitigate seed germination problems. Additionally, this research demonstrated that Zn-priming is an efficient option for agronomic biofortification of maize grain compared to soil application of recommended dose. Zn-priming in 0.5 % zinc sulfate for 24 h increased Zn content of maize grain by 15 %. Thus, Zn priming has an even greater advantage for improving animal feed quality, and consequently improve human nutrition. This improvement in seed quality was associated with yield increase of 47 %. However, further research utilizing other micronutrients, seed types, micronutrient concentrations are necessary to confirm the technical feasibility of this technique. Also, we recommend evaluating the financial feasibility of nutripriming and comparing it to other fertilization methods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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