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Zinc doses, sources and application times: seed physiological potential and flooded rice yield

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Abstract

Zn is the most deficient micronutrient in soils worldwide and the most important and limiting micronutrient essential for rice growth and yield. It can be applied in the soil for rice cultivation or by coating seeds or leaves of plants. However, only a small amount of Zn can be applied by coating seeds or plants. We evaluated the effect of dose, source and application time of zinc (Zn) on germination, cold test (germination), seed physiological potential and flooded rice yield. The rice cultivar was Puitá INTA Cl, and the laboratory experimental design was completely randomized with a factorial 6 × 2 scheme. Six Zn doses were assessed: 0, 50, 100, 200, 400 and 800 g ha^{-1}, using two Zn sources such as $ZnSO_4.7H_2O$ and $ZnCl_2$. After treating the seeds, germination, seedling length, seedling dry matter, cold test, accelerated ageing and seedling emergence were evaluated. Seven Zn doses were used in the field: 0, 50, 100, 200, 400, 800 and 1600 g ha^{-1} on seeds and at the V_4 and V_{11} vegetative stages. The results showed that the Zn rice seed treatment did not change germination or seedling emergence. The seedling dry mass and germination at low temperatures (cold test) are the physiological potential parameters that benefited when using $ZnSO_4.7H_2O$. Foliar application of $ZnCl_2$ at the V_{11} vegetative stage caused leaf damage when used at a dose of 400 g ha^{-1}, and caused more intense damage at 1600 g ha^{-1}. Treating rice seeds by coating with $ZnCl_2$ provided 6.4% higher grain yield compared to that of $ZnSO_4.7H_2O$. Applying Zn by coating the seeds promoted 5.8% higher rice grain yield and increased grain yield 7.0% by spraying at the V_{11} stage compared with that at the V_4 vegetative stage. The principal components analysis by seed treatment condition showed that the cold test and accelerated ageing had the highest contributions to the first and third components and acted in opposition to grain yield. Yield had the highest contribution to the second component and was the third component that most influenced the first and third components.

Keywords: Zn, emergence, dry mass, productivity, *Oryza sativa* L.
Abbreviations: $ZnSO_4.7H_2O$_ Zinc sulphate heptahydrate, $ZnCl_2$_ Zinc chloride, PCA_ Principal component analysis.

Introduction

Zinc (Zn) is an essential plant micronutrient and is involved in growth and metabolism, including enzyme activation, metabolism of carbohydrates, lipids, nucleic acids, gene expression and regulation, protein synthesis and reproductive development of plants (Marschner, 1995). Zn is the most deficient micronutrient in soils worldwide (Cakmak, 2002; Shivay et al., 2008), as more than 30% of soils have low Zn availability (Alloway, 2008), Zn deficiencies occur in countries on various continents, reflecting decreased yield and reduced rice grain nutritional quality (Alloway, 2008). Fertilizers containing Zn are used to correct Zn deficiency, but these are expensive (Mustafa et al., 2011). Flood-irrigated rice is highly sensitive to Zn deficiency, but Zn is the most important and limiting micronutrient for rice growth and yield (Rehman et al., 2012). Applying Zn to the soil and treating seeds or plants increase plant yields (Naik and Das, 2007; Shivay et al., 2008; Rehman et al., 2012). Applying Zn to seeds or leaves can be performed in small quantities, reducing the costs compared to soil applications, which require substantial Zn doses to fulfill crop needs. Mustafa et al. (2011) found that foliar application of 0.5% Zn 75 days after transplanting promotes greater flooded rice yield; similar values were obtained by applying 25 kg ha^{-1} $ZnSO_4$ to the soil. Among existing Zn sources, the sulphate form of Zn ($ZnSO_4.7H_2O$) has been the most widely used and recommended Zn source for applications to soil, seeds or spraying on plants (Shivay et al., 2008; Boonchuay et al., 2013; Kabeya and Shankar, 2013), as it has high solubility in water and is lower in cost compared to other sources (Alloway, 2008; Shivay et al., 2008). $ZnSO_4.7H_2O$ and Zn chloride ($ZnCl_2$) are more soluble Zn sources, which is why they were tested in this study. Zn uptake by leaves is related to the accompanying ions and foliage morphology, as reported for beans and coffee. Zn is partly retained by cuticles on the cell wall when $ZnSO_4.7H_2O$ is used as the source (Franco et al., 2005). Furthermore, a greater number of anions are present in $ZnSO_4.7H_2O$ compared to that in $ZnCl_2$, which makes it more toxic; thus, reducing plant growth when used at high levels (Aery and Sarkar, 2012). High yields of flood-irrigated rice have been obtained (>10 t ha^{-1} grains) in Brazilian soils, resulting in greater amounts of Zn needed for crops. This result has stimulated the development of several products with macro and micronutrients for soil and seed applications and a spray for plants. All of these products are aimed at improving plant development and yield. Among micronutrients, Zn provides many benefits to rice plants (Mustafa et al., 2011). Zn availability is reduced in flooded soils due to precipitation of

$Zn(OH)_2$ as a result of increased pH. $ZnCO_3$ precipitates because of CO_2 accumulation due to organic matter decomposition and precipitation of ZnS (Camargo et al., 1999). Thus, further studies are essential to assess doses, sources and times for Zn application to flooded rice. $ZnSO_4.7H_2O$ is used widely worldwide as a source of Zn for rice. However, it is highly soluble and contains toxic anions (Aery and Sarkar, 2012) compared to those of $ZnCl_2$. For this reason, $ZnCl_2$ may result in more efficient uptake by foliar application. Our study hypothesis was that the dose and $ZnCl_2$ source for Zn applications to rice seeds or at the V_4 and V_{11} stages promotes different effects on rice, germination, growth and yield compared to those of $ZnSO_4.7H_2O$. We evaluated the effect of dose, source and time of Zn application on germination, seed physiological potential and flooded rice yield.

Results and Discussion

Germination and physiological potential

Rice seed germination was not affected by applying Zn from different sources (Table 1). This result agrees closely with those reported by Rehman et al. (2012), who stated that coating rice seeds with Zn had no effect on germination. Rufino et al. (2013) and Tavares et al. (2013) also did not observe any differences in rice seed germination following treatment with different doses of formulated products containing Zn. However, most of the evaluated parameters related to seed physiological potential changed due to Zn seed treatment. Seedling length was influenced by Zn dose and source; however, no interaction was detected between these factors. Applying Zn reduced seedling growth (Fig. 1), which agreed with the study of Tavares et al. (2013), who found that seeds treated with 0.12–0.25 g of Zn kg^{-1} seed produce the smallest seedlings; these dosages were less than the lowest Zn dose used in our study (0.5 g kg^{-1} seed). According to Li et al. (2012), this result may have occurred because excess Zn interferes with cellular metabolism, resulting in inefficiencies in cellular differentiation and expansion. Ozturk et al. (2006) reported high Zn mobilization in wheat seeds, which accumulated in the embryo and aleurone layer and in the primary roots and coleoptile during the first 36 h of germination. In contrast, considering that equal amounts of Zn were applied from both sources, divergent responses may have occurred because different anion amounts are present in the two sources; $ZnSO_4.7H_2O$ contains more anions than $ZnCl_2$ (SO_4^{2-} *vs.* Cl^-), which is toxic and reduces seedling growth at high doses (Aery and Sarkar, 2012). This would explain the greater reduction in plant length following the application of $ZnSO_4.7H_2O$. Moreover, Li et al. (2012) treated wheat seeds with Zn and found high Zn concentrations close to the roots, which increased hydrogen peroxide levels and decreased cell viability and was directly related to reduced root growth.

El-Ghamery et al. (2003) observed that wheat seeds imbibed in solutions with concentrations greater than 25 mg L^{-1} $ZnSO_4.7H_2O$ suffered toxic cell effects in the first 24 h of germination, with inhibited cell division in the radicle region. Seedling dry mass using $ZnSO_4.7H_2O$ was higher than 100% compared to that in seeds without treatment, which agreed with the results of Rozane et al. (2008), who found that applying Zn (8 g kg^{-1} seeds) in the form of $ZnSO_4.7H_2O$ provided greater rice seedling dry mass production. However, Tavares et al. (2013) reported that treating rice seeds with 0–0.25 g kg^{-1} Zn did not increase seedling dry mass; this was also reported by Bonnecarreré et al. (2003) working with the rice cultivars BR-IRGA 410, EMBRAPA 7-TAIM and IRGA 417 who applied 0–1.33 g kg^{-1} Zn to seeds using $ZnSO_4.7H_2O$. These results suggest that higher doses of $ZnSO_4.7H_2O$ provide for greater seedling dry masses. However, $ZnCl_2$ only slightly decreased seedling dry matter in a linear fashion. Yemeni and Al-Helal (2003) found that rice seeds exposed to 0.5 and 100 mM $ZnCl_2$ during the first 96 h had inhibited germination, with a significant reduction in initial seedling growth. Stanković et al. (2011) reported that wheat seeds exposed to $ZnCl_2$ solutions had reduced seedling root and shoot length that were directly proportional to increased Zn concentration, with effects shown from $ZnCl_2$ doses of 0.01 M. Therefore, it was concluded that $ZnCl_2$ had a negative effect on seedling growth, which was also observed by Aery and Sarkar (2012) for soybean seeds with $ZnCl_2$ doses of 10–1000 µg mL^{-1}. The concentrations used by the latter group were similar to those used in our study. However, is important to note that unlike the above studies, the rice seeds used in our study had 13% moisture at the time of Zn treatment and were subjected to germination later and not exposed to Zn solutions during soaking, which enhances Zn uptake by seeds. It is possible that $ZnCl_2$ had a negative effect on seedling growth because it can alter cellular respiration, which is very sensitive to the presence of excess metals (Stanković et al., 2011). When Zn accumulates in the mitochondrial cytoplasm, the Krebs cycle and electron transport chain of oxidative phosphorylation are inhibited (Munzuroglu and Geckil, 2002), thereby reducing ATP formation. Accelerated ageing tests showed the negative effects of treating seeds with Zn from both sources (Fig. 1). The reduction of germination in seed exposed to accelerated ageing indicated a negative effect of seed treatment on conservation potential during storage, as germination values were less than 20% at a dose of 800 g ha^{-1} (Fig. 1), causing a lack of seed viability. It is possible that the doses used caused Zn to accumulate in cells because larger quantities of this element often induce oxidative damage by initiating lipid peroxidation and degradation of other compounds (Malavolta, 2006). Albuquerque et al. (2010) explained that excess Zn can affect normal plant growth and metabolism; thus, causing phytotoxic effects.

Solanki et al. (2011) observed a 17% reduction in *Vigna mungo* germination when seeds were exposed to solutions containing 1.5 mM $ZnSO_4.7H_2O$ during the entire germination period; this dose was lower than those used in our study. For example, at the lowest Zn dose of 50 g ha^{-1}, the seed treatment solution had a concentration of 130 mM, which may account for the reduction in the accelerated ageing tests, as the high temperature may have enhanced treatment effects. Differences between Zn dose and source were observed in the cold test results. $ZnSO_4.7H_2O$ improved seed performance, with a greater effect seen at a dose of 283 g ha^{-1}. However, $ZnCl_2$ caused negative effects and a slight linear decrease. These results are important, because temperatures at crop sowing time are low in many regions where rice is cultivated, as in southern Brazil and often reduce plant germination and establishment. The optimum temperature for rice seed germination is 25°C, and lower temperatures can disrupt the process (Sharifi, 2010; Mertz et al., 2009). This is because low temperatures alter the imbibition pattern (Bewley et al., 2013) and disrupt homeostasis in rice by increasing the accumulation of reactive oxygen species, reducing antioxidant activity (Bhattacharjee, 2013), causing mitochondrial damage (Yin et al., 2009) and disrupting primary root growth (He and Yang,

Table 1. Mean values of seed germination and seedling emergence of rice seed treated with Zn doses and sources.

Zn doses	Germination (%)		Seedling emergence (%)	
(g ha^{-1})	$ZnSO_4.7H_2O$	$ZnCl_2$	$ZnSO_4.7H_2O$	$ZnCl_2$
0	82^{Aa}	82^{Aa}	88^{Aa}	88^{Aa}
50	89^{Aa}	82^{Aa}	92^{Aa}	96^{Aa}
100	81^{Aa}	86^{Aa}	92^{Aa}	96^{Aa}
200	82^{Aa}	87^{Aa}	100^{Aa}	92^{Aa}
400	84^{Aa}	86^{Aa}	92^{Aa}	84^{Aa}
800	84^{Aa}	85^{Aa}	84^{Aa}	96^{Aa}
CV (%)	5.0		7.6	

Means followed by same capital letter in a line and lower case in a column were not difference according to Tukey's test at an error probability of 5%. CV – Coefficient of variation.

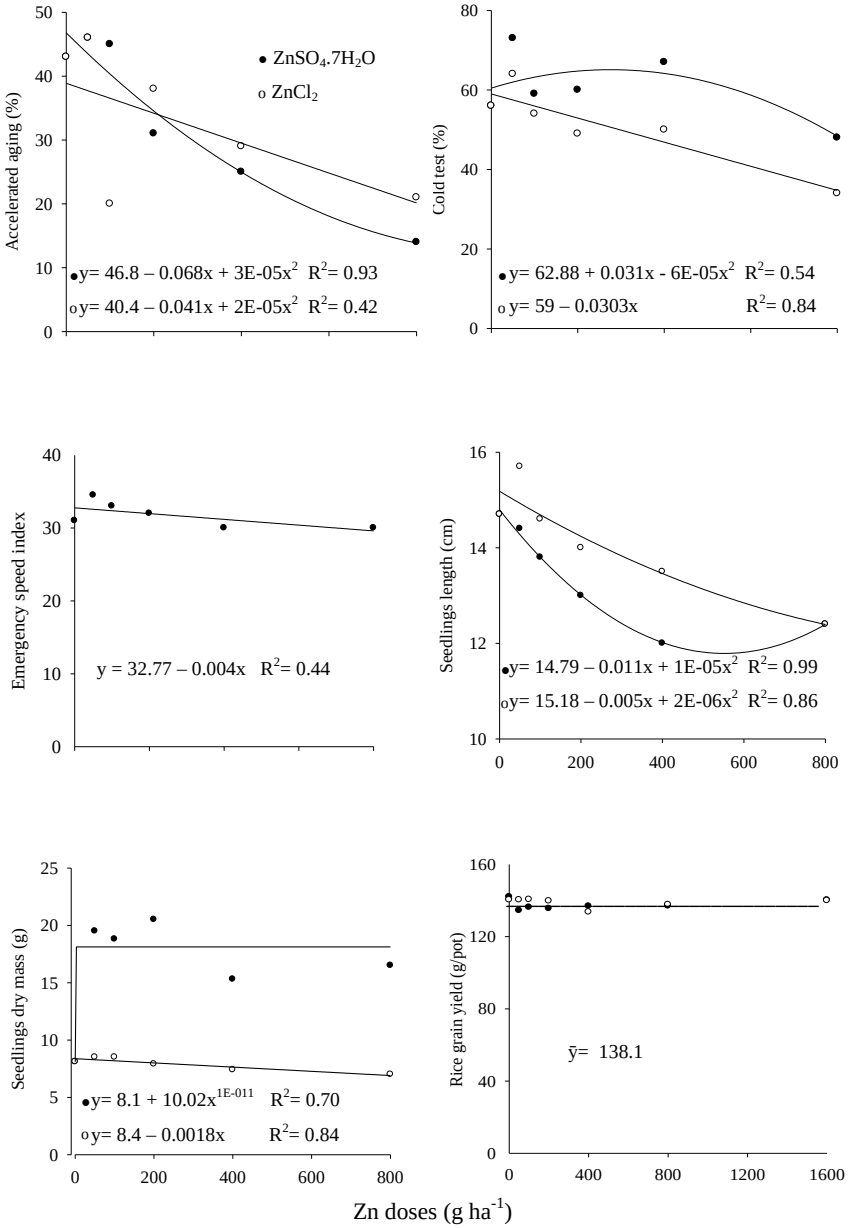

Fig 1. Accelerated aging, cold test, emergency speed index, seedling length, seedling dry mass (laboratory) and flooded rice grain yield (field).

2013). Therefore, a treatment that allows better seed performance under stress conditions, such as cold temperatures, is desirable. Studies have extensively documented the importance of Zn for maintaining cell membrane integrity, particularly that of root cells (Cakmak, 2000). Zn may act on seed coat tissue cell membranes by regulating exchange with the external environment and promoting germination. Thus, Zn may provide more efficient membrane reorganization at low temperatures, allowing seeds to undergo water absorption and more rapid reserve mobilization, thereby permitting more rapid germination under these conditions.

Seedling emergence

No difference in seedling emergence was observed after applying the different Zn doses and sources (Table 1). Nevertheless, the emergence speed index showed small linear decreases with increasing Zn dose (Fig. 1). The highest seedling emergence speed is important, as it allows for rapid establishment of field plants and reduces interference by weeds. The increased competitive ability of plants is associated with early emergence (Bennett and Shaw, 2000), as plants with a high emergence speed and early growth take priority over environmental resource use and generally have an advantage (Firbank and Watkinson, 1985). Furthermore, rapid seedling emergence provides an advantage, as plants are exposed to fewer stressors that may lead to reduced growth and plant establishment.

Growth and yield of flooded rice

Rice shoot dry mass, panicle number and 100 grain mass were not different according to Zn dose, source, application time or their interactions (Table 2). Rice grain yield did not differ as a function of Zn dose or dose interactions with time of application and source (Table 2, Fig. 1). However, treating seeds with Zn at vegetative stage V_{11} resulted in a 7.0% higher rice yield compared to that at vegetative stage V_4 (Table 3). This result may have occurred due to the small leaf area of rice plants at the V_4 stage (four leaves), which leads to absorption of only a small amount of Zn applied by spray in comparison to that for the other two application methods. Nevertheless, the Zn sources differed only by seed treatment application, with $ZnCl_2$ showing 6.4% higher rice grain yield compared to that of $ZnSO_4.7H_2O$. The small responses of rice to Zn application and the lack of a response to Zn dose, even after using a high nitrogen level and phosphorus and potassium fertilizer to obtain high yields, may have occurred because the soil used provided reasonable Zn levels to rice (1.2 mg dm^{-3}, as measured by chemical analysis), according to Tedesco et al. (2004) for drained soil, and the average organic matter content in this soil (2.3%). This certainly supplied the Zn needs for rice culture, even in a medium textured soil with 19% clay originating from a natural area under native vegetation. According to Tedesco et al. (2004), Zn deficiency in Brazil is more characteristic of sandy soils with lower organic matter content, which offer only small amounts of Zn to plants. In contrast, Zn concentration decreases in flooded soils (Camargo et al., 1999; Mustafa et al., 2011), which reduces Zn availability due to the precipitation of $Zn(OH)_2$ as a result of increased pH, and precipitation of $ZnCO_3$ because of CO_2 accumulation due to organic matter decomposition and ZnS precipitation (Camargo et al., 1999). Thus, higher Zn concentrations in flooded soils are needed to supply rice demand, which is why rice is often Zn deficient and has decreased yield (Rehman et al., 2012). Yan (2003) reported that the minimum Zn concentration necessary for full development of irrigated rice in flooded soil is 1.5 mg kg^{-1}. Boonchuay et al. (2013) also found no increase in shoot dry mass, panicle number or irrigated rice yield when $ZnSO_4.7H_2O$ was applied during various reproductive stages following panicle differentiation in soil with Zn content of 1.05 mg kg^{-1}. Shivay et al. (2008) observed higher rice yields after applying $ZnSO_4.7H_2O$ when soil had a Zn content of 0.68 mg kg^{-1}. These results suggest that the small responses to Zn application seen in our study may have been due to satisfactory Zn levels in the soil, which allowed for adequate rice development. It was interesting that foliar application of 400 g ha^{-1} $ZnCl_2$ at the V_{11} vegetative stage caused leaf damage, with greater intensity at 1600 g ha^{-1}, as illustrated in Fig. 2; this was probably due to toxicity, which caused reduced rice yield.

Principal components analysis (PCA)

The first three main components explained 79.23% of the variability in response to dose and source of Zn (factor 1 = 44.62% + factor 2 = 20.64% + factor 3 = 13.97%) (Fig. 3A). Thus, the information contained in the 10 variables measured under different doses and sources of Zn can be explained satisfactorily based on the analysis of the first three PCA components, as the number of components that explain 0–90% of the total variance must be considered (Ferreira 2008). The coordinates of the variables in the first three components indicated a differential pattern of response to Zn dose and source. The cold and accelerated ageing tests were the highest contributors to the first and third components (Fig. 3B–D and Table 4), acting in the opposite direction to yield, which contributed the most to the second component. Thus, the cold and accelerated ageing tests as well as grain yield were the variables with the highest contribution to variability in relation to Zn dose and source.Considering the correlation scores among the variables in each component (Table 4) and the graphical analysis of the components in pairs, Fig 3B-D shows that grain yield responded similarly to Zn, but in greater magnitude than the response of shoot dry mass. Therefore, the variables cold test, accelerated ageing and seedling dry weight showed response patterns in opposite directions, according to the component considered. The remaining variables (germination, seedling length, seedling dry mass, seedling emergence, panicle number and 100 grain mass) did not contribute significantly to the main components, indicating little contribution to the variability in response to the Zn treatment by seeds (doses and sources).

Materials and Methods

Location and plant material

The experiment was carried out at the geographic coordinates 29º 12’ 28’’ S and 56º 18’ 28’’ W, at an altitude of 64 m, with the flood-irrigated rice cultivar Puitá INTA Cl in the laboratory and in the field.

Laboratory experimental design

The experimental design was completely randomized in a 6 × 2 factorial scheme with four replicates. Six doses of Zn were used, such as 0, 50, 100, 200, 400 and 800 g ha^{-1} (considering 100 kg ha^{-1} of seeds), along with two Zn

Table 2. ANOVA on data generated using seven Zn doses, three times of application (seed coating treatment, V4 and V11 stages of rice growth, and two Zn sources ($ZnSO_4.7H_2O$ and $ZnCl_2$), three replicates and their interaction in field on panicle number/pot, shoot dry mass, 100 grains mass, and rice yield/pot.

Source of variation	Degree of freedom	Mean square			
		Panicle number	Shoot dry mass	100 grains mass	Rice yield/pot
Doses (A)	6	38.03^{ns}	70.36^{ns}	0.003^{ns}	49.21^{ns}
Application times (B)	2	29.78^{ns}	185.41^{ns}	0.008^{ns}	861.09^{**}
Sources (C)	1	2.29^{ns}	2.43^{ns}	0.020^{ns}	99.73^{ns}
A x B	12	23.19^{ns}	88.60^{ns}	0.007^{ns}	120.22^{ns}
A x C	6	16.64^{ns}	68.20^{ns}	0.008^{ns}	80.16^{ns}
B x C	2	81.34^{ns}	186.43^{ns}	0.018^{ns}	543.48^{*}
A x B x C	12	74.52^{ns}	34.52^{ns}	0.008^{ns}	208.98^{ns}
Error	84	69.74	90.00	0.008	130.12
CV (%)		9.88	6.87	3.62	8.95

^{ns} No significance at $p \leq 0.05$. * Significance at $p \leq 0.05$. ** Significance at $p \leq 0.01$.

Fig 2. Rice plants at one and seven days, respectively, after the application of 1600 g ha^{-1} $ZnCl_2$ in V_{11} vegetative stage.

sources, such as zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and zinc chloride ($ZnCl_2$).

Germination, physiological potential and emergence

Seeds were treated by applying Zn diluted in 5 mL water, which was distributed throughout 50 g seed samples packed in 500 mL transparent plastic bags. Then, the seeds were homogenized and transferred to a plastic tray for drying, where they remained for 50 min. After treatment, the seeds were evaluated for germination and physiological potential, in accordance with the methodology described below: (a) Germination test: four replicates of 50 seeds were used for each treatment and were distributed in paper towel rolls, consisting of three sheets moistened with distilled water, with a weight equivalent to 2.5 times dry paper (Brasil, 2009). The seeds were wrapped in a polyethylene bag to prevent water loss. Then, the rolls containing the seeds were transferred to a 25°C germination chamber for 14 days, and evaluations were performed on days 5 and 14 in accordance with the Brazilian Rules for Seed Analysis (Brasil, 2009); (b) Seedling length: four replicates of 20 seedlings per treatment were used as obtained from the germination test and were measured manually (Nakagawa, 1999); (c) Seedling dry mass: 20 seedlings were transferred to paper bags and incubated at 65ºC for 72 h after measurements to obtain seedling dry mass (Nakagawa, 1999); (d) Cold test: a procedure similar to the standard germination test, described above, was carried out, differing only by maintaining the seeds in a 10°C germination chamber for 48 h. Then, the seeds were transferred to a 25ºC chamber and evaluated 14 days after the start of the test, according to the Brazilian Rules for Seed Analysis (Brasil, 2009); (e) Accelerated ageing: this test was performed with four replicates per treatment, with a thin layer of seeds distributed on a metal screen coupled to transparent plastic boxes (11 × 11 × 3.5 cm) containing 40 mL water. The boxes were maintained in a Biochemical Organism Development chamber at 41°C for 48 h (Marcos Filho, 1999). After, four replicates of 50 seeds were subjected to the germination test using the methodology described above, they were evaluated on day 5 after sowing, according to the Brazilian Rules for Seed Analysis (Brasil, 2009); (f) Seedling emergence: four replicates of 25 seeds per treatment were sown in pots with 7 L soil, at a depth of 3 cm and irrigated twice daily. Daily evaluations were made by counting the number of emerged seedlings up to 14 days after sowing.

Table 3. Rice grain yield as a function of Zn application time and sources.

Application time	Rice grain yield, g/pot		
	Zn sources	$ZnSO_4.7H_2O$	$ZnCl_2$
Seed	140.4 a*	136.1 Bb	144.8 Aa
Vegetative stage-V_4	132.7 b	131.0 Ab	134.6 Ab
Vegetative stage-V_{11}	142.0 a	145.2 Aa	138.7 Aab
CV (%)		8.9	

*Means followed by same capital letter in a line and lower case in a column were not difference according to Tukey's test at an error probability of 5%.

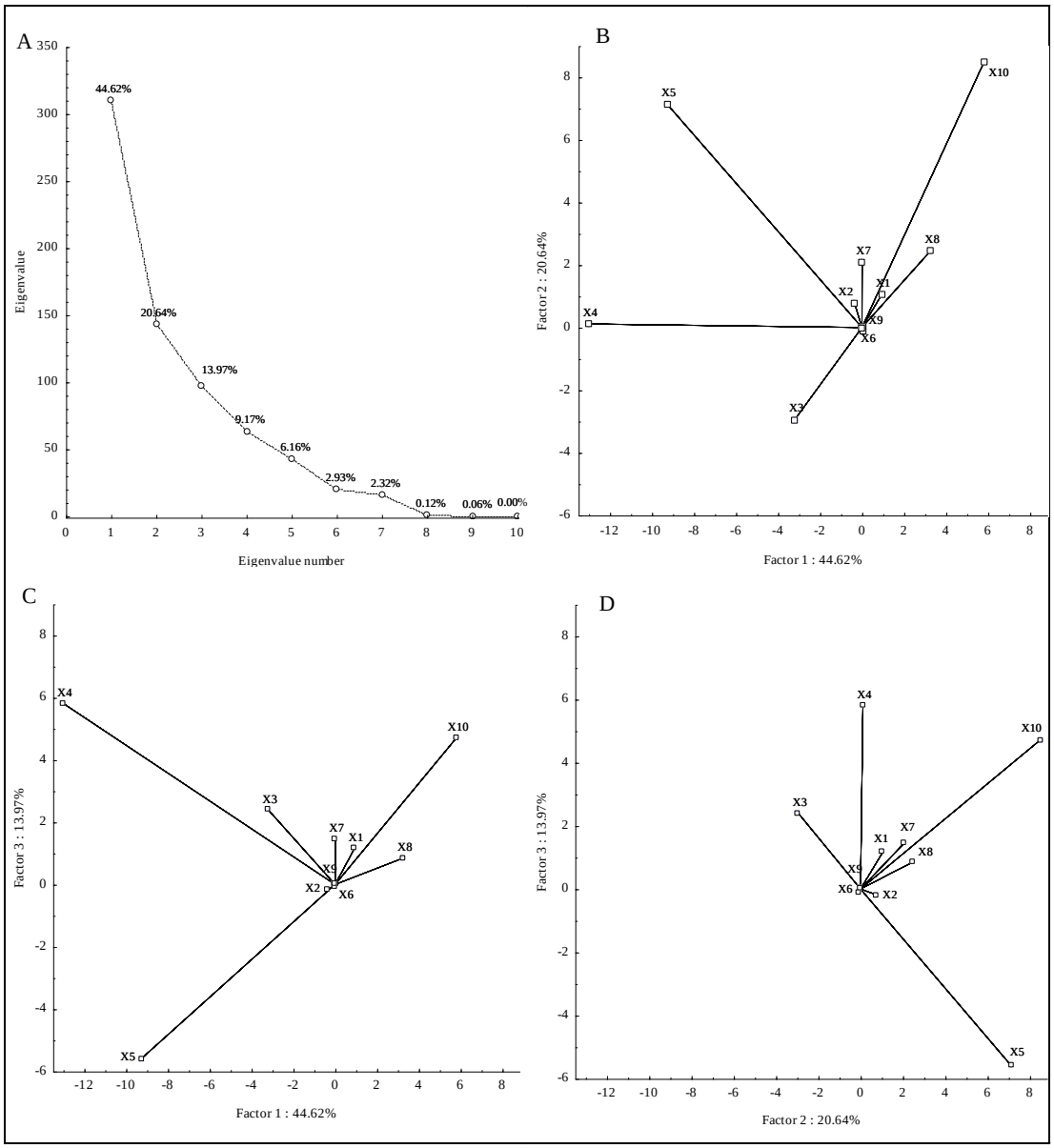

Fig 3. Percentage contribution of each principal component, based on its eigenvalue (A); graphical representation of the relationship between the first and second (B), the first and third (C) and second and third (D) factor of the principal component analysis, based on the analysis of covariance. X1 = Germination; X2 = Seedling length; X3 = Seedling dry mass; X4 = Cold test; X5 = Accelerated aging; X6 = Seedling emergence; X7 = Panicle number; X8 = Shoot dry mass; X9 = 100 grains mass; X10 = Rice grain yield.

Table 4. Coordinates, contribution of each variable and correlations between variables in the first three factors of the principal component analysis (PCA) performed on the basis on covariance, considering n = 36 observations (six doses x two sources of Zn x three replicates), depending on the Zn application by seed treatment.

Variable[(1)]	Coordinates of the variables			Variable contribution			Variable correlations		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
X1	0.93	1.05	1.16	0.00	0.01	0.01	0.20	0.22	0.25
X2	-0.35	0.77	-0.19	0.00	0.00	0.00	-0.27	0.60	-0.15
X3	-3.19	-2.94	2.39	0.03	0.06	0.06	-0.42	-0.38	0.31
X4	-13.00	0.11	5.81	0.54	0.00	0.35	-0.90	0.01	0.40
X5	-9.26	7.13	-5.59	0.28	0.35	0.32	-0.71	0.55	-0.43
X6	0.03	-0.10	-0.10	0.00	0.00	0.00	0.04	-0.15	-0.14
X7	0.01	2.09	1.46	0.00	0.03	0.02	0.00	0.29	0.21
X8	3.27	2.45	0.85	0.03	0.04	0.01	0.46	0.35	0.12
X9	0.03	0.01	0.01	0.00	0.00	0.00	0.44	0.16	0.15
X10	5.81	8.49	4.71	0.11	0.50	0.23	0.50	0.73	0.41

(1) X1 = Germination; X2 = Seedling length; X3 = Seedling dry mass; X4 = Cold test; X5 = Accelerated aging; X6 = Seedling emergence; X7 = Panicle number; X8 = Shoot dry mass; X9 = 100 grains mass; X10 = Rice grain yield.

Field experimental setup

Ultisol, with a medium texture and 190 g kg^{-1} clay, was used for cultivation. The soil was collected from the 0–20 cm layer of native vegetation and passed through a 4 mm sieve; the chemical characteristics were: pH H_2O = 5.4; P = 1.5 mg L^{-1}; K = 0.102 $cmol_c$ dm^{-3}; Ca = 2.9 $cmol_c$ dm^{-3}; Mg = 1.0 $cmol_c$ dm^{-3}; Zn (DTPA) = 1.2 mg dm^{-3}; Al = 0.2 $cmol_c$ dm^{-3}; basis saturation = 50%; organic matter = 2.3%. The climate was Cfa, humid subtropical without a dry season and a hot summer (Peel et al., 2007).

Experimental design

The experimental design was completely randomized in a 7 × 3 × 2 factorial scheme, with three replicates consisting of pots containing 7 L soil. Seven Zn doses were used: 0, 50, 100, 200, 400, 800 and 1600 g ha^{-1}, along with three application times, such as the seed and the V_4 and V_{11} vegetative stages (Counce et al., 2000); two Zn sources were used: $ZnSO_4.7H_2O$ and $ZnCl_2$.

Rice cultivation

Sowing fertilization was performed using N (50 kg ha^{-1}) in the form of urea; fertilization was performed with 350 kg ha^{-1} P as triple superphosphate and 300 kg ha^{-1} K in the form of potassium chloride. P and K fertilization with was five times greater in pots than that recommended for the field, and the fertilizer was passed through a Willey mill and homogenized in pot soil. The total N applied was 180 kg ha^{-1}. On October 9, 2013, eight seeds were sowed per pot, in a line that was 3 cm deep. After 14 days, the pots were thinned, leaving two plants per pot. Twenty days after sowing at stage V_3/V_4 (Counce et al., 2000), 50% (65 kg ha^{-1}) of the remaining N was applied, and 4 cm water was applied above the surface the next day and maintained constantly by several daily irrigations until rice harvest. The other 50% (65 kg ha^{-1}) N was applied 40 days after sowing at the vegetative stage V_{10} (Counce et al., 2000) in the form of urea. Two weekly casters of pots were performed during rice cultivation. Zn was applied by seed treatment (considering 100 kg ha^{-1} seeds), with a nozzle array and liquid sprayer doses of 160 L ha^{-1} during vegetative stages V_4 and V_{11} (Counce et al., 2000) and at 25 and 44 days after sowing. Applications were made in the morning at 10:00 h.

Yield and growth evaluation

Panicle number, shoot dry mass, 100 grain mass and rice grain yield per pot were evaluated at harvest, and grain moisture was calculated and adjusted to 12% to determine yield.

Statistical analysis

Analysis of variance was performed according to the completely randomized experimental design in a factorial scheme for each variable measured in the laboratory and field. Six doses and two sources of Zn with four replicates were used in the laboratory, whereas six doses, three application times and two sources of Zn with three replicates were used in the field. The doses were split within each Zn source or application time when interactions between levels of factors doses or sources (in laboratory) or doses, application times and sources (in field) were significant ($p \leq 0.05$) by fitting a regression model for each source or application time. When the interaction was not significant ($p \geq 0.05$), the main effects of source, dose and application time of Zn were assessed separately. Tukey's test was employed for qualitative factors (Zn source and applied time), and adjusted regression equations were used for quantitative factors (doses) when the interaction was significant ($p \leq 0.05$) PCA was performed considering 10 variables evaluated in 36 experimental units with Zn application by seed treatment (6 Zn doses × 2 Zn sources × 3 replicates). The PCA was performed based on the covariance method, as described by Ferreira (2008). Statistical analyses were performed using Statsoft Statistica for Windows software package (Statsoft, 2005) and the Microsoft Office Excel application.

Conclusion

Treating rice seeds with Zn did not change the germination rate or seedling emergence. Among the parameters used to assess the physiological potential of rice seeds, seedling dry mass and germination at low temperatures (cold test) benefited from the use of $ZnSO_4.7H_2O$. Treating rice seeds with $ZnCl_2$ provided 6.4% higher grain yield compared to that of $ZnSO_4.7H_2O$. Applying Zn promoted 5.8% higher rice grain yield by coating seeds and 7.0% by spraying at the V_{11} stage compared to that at the V_4 vegetative stage. Use of a higher Zn dose did not affect germination, seedling emergence or grain yield up to1600 g ha^{-1}.

The first three main components of the PCA by seed treatment condition explained 79.23% of the variability in response to Zn dose and source. The cold test and accelerated ageing tests were the highest contributors to the first and third components and acted in opposite directions to grain yield. Yield had the highest contribution to the second component and was the third component that most influenced the first and third components.

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References

Aery NC, Sarkar S (2012) Metal species vis-à-vis seed germination and early seedling growth responses in soybean. J Chem Bio Phy Sci Sec B. 2:763-769.

Albuquerque KAD, Oliveira JA, Silva PA, Veiga AD, Carvalho BO, Alvim PO (2010). Storage and quality of tomato seeds enriched with micronutrients and growth regulators. R Ci Agrotec. 34:20-28.

Alloway BJ (2008) Zinc in soils and crop nutrition. (2nd ed.). IZA publications. International zinc association, Brussels. 135p.

Bennett AC, Shaw DR (2000) Effect of *Glycine max* cultivar and weed control on weed seed characteristics. Weed Sci. 48:431-435.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds, physiology of development, germination and dormancy. Springer, Lexington. 392p.

Bhattacharjee S (2013) Heat and chilling induced disruption of redox homeostasis and its regulation by hydrogen peroxide in germinating rice seeds. Physiol Mol Biol Plant. 19:119-207.

Bonnecarrère RAG, Londero FAA, Santos O, Schmidt D, Pilau FP, Manfron PA, Dourado Neto D (2003) Answer of genotypes of rice irrigated to the zinc application. R Fac Zootec Vet Agron. 10:214-222.

Boonchuay P, Cakmak I, Rerkasem B, Prom-U-Thai C (2013) Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. Soil Sci Plant Nutr. 59:180-188.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento (2009) Regras para análise de sementes. MAPA/ACS, Brasília. 395p.

Cakmak I (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol. 146:185-205.

Cakmak I (2002) Plant nutrition research: priorities to meet human needs for food in sustainable ways. Plant Soil. 247:3-24.

Camargo FAO, Santos GA, Zonta E (1999) Alterações eletroquímicas em solos inundados. Ci Rural. 29:171-179.

Counce PA, Keisling TC, Mitchell AJ (2000) A uniform, objective, and adaptive system for expressing rice development. Crop Sci. 40:436–443.

El-Ghamery AA, El-Kholy A, El-Yousser A (2003) Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutat Res. 537:29-41.

Ferreira, D.F. Estatística multivariada. 1. ed. Lavras, UFLA, 2008. 662p.

Firbank LG, Watkinson AR (1985) On the analysis of competition within two-species mixtures of plants. J Appl Ecol. 22:503-517.

Franco IAL, Martinez HEP, Zabini AV, Fontes PCR (2005) Translocação e compartimentalização de Zn aplicado via $ZnSO_4$ e ZnEDTA nas folhas de cafeeiro e feijoeiro. Ci Rural. 35:332-339.

He D, Yang P (2013) Proteomics of rice seed germination. Front Plant Sci. 4: 1-9.

Kabeya MJ, Shankar AG (2013) Effect of different levels of zinc on growth and uptake ability in rice zinc contrast lines (*Oryza Sativa* L.). Asian J Plant Sci Res. 3:112-116.

Li X, Yang Y, Zhang J, Jia L, Qiaoxia L, Zhang T, Qiao K, Ma S (2012) Zinc induced phytotoxicity mechanism involved in root growth of *Triticum aestivum* L. Ecotox Environ Safe. 86:198–203.

Malavolta E (2006) Manual de nutrição mineral de plantas. Agronômica Ceres, São Paulo. 631p.

Marcos Filho J (1999) Accelerated aging test. In: Krzyzanowski FC, Vieira RD, França-Neto JB (Ed.). Seed vigor: concepts and tests. Abrates, Londrina. p.1-24.

Marschner H (1995) Mineral nutrition of higher plants. Academic, San Diego. pp.889.

Mertz LM, Henning FA, Soares RC, Baldiga RF, Peske FB, Moraes DM (2009) Physiological changes in rice seeds exposed to cold in the germination phase. R Bras Sementes. 31:262-270.

Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. Arch Environ Cont Tox. 43:203-213.

Mustafa G, Ehsanullah, Akbar N, Qaisrani SA, Iqbal A, Khan HZ, Jabran K, Chattha AA, Trethowan R, Chattha T, Atta BM (2011) Effect of zinc application on growth and yield of rice (*Oryza sativa* L.). Int J Agro Vet Med Sci. 5:530-535.

Naik SK, Das, DK (2007) Effect of split application of zinc on yield of rice (*Oryza sativa* L.) in an inceptisol. Arch Agron Soil Sci. 53:305-313.

Nakagawa J (1999) Seed vigor tests based in seedling evaluation. In: Krzyzanowski FC, Vieira RD, França-Neto JB (Ed.). Seed vigor: concepts and tests. Abrates, Londrina. pp.2:1-2:21.

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun HJ, Sayers Z, Cakmak I (2006) Concentration and localization of zinc during seed development and germination in wheat. Physiol Plantarum. 128:144–152.

Peel MC, Finlayson BL, Mcmahon, TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sc. 11:1633–1644.

Rehman H, Aziz T, Farooq M, Wakeel A, Rengel Z (2012) Zinc nutrition in rice production systems: a review. Plant Soil. 361:203-226.

Rozane DE, Prado RM, Romualdo LM, Simões RR (2008) Response in rice cv. brs Soberana seedlings to addition of zinc to seeds. Ci Agrotec. 32:847-854.

Rufino CA, Lemes ES, Tavares LC, Villela FA (2013) Physiological quality of rice seeds treated with macro and micronutrients. R Fac Agron. 112:11-17.

Sharifi P (2010) Evaluation on sixty-eight rice germplasms in cold tolerance at germination. Rice Sci. 17:77-81.

Shivay YS, Kumar D, Prasad R, Ahlawat IPS (2008) Relative yield and zinc uptake by rice from zinc sulphate and zinc oxide coatings onto urea. Nutr Cycl Agroecosys. 80:181–188.

Solanki R, Anju P, Dhankhar R (2011) Zinc and copper induced changes in physiological characteristics of *Vigna mungo* (L.). J Environ Biol. 32: 747-751.

Stanković M, Topuzović M, Marković A, Pavlović D (2011) Influence of zinc (Zn) on germination of wheat (*Triticum aestivum* L.). Biotechnol Biotec Eq. 24:236-239.

Statsoft. Statistica 7.0 Sofware. Tucksa, USA, 2005

Tavares LC, Fonseca DAR, Brunes AP, Rufino CA, Meneguello GE, Barros AAS (2013) Performance of rice seeds treated with zinc, boron and molybdenium. J Seed Sci. 35:532-539.

Tedesco MJ, Gianello C, Anghinoni I, Bissani CA, Camargo FAO, Wiethölter S (2004) Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. (10^{th} ed.). Sociedade brasileira de ciência do solo, Porto Alegre. 401p.

Yan XX (2003) Research on genotypic difference in zinc nutrition of rice. Guangdong Trace Elem Sci. 10:6-9.

Yemeni A, Al-Helal AA (2003) Effect of zinc chloride and lead nitrate on seed germination and early seedling growth of rice and alfalfa. J King Saud Univ Sci. 15:39-47.

Yin G, Sun H, Xin X, Quin G, Liang Z, Jing X (2009) Mitochondrial damage in the soybean seed axis during imbibition at chilling temperatures. Plant Cell Physiol. 50:1305-1318.