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Optimization of Zinc seed priming treatments for improving the germination and early seedling growth of *Oryza sativa*

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Abstract:

Background: Seed priming is a technique that is used to regulate seed germination through temperature and moisture content management, which helps to increase the seed germinating potential. Zinc (Zn) is an essential micronutrient for rice growth and development. Lab and pot experiments were conducted to explore the potential of seed priming with Zn for improving the germination and early seedling growth of fine grain rice.

Methods: Zinc was applied as seed priming. For priming, seeds of fine rice cultivars Super Basmati and Shaheen Basmati were soaked in 0.05%, 0.1%, 0.5% and 1% aerated Zn solutions (w/v); while untreated dry seeds and water soaked seeds were taken as control.

Results: Seed priming with 0.1% and 0.5% Zn solution not only reduced the time for 50% germination but it also decreased the mean germination time. Moreover, seed priming with 0.1% and 0.5% Zn solution improved the germination vigour, germination index and final germination percentage in both the tested cultivars. Similarly, plumule length, radicle length, seedling dry weights and vigour index were also improved by seed priming with 0.1 and 0.5% Zn solution. Application of 0.1% Zn solution also improved tillering, leaf emergence, leaf elongation and chlorophyll content. However, increase in concentration application of Zn from 0.1% to 0.5% was toxic for seeds of both cultivars.

Conclusion: In conclusion, rice seeds may be primed with 0.5% Zn solution used to improve the germination and early seedling growth.

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Key words:
Chlorophyll content, Development, Growth, Seed priming, Zinc, Rice

Introduction

Rice (*Oryza sativa* L.) is the leading staple food in Asia, and provides 35-80% of total metabolic energy to almost half the world's population [1]. As 55% of total rice area is irrigated, which accounts for 75% of rice production, the future food security of most of the world's population depends on it. Global food security is challenged due to increasing food demand with growing population and declining available irrigation water. This demands development of strategies for economizing water use without yield reduction [2]. Some technologies like aerobic rice, which involves growing rice under conditions similar to other cereals like wheat and maize, have been developed [3]. Although, aerobic culture is an attractive option of water-wise rice production, some constraints including poor crop stand high weed infestation and panicle sterility are hindering its wide scale adoption [4]. For optimal growth and development, micronutrients are required by crop plants. Improving plant micronutrient status in situations where there is inadequate supply of micronutrients in soil would increase yield. This, however, requires application of higher doses of fertilizer to soils because of low nutrient-use efficiency [5].

Micronutrients are involved in the key physiological processes of photosynthesis and respiration [6] and their deficiency can impede these vital physiological processes thus limiting yield gain. For example, zinc (Zn) deficiency is a major yield-limiting factor in several Asian countries [7]. Similarly Zn supply is considered as an important factor in reproduction process. According to Brown *et al.*, [8] formation of male and female reproductive organs and pollination process are disturbed by Zn deficiency which may be attributed to the reduction of Indol acetic acid (IAA) synthesis. Marschner [9] declared that following Zn deficiency, reduction in RNA-polymerase activity and increase in RNA destruction can severely reduce the seed protein content. Studies have revealed that, B and Zn are interestingly related with each other. Hosseini *et al.*, [10] reported that there was a significant effect of Zn on corn growth and tissue nutrient concentration which were rate dependent. In general, the effect was antagonistic in nature on nutrient concentration and synergistic on plant growth. The purpose of this research was to study the effect of Zn priming of rice seed on growth and development of rice at early growth stage in lab and green house conditions.

Methods

This study was conducted in Allelopathy Laboratory, Department of Agronomy, University of Agriculture Faisalabad, Pakistan during 2012-13. Seeds of fine grain aromatic rice (*Oryza sativa* L.) cultivars Super Basmati and Shaheen Basmati, used in this study were obtained from Rice Research Institute, Kala Shah Kakoo, Pakistan and Soil Salinity Research Institute, Pindi Bhattian, Hafizabad, Pakistan, respectively. For priming, a weighed quantity (100 g) of seeds was soaked in aerated solution 0.05, 0.1, 0.5 and 1% Zn for 24 hours (h) keeping seed to volume ratio 1:5 (w/v); while seeds soaked in water (hydropriming) for 24 h and untreated seeds were taken as control. Hydrated zinc sulphate was used for Zn priming treatments. Primed seeds were given three surface washings with distilled water and dried closer to the original moisture level with forced air, after which treated and untreated seeds were sealed in polythene bags and stored in a refrigerator at 7 ± 1°C until use.

Treated and untreated seeds were sown in moist sand in petri plates, and were placed at 27°C in an incubator. Seven seeds were sown in each patri plates. Seedling emergence was observed daily according to the Association of Official Seed Analysts method (AOSA 1990) until a constant count was achieved. The time to 50% emergence (E50) was calculated [4]. Mean Emergence time (MET) was calculated according to the equation of Ellis and Roberts [11]. Emergence index (EI) was calculated according to the Association of Official Seed Analysts [12]. Emergence energy (EE) was recorded on the 4^{th} day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested [13]. On 10^{th} day after sowing, the seedlings were tested for vigour after careful removal. Radicle and plumule lengths were measured on a scale of five random seedlings per replicate and then averaged. To measure seedling dry weight, seedlings were dried at 70°C in electric oven till constant weight was achieved. A pot experiment was conducted at Agronomy Research Area, University of Agriculture Faisalabad during 2012-13 to explore the influence of Zn priming on growth and chlorophyll contents of rice cultivars. For priming, same procedure was followed as described for petri dish experiments. Treated and untreated seeds were sown in soil-filled earthen pots (45 × 30 cm) placed in a net house under natural conditions on July 25, 2012. Ten seeds were sown in each pot and plants were thinned to five plants per pot 1 week after the emergence. Number of leaves and number of tillers were counted daily to determine the rate of leaf and tiller emergence. Starting from the seedling emergence, length of each individual leaf was measured daily to derive leaf emergence and elongation rates [14]. Leaf chlorophyll (Chl) content was determined [15]. For this purpose, pigments were extracted in 80% acetone and determined in the same solvent. The concentration of each Chl was determined by measuring the absorbance (A) of the extract at the major red absorption maxima of Chl-a (663 nm) and Chl-b (645 nm) and values were inserted into the standard equations. The data was analyzed using MSTATC package and treatment means were compared using least significance difference (LSD) test at 5% probability [16].

Results

Rice seed priming with Zn significantly improved the germination energy, germination index and final germination percentage; however rice seed priming significantly reduced T50 and MGT. In all cases both cultivars behaved alike, showing no-significant ($P > 0.05$) interaction of seed priming and cultivars. Seed priming with 0.1 and 0.5% zinc solution improved the germination energy, germination index and final germination percentage (Table 1).

However seed priming beyond 0.5% has not improved final germination index and final germination percentage. The germination of rice is problematic in an effort to improve germination of rice cultivars, seed priming with 0.1-0.5% Zn solution was found suitable. Similarly, seed priming with 0.5% significantly reduced T50 and MGT (Table 1).

Seed priming with Zn significantly ($p \leq 0.05$) affected the radicle length, plumule length, seedling length, seedling dry weight and vigour index. Cultivars difference was only significant for plumule length and seedling dry weight. While the interaction of seed priming with cultivars was significant for plumule length and seedling dry weight (Table 2). Maximum plumule, radicle, seedling lengths, seedling fresh weight and vigour index was observed from seed priming with 0.1 and 0.5% Zn solution compared with control (Table 3). However, seed priming with 1% Zn solution was toxic for radicle and plumule length and seedling dry weight. Maximum plumule length and seedling dry weight was noted in Super basmati. Analysis of variance (ANOVA) showed that seed priming treatments were highly significant, varieties were significant and seed priming treatments × varieties interactions were non-significant for Chl-a, Chl-b and total chlorophyll content (Table 4).

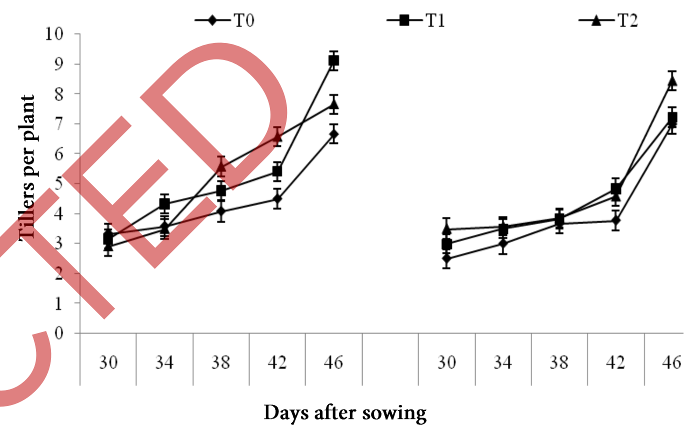

Figure 1. Influence of seed priming with zinc on number of tillers per plant of rice i.e. Super basmati and Shaheen basmati ± S.E. (T0 = Hydropriming; T1 = 0.1% Zn; T2 = 0.5% priming in respective Zn solutions) in pot culture

Likewise, seed priming with Zn significantly improved the chlorophyll concentrations in rice leaves. However, priming in 0.1 % Zn solution was better followed by priming in 0.5 % Zn solution with similar trend in the tested cultivars (Table 5). Pot experiment results showed that seed priming with Zn improved the leaf emergence rate, leaf elongation, tiller emergence and plant height of both rice cultivars. In case of leaf emergence, priming with 0.1% Zn solution showed better leaf emergence than all over other (Fig 1). Regarding leaf elongation, maximum leaf elongation rate was observed in priming with 0.1% Zn solution, followed by priming with 0.5% Zn solution; however, the trend was similar in both cultivars (Fig. 2). Seed priming improved the tillering, maximum tiller emergence was noted in priming with 0.5% Zn solution, and similar trend was observed in Shaheen basmati (Fig. 3).

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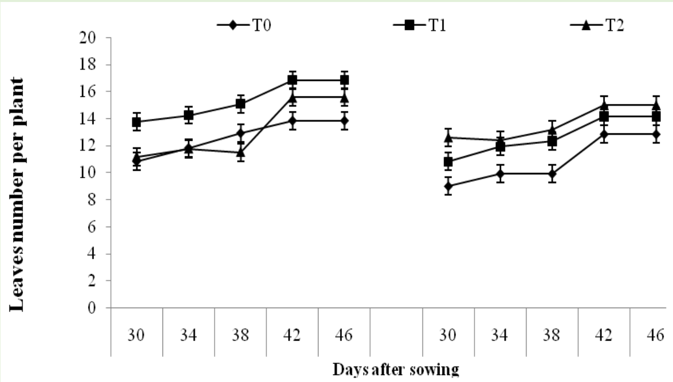

Figure 2: Influence of seed priming with zinc on number of leaves per plant of rice i.e. Super basmati and Shaheen basmati ± S.E. (T0 = Hydropriming; T1 = 0.1% Zn; T2 = 0.5% priming in respective Zn solutions) in pot culture

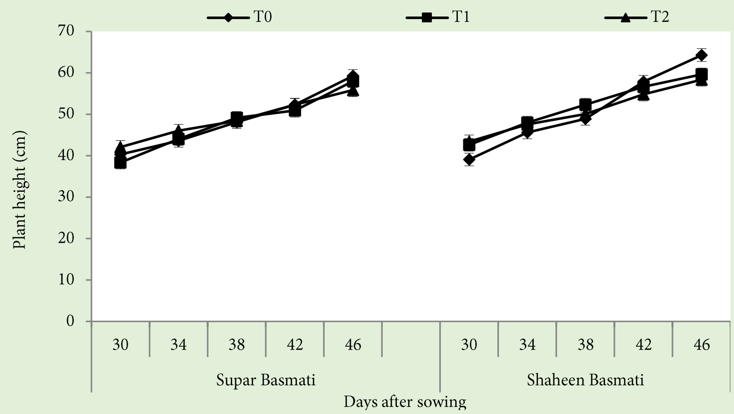

Figure 3: Influence of seed priming with zinc on plant height of rice i.e. Super basmati and Shaheen basmati ± S.E. (T0 = Hydropriming; T1 = 0.1% Zn; T2 = 0.5% priming in respective Zn solutions) in pot culture

Osmopriming with-	T_{50} (days)			MGT (days)			GI			GE (%)			FGP (%)		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
Control	3.81	3.34	3.58 A	4.20	4.03	4.12 A	3.28	3.50	3.39 CD	50.00	60.71	55.36 B	89.29	89.29	89.29 B
Hydropriming	3.54	3.03	3.29 AB	3.88	3.54	3.71 BC	3.77	4.37	4.07 BC	67.86	71.43	69.64 A	92.86	96.43	94.64 AB
0.05% Zn	3.12	3.13	3.12 BC	3.68	3.70	3.69 BC	4.11	4.18	4.15 B	78.57	71.43	75.00 A	100.00	96.43	98.21 A
0.1% Zn	2.63	2.58	2.60 D	3.23	3.22	3.22 D	4.85	5.04	4.95 A	82.14	75.00	78.57 A	92.86	92.86	92.86 A
0.5% Zn	3.01	2.78	2.90 C	3.62	3.57	3.60 C	4.24	4.75	4.49 AB	71.43	75.00	73.21 A	96.43	100.00	98.21 A
1% Zn	3.67	3.20	3.43A	3.97	3.87	3.92 AB	2.91	3.22	3.06 D	49.99	64.29	57.14 B	78.57	82.14	80.36 C
Mean	3.30	3.01		3.76	3.65		3.86	4.18		66.67	69.64		91.67	92.86	

Means not sharing the same letter for a parameter don't differ significantly at p = 0.05

T50 = Time to 50% germination; MGT = Mean germination time; GE = Energy of germination; GI = Germination index; FGP = Final germination percentage; V1 = Super Basmati; V2 = Shaheen Basmati

Table 1: Influence of seed priming with zinc on germination of rice cultivars

Source of variation	DF	Mean sum of square				
		Plumule length	Radicle length	Seedling length	Seedling dry weight	Vigour index
Zinc (Zn)	5	26.001**	13.897**	76.128**	5.421**	1278362**
Rice cultivars (C)	1	10.083**	4.575**	28.244**	2.520*	146348*
Zn × C	5	0.787*	0.112^{ns}	0.957^{ns}	0.020*	18839^{ns}
Error	36	0.428	0.292	0.665	0.423	38081

DF = Degree of freedom; * = Significant at p 0.05; ** = Significant at p = 0.01

Table 2: Analysis of variance for influence of Zn seed priming on early seedling growth of rice

Osmo-priming with	Plumule length (cm)			Radicle length (cm)			Seedling length (cm)			Seedling dry weight (mg)			Seedling vigour		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
Control	13.48 g	12.36 h	12.92 D	10.82	9.87	10.35 E	24.30	22.23	23.26E	80.0 ef	75.0 f	77.5D	2454.00	2170.07	2312.04 D
Hydro-priming	14.44 ef	14.11 fg	14.27 C	11.68	11.10	11.39 D	26.12	25.21	25.66D	87.5 b-e	82.5 def	85.0C	2400.00	2419.43	2409.71 C
0.05% Zn	15.68 d	15.11 de	15.39 B	12.49	12.17	12.33 C	28.17	27.28	27.72C	90.0 bcd	85.0 cde	87.5BC	2866.00	2816.50	2841.25 B
0.1% Zn	18.70 a	16.63 c	17.67 A	13.73	12.89	13.31 B	31.35	29.52	30.44B	95.0 ab	92.5 abc	93.8AB	3301.00	2916.61	3108.80 B
0.5% Zn	17.62 b	16.66 c	17.14 A	14.25	13.79	14.02 A	32.95	30.45	31.70A	100.0 a	95.0 ab	97.5A	3314.00	3176.79	3245.39 A
1% Zn	14.81 def	14.34 efg	14.57 C	12.84	12.29	12.57 C	27.65	26.63	27.14C	80.0 ef	75.0 f	77.5D	2379.43	2172.25	2275.84 D
Mean	15.78 A	14.87 B		12.64	12.02		28.42	26.89		88.8 A	84.2 B		2785.74	2611.94	

Means not sharing the same letter for a parameter don't differ significantly at p = 0.05
V1 = Super Basmati; V2 = Shaheen Basmati

Table 3: Influence of seed priming with zinc on the stand establishment of rice cultivars

Source of variation	DF	Mean Sum of Square		
		Chlorophyll a	Chlorophyll b	Chlorophyll T
Seed priming (SP)	2	44.107**	99.668**	274.791**
Varieties (V)	1	14.311*	7.144*	41.678*
SP×V	2	0.636^{NS}	1.197^{NS}	0.181^{NS}
Error	12	6.393	5.8679	9.021
Total	17			

DF = Degree of freedom; * = Significant at p 0.05; ** = Significant at p 0.01; NS = Non-significant

Table 4: Analysis of variance for chlorophyll a, chlorophyll b and total chlorophyll contents of rice cultivars

Osmo-priming with	Chlorophyll a ($\mu g\ L^{-1}$)			Chlorophyll b ($\mu g\ L^{-1}$)			Total Chlorophyll ($\mu g\ L^{-1}$)		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
Control	20.35	19.26	19.8 B	6.56	4.27	5.4 B	26.91	23.53	25.2 C
0.1% Zn	26.16	23.78	24.9 A	13.81	13.12	13.5 A	39.97	36.90	38.4 A
0.5% Zn	24.77	22.88	23.8 A	10.94	10.14	10.5 A	35.71	33.03	34.4 B
Mean	23.76	21.97		10.44	9.18		34.20	31.15	

Means not sharing the same letter for a parameter don't differ significantly at p 0.05 V1 = Super Basmati; V2 = Shaheen Basmati

Table 5: Influence of seed priming with zinc on the chlorophyll contents of rice cultivars

Discussion

The study reveals that seed priming with Zn improved the leaf emergence rate, leaf elongation, tiller emergence and plant height of both rice cultivars. Seed priming minimizes the time between sowing and emergence of seedling and also regulates emergence [13]. Primed seeds exhibit rapid and uniform germination accomplished with better germination percentage as compared to non-primed seeds [17-21]. Zinc is an important micronutrient and is involved in a number of physiological processes of plant growth and metabolism including protein synthesis, enzyme activation, carbohydrates, lipids, auxins and nucleic acids metabolism, gene expression and regulation and pollen formation [22,23]. The addition of Zn in the priming medium stimulated the seedling emergence and seedling establishment. Seed priming with 0.5% and 0.1% Zn solution improved the seedling emergence, reduced MGT and T_{50} in lab study due to function of Zn in protein synthesis, enzyme activation [6], and IAA synthesis [23]. However, concentrations of Zn are very important for seed germination. Seed priming with 1% Zn solution took maximum time for 50% germination, MGT owing to toxicity. Seed priming with 0.5% and 0.1% improved the root and shoot length and seedling dry weight these results were in accordance with Weisany *et al*., [24] who concluded that zinc application improved the shoot length, root fresh and dry weight and shoot fresh and dry weight under saline condition. However, Malik *et al.* [25] reported that length of roots and shoots, the fresh and dry matter production decreased with increasing zinc levels for red amaranth. Seed priming improves the germination resulting in earlier radicle and plumule emergence as compared to non-primed seeds by improving water uptake and metabolic activities [13-15]. Seed priming with low concentrations of Zn resulted in early seedling emergence which helped in the leaf elongation, leaf expansion, increase in plant height, number of leaves and number of tillers as Zn is involved in cell division and IAA metabolism [26] found that Zn in newly-developed radicles and coleoptiles during seed germination was much higher (up to 200 $mg\ kg^{-1}$ seed) thus highlighting the involvement of Zn in physiological processes during early seedling development, possibly in protein synthesis, cell elongation membrane function and resistance to abiotic stresses [27-29].

Seed priming with Zn may improve the germination and early seedling growth of rice if applied at optimized concentration. Seed priming with 0.1% and 0.5% Zn solution improved the seed germination and early seedling development while reduced the time to 50% emergence, mean emergence time and improved the germination energy, germination index and seedling vigor. In case of pot experiment, 0.1% Zn solution treatment improved tillering, leaf emergence, leaf elongation and chlorophyll content. So we recommend rice seed priming with 0.1% Zn.

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