



Full Length Article

Zinc Seed Coating Improves Emergence and Seedling Growth in *Desi* and *Kabuli* Chickpea Types but Shows Toxicity at Higher Concentration

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Abstract

Chickpea is very sensitive to zinc (Zn) deficiency that can be corrected by Zn application in several ways to various crops including seed coating. However, the optimization of Zn seed coating in chickpea is needed prior to its wide application under field conditions. This study was comprised of four experiments in petri plates and sand filled pots for optimization of Zn seed coating concentration in *desi* and *kabuli* chickpea types. In first two experiments, Zn was applied using different seed coating concentrations *viz.*, 5, 50, 100 and 200 mg Zn kg⁻¹. No Zn was taken as control. High Zn concentrations (100 and 200 mg Zn kg⁻¹) indicated Zn toxicity and inhibited the seed germination. In third and fourth experiments, Zn was applied at 5, 20, 35 and 50 mg Zn kg⁻¹ with non-coated seeds as control. These studies were carried out in sand filled pots and petri plates. In petri plates, seeds were uniformly spread between the two layers of moist filter paper while seeds were sown in small sand filled (500 g) pots. In experiments, emergence/germination rate, emergence/germination index, root and shoot lengths improved with Zn seed coating (5 mg Zn kg⁻¹). Moreover, seedling dry weight and number of secondary roots per plant in both chickpea types (*desi* and *kabuli*) improved with Zn seed coating at 5 mg Zn kg⁻¹ compared with other Zn seed coating concentrations. However, Zn seed coating above 5 mg kg⁻¹ suppressed the germination/emergence and seedling growth of both chickpea types indicating Zn toxicity. In conclusion, seed coating with 5 mg Zn kg⁻¹ was best treatment in improving the emergence/germination and early seedling growth in both chickpea types. © 2019 Friends Science Publishers

Keywords: Emergence; Secondary roots; Seedling dry weight; Zinc application; Zinc toxicity

Introduction

Zinc (Zn) is vital micro-element involved in variety of plant biochemical processes including respiration, chlorophyll biosynthesis and photosynthesis (Nishizawa, 2005). Auld (2001) reported involvement of Zn in various metabolic functions such as nucleic acid, protein, carbohydrates and lipid formation. Deficiency of Zn adversely affects the fertilization and pollen functionality (Pandey *et al.*, 2006) which leads towards the reproductive impairment in plants (Pandey *et al.*, 2009). Deficiency of Zn is among the major reasons responsible for reduction in chickpea (*Cicer arietinum* L.) yield (Ahlawat *et al.*, 2007; Singh, 2008) as soils of many chickpea growing countries are deficit in plant available Zn (Khan *et al.*, 2000; Roy *et al.*, 2006; Ahlawat *et al.*, 2007).

More than 70% of Pakistani soils are prone to Zn deficiency (Hamid and Ahmad, 2001; Maqsood *et al.*, 2015). Calcareous nature of soil, high pH, less organic matter, waterlogging (Alloway, 2009) and coarse soil texture are the major factors causing Zn deficiency. In Pakistan, chickpea is mostly grown in arid lands of Pothohar and rainfed lands of

Thal which are Zn deficient (FAO, 2017).

Zinc deficiency not only limits the chickpea productivity but its application above the required level also impairs the plant growth and development. For instance, Zn concentration of ≤ 10 mM in the germination medium decreased the germination by 75% and caused reduction in zeatin contents and gibberellic acid in chickpea (Atici *et al.*, 2005). Zn applied above the required level behaves as heavy metal (Ali *et al.*, 1999, 2000) and cause toxicity. In wheat, Zn application at higher rate (300 mg L⁻¹) in solution culture inhibited the shoot and root development (Glińska *et al.*, 2016). Rehman and Farooq (2016) reported that Zn application through seed coating with higher Zn concentration *i.e.* > 1500 mg kg⁻¹ seed inhibited the seedling growth of wheat.

Zinc can be delivered to plants through various methods including, seed treatments, foliage and soil application to meet the crop requirements (Johnson *et al.*, 2005). Each application method has its own pros and cons. It is hard to achieve uniform application of micronutrients when applied to soil (Johnson *et al.*, 2005). In a recent study,

Haider *et al.* (2018) indicated that foliage applied Zn improved the growth, yield and grain Zn concentration in mungbean (*Vigna radiata* (L.) Wilczek). In another study, foliar application of Zn at reproductive phase improved the yield and related traits of chickpea (El-Habbasha and Magda, 2013). However, the repeated foliage applications may increase the production cost (Farooq *et al.*, 2012).

Micronutrients can also be supplied as seed treatments via seed priming and coating. In this method, coating of seeds with specific nutrients for specific period or dipping into solution is an easy and cost-effective method of micronutrient application (Farooq *et al.*, 2009). This helps to improve the seedling emergence and crop growth (Farooq *et al.*, 2012). Seed treatments require very small amount of nutrient, hence considered cost-effective and enhance nutrients availability to the germinating seeds (Singh *et al.*, 2005).

Seed coating develop a ready covering of nutrients around the germinating seeds to ensure the continuous supply of nutrients to the plants (Taylor and Herman, 1990) during early stages of growth. Seed coating with Zn results in better crop stand, improved crop productivity (Shivay *et al.*, 2008; Farooq *et al.*, 2012) and Zn concentration in grain. However, the higher concentration of Zn may suppress the germination (Jain *et al.*, 2010; Glińska *et al.*, 2016; Rehman and Farooq, 2016).

Seed coating can be pragmatic and cost-effective method of Zn application in field crops (Baudet and Peres, 2004; Farooq *et al.*, 2018a), however, requires optimization before field application. To the best of our knowledge, no information is available on the optimization of Zn seed coating concentrations in chickpea. Therefore, in present study the optimization of Zn seed coating concentrations was investigated to enhance seedling growth and stand establishment of *desi* and *kabuli* chickpea types.

Materials and Methods

Plant Material

Seeds of *kabuli* chickpea *i.e.*, Noor-2013 were collected from Ayub Agricultural Research Institute and *desi* chickpea *i.e.*, NIAB-CH-2016 was obtained from the Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Experimental Design and Treatment Details

This study was consisted of four independent experiments conducted during 2016. The experiments were designed in completely randomized design (CRD) under factorial arrangement and repeated four times. The experiments were carried out in petri plates (150 × 90 mm) and in sand filled pots (500 g). The first two experiments were pre-optimization experiments and the Zn was applied at 5, 50, 100 and 200 mg Zn kg⁻¹ for both petri plates and sand filled pots. In experiment, conducted in petri plates, both

chickpea types seeds were uniformly placed between the layers of moist filter papers. In the second experiment, seeds of both chickpea types, were sown in plastic pots filled with sand (500 g pot⁻¹). At these Zn concentrations (50, 100 and 200 mg Zn), there was no germination in both chickpea types except for 5 mg Zn kg⁻¹ in both media (data not given).

Based on pre-optimization experiments, Zn concentration levels were modified to 5, 20, 35 and 50 mg Zn kg⁻¹ for 3rd and 4th optimization experiments. For both chickpea types, seeds at 12% moisture contents were coated using ZnSO₄.7H₂O (33%) as Zn source. In all experiments, non-coated seeds were taken as control. To adhere Zn to the seed surface, Arabic gum was used as a source. Eight seeds were sown in each petri plate or sand filled pots.

Throughout the experimentation, the petri plates were kept at temperature of 25 ± 2°C. In petri plates, seeds with 2 mm radicles (length) were counted as germinated. While in pots, plumule appearance above the sand was scored as seedling emergence. After the constant germination/emergence, the seedlings were harvested for growth traits and seedling Zn concentration determination.

Stand Establishment and Plant Growth

The experiments were visited daily to record germination/emergence count till constant scores. Mean germination/emergence time (MGT/MET) was calculated using the formula of Ellis and Roberts (1981). The emergence index/germination index (EI/GI), were calculated using the formula of Association of Official Seed Analysts (AOSA) (1983). Fifteen days after sowing, seedlings were harvested to record the seedling shoot, root length, number of secondary roots and seedling dry weight. The seedling shoot and root length was measured with measuring tap while the numbers of secondary roots were counted from the same plants to record the numbers of secondary roots. Seedling dry weight was determined by drying in oven at 70°C till constant weight.

Seedling Zinc Concentration

Roots and shoots samples were oven dried, ground, and digested in di-acid (HClO₄:HNO₃ 1:2 v/v) on a digestion plate, and Zn concentration was estimated by atomic absorption spectrophotometer (Perkin Elmer, CA, USA) following Prasad *et al.* (2006).

Statistical Analysis

The experimental data were subjected to the analysis of variance technique using statistical package Statistix 8.1 (Analytical Software, USA). The difference among treatments means were compared using least significant difference (LSD) test at 5% probability level (Steel *et al.*, 1997).

Results

Petri Dishes Experiments

Zinc seed coating with different concentrations significantly affected the germination index, mean germination time, number of secondary roots, seedling dry weight and shoot length of both (*kabuli* & *desi*) chickpea types (Table 1 and 2). Moreover, the interaction of Zn seed coating \times chickpea types was significant for root length only. However, chickpea types significantly differed for mean germination time, germination index, shoot length and numbers of secondary roots per plant (Table 1 and 2). Seed coating with 5 mg Zn reduced the mean germination time in both chickpea types (Table 1). Among chickpea types, *kabuli* chickpea took less time to germinate compared with *desi* chickpea (Table 1). Highest germination index was recorded with Zn seed coating at 5 mg in both chickpea types. Among the chickpea types, highest germination index was recorded in *kabuli* than *desi* chickpea (Table 1).

Maximum shoot length was recorded with 5 mg Zn while minimum was recorded for Zn seed coating with 50 mg Zn (Table 1). Among the chickpea types, maximum shoot length was recorded in *desi* chickpea. The maximum root length was recorded with 5 mg Zn in *desi* chickpea while; minimum was with 50 mg Zn in the same chickpea (Table 2). The maximum numbers of secondary roots per plant were recorded with 5 mg Zn and minimum were with 35 and 50 mg Zn in both chickpea types (Table 2). However, among the chickpea types, the higher numbers of secondary roots were recorded for *desi* chickpea (Table 2). The maximum seedling dry weight was recorded with seed coating of 5 mg Zn which was similar with control; while, minimum was noted with seed coating of 50 mg Zn in both chickpea types (Table 2).

The maximum seedling Zn concentration was measured with seed coating of 50 mg Zn which was toxic for both chickpea types (Fig. 2a). While at 5 mg Zn both chickpea types performed best (Fig. 2a). However, minimum seedling Zn concentration was recorded for no Zn coating in both chickpea types (Fig. 2a).

Sand Filled Pots Experiments

Zinc seed coating with different concentrations significantly affected the mean emergence time, emergence index, root length, numbers of secondary roots per plant and seedling dry weight of both chickpea types (Table 3 and 4). Zinc seed coating at 5 mg reduced the mean emergence (Table 3 and 4), had highest emergence index while minimum emergence index was observed with 50 mg Zn in both chickpea types (Table 3).

Moreover, the interaction of Zn seed coating \times chickpea types was significant for shoot length only. Maximum shoot length was recorded with seed coating of 5 mg Zn, while minimum was with seed coating of 50 mg Zn in *kabuli* chickpea (Table 3). However, chickpea types significantly differ for root length and numbers of secondary roots.

The maximum root length was recorded with seed coating of 5 mg Zn while, minimum was with seed coating of 50 mg Zn in both chickpea types (Table 4). However, among the chickpea types, the maximum root length was recorded for *desi* chickpea. The maximum numbers of secondary roots per plant were recorded with seed coating of 5 mg Zn and minimum for 50 mg Zn in both chickpea types (Table 4). Among chickpea types, more numbers of secondary roots per plant were noted for *desi* chickpea (Table 4). The highest seedling dry weight was recorded with seed coating of 5 mg Zn while, minimum was noted with seed coating of 50 mg Zn in both chickpea types (Table 4).

Correlation analysis indicated a highly positive correlation of emergence/germination index, root and shoot lengths and numbers of secondary roots with seedling dry weight of both chickpea types grown in petri plates or sand filled pots under different Zn seed coating concentrations (Table 5). However, mean emergence/germination time had highly negative association with seedling dry weight of both chickpea types, either grown in petri plates or sand filled pots, under different Zn seed coating concentrations (Table 5). The highest seedling Zn concentration was measured with seed coating of 50 mg Zn (toxic for both chickpea types Fig. 1 and 2b) while minimum was with no Zn seed coating treatment in both chickpea types (Fig. 2b).

Discussion

Zinc supply at supra and sub-optimal levels affects the plant growth due to toxicity or deficiency problems, respectively (Noulas *et al.*, 2018). In chickpea, the threshold for Zn deficiency is 25 mg kg⁻¹ (Takkur and Walker, 1993) while toxicity has not been reported. In this study, Zn seed coating concentrations were optimized for potential improvement in seedling emergence and early seedling growth of *kabuli* and *desi* chickpea types. Zinc seed coating fostered the germination/emergence and seedling growth of chickpea (Table 1 and 2). Seed coating helps in the formation of nutrients enrich layer around the emerged seedling and make easier availability of nutrient (Taylor and Herman, 1990; Sousa *et al.*, 2017) during initial stage of seedling growth; by minimizing the soil contact with nutrient and form uniform and vigorous plant stand (Sousa *et al.*, 2017). Zinc improved the root and shoot length due to its involvement in germination metabolism (Alloway, 2003; Haider *et al.*, 2018) as observed in this study (Table 1 and 2 and Fig. 1). During germination, the adequate supply of Zn in coleoptiles and radicles stimulated the germination metabolism (Ozturk *et al.*, 2006).

Seed coating with Zn improved the seedling growth (Table 1 and 2; Khan *et al.*, 2000) due to continuous supply of Zn for growth cascades (Cakmak, 2000; Palmer and Guerinot, 2009) and increase in auxin level in roots (Pandey *et al.*, 2010). Zinc augments the auxin and its ample supply regulates the growth promotion (Alloway, 2003; Prasad *et al.*, 2012).

Table 1: Effect of zinc seed coating levels on mean germination time, germination index and shoot length of chickpea types (petri plate experiment)

Zn seed coating	Mean germination time (days)			Germination index			Shoot length (cm)		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
Control	2.40	3.55	2.98 C	2.00	1.25	1.63 B	2.12	3.68	2.90 B
SC (5 mg kg ⁻¹ seed)	2.20	2.95	2.58 D	2.25	1.91	2.08 A	2.90	5.32	4.11 A
SC (20 mg kg ⁻¹ seed)	2.83	4.05	3.44 B	1.70	1.13	1.42 BC	2.39	2.63	2.51 BC
SC (35 mg kg ⁻¹ seed)	3.08	4.50	3.79 B	1.09	0.83	0.96 D	1.85	2.52	2.18 BC
SC (50 mg kg ⁻¹ seed)	3.76	4.75	4.26 A	1.54	0.67	1.10 CD	1.66	1.31	1.48 C
Mean (C)	2.86 B	3.96 A		1.72 A	1.16 B		2.18 B	3.09 A	
LSD ($p \leq 0.05$)		C=0.22; T=0.35			C=0.25; T=0.40			T=1.09; C=0.69	

Means sharing the same letter, within a column or row, did not differ significantly from each other at $p \leq 0.05$

SC=Seed coating; Mean (T)=Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 2: Effect of zinc seed coating levels on root length, number of secondary roots per plant and seedling dry weight of chickpea types (petri plate experiment)

Zn seed coating	Root length (cm)			Numbers of secondary roots per plant			Seedling dry weight (mg)		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
Control	2.68 cd	6.29 b	4.49	08	10	09 B	513	535	524 AB
SC (5 mg kg ⁻¹ seed)	4.95 b	8.45 a	6.70	10	12	11 A	645	588	616 A
SC (20 mg kg ⁻¹ seed)	3.16 c	3.12 c	3.14	08	10	09 B	403	508	455 BC
SC (35 mg kg ⁻¹ seed)	1.48 de	1.84 cde	1.66	04	06	05 C	374	476	425 BC
SC (50 mg kg ⁻¹ seed)	1.71 cde	1.03 e	1.37	05	04	04 C	362	345	353 C
Mean (C)	2.80	4.15		07 B	08 A		459	490	
LSD ($p \leq 0.05$)		T×C=1.62			T=1.67; C=1.05			T= 118	

Means sharing the same letter, within a column or row, did not differ significantly from each other at $p \leq 0.05$

SC=Seed coating; Mean (T)=Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 3: Effect of zinc seed coating levels on mean emergence time, germination index and shoot length of chickpea types (sand filled pot experiment)

Zn seed coating	Mean emergence time (days)			Emergence index			Shoot length (cm)		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
Control	3.58	4.05	3.81 B	1.30	1.43	1.36 B	28.61 ab	26.49 bcd	27.55
SC (5 mg kg ⁻¹ seed)	3.15	3.45	3.30 C	1.73	1.70	1.72 A	30.85 a	29.08 ab	29.96
SC (20 mg kg ⁻¹ seed)	4.05	3.96	4.00 B	1.23	1.55	1.39 B	24.21 de	27.10 bc	25.65
SC (35 mg kg ⁻¹ seed)	4.19	4.06	4.12 B	1.16	1.33	1.24 BC	25.59 cd	24.78 cde	25.19
SC (50 mg kg ⁻¹ seed)	4.84	4.66	4.75 A	1.07	1.03	1.05 C	17.09 f	22.27 e	19.68
Mean (C)	3.96	4.04		1.30	1.41		25.27	25.94	
LSD ($p \leq 0.05$)		T=0.38			T=0.21			C×T=2.70	

Means sharing the same letter, within a column or row, did not differ significantly from each other at $p \leq 0.05$

SC=Seed coating; Mean (T)=Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 4: Effect of zinc seed coating levels on root length, numbers of secondary roots per plant and seedling dry weight of chickpea types (sand filled pot experiment)

Zn application	Root length (cm)			Numbers of secondary roots			Seedling dry weight (mg)		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
Control	15.15	17.30	16.22 B	16	18	17 B	585	523	554 BC
SC (5 mg kg ⁻¹ seed)	17.42	21.30	19.36 A	19	24	22 A	643	573	608 A
SC (20 mg kg ⁻¹ seed)	16.51	20.56	18.53 A	16	22	19 AB	579	558	568 AB
SC (35 mg kg ⁻¹ seed)	14.87	16.52	15.70 B	16	21	18 B	533	555	544 BC
SC (50 mg kg ⁻¹ seed)	13.75	16.15	14.95 B	15	18	16 B	488	528	508 C
Mean (C)	15.54 B	18.37 A		16 B	20 A		565	547	
LSD ($p \leq 0.05$)		C= 1.19; T=1.89			C=1.89; T= 2.99			T=55.38	

Means sharing the same letter, within a column or row, did not differ significantly from each other at $p \leq 0.05$

SC=Seed coating; Mean (T)=Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

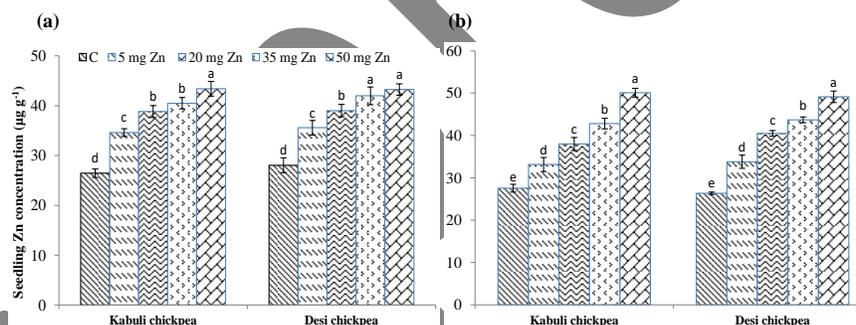
Improvement in seedling dry weight of both chickpea types with different Zn seed coating concentrations might be due to significant improvement in emergence/germination index, root and shoot lengths, and number of secondary roots

(Table 5). Nonetheless, this improvement in seedling dry weight was also linked with significant decrease in mean emergence/germination time of both chickpea types under varying Zn seed coating concentrations (Table 5).

Table 5: Correlation of some important stand establishment and growth traits with seedling dry weight of chickpea types in petri plate and sand filled pot experiments (n=4)

Variables	Petri plate experiment		Sand filled pot experiment	
	<i>kabuli</i> chickpea	<i>desi</i> chickpea	<i>kabuli</i> chickpea	<i>desi</i> chickpea
Mean emergence time (days)	-0.86**	-0.90**	-0.97**	-0.77*
Germination index	0.87**	0.87**	0.91**	0.70*
Shoot length (cm)	0.85**	0.92**	0.90**	0.65*
Root length (cm)	0.89**	0.86**	0.93**	0.75*
Numbers of secondary roots	0.88**	0.94**	0.96**	0.99**

** = Significant at $p \leq 0.01$; * = Significant at $p \leq 0.05$

**Fig. 1:** Influence of different Zn seed coating concentrations (5, 20, 35 and 50 mg kg⁻¹ seed) on the seedling root growth of chickpea types (*kabuli* and *Desi*) in sand filled pots**Fig. 2:** Influence of different Zn seed coating concentrations (5; 20; 35 and 50 mg kg⁻¹) in desi and kabuli chickpea seedling; (a) Petri plate experiment, (b) Sand filled pots; columns show the mean value while bars show standard deviation. Columns labeled with different alphabets show significant differences among among different Zn seed coating concentrations at ($p \leq 0.05$)

Desi chickpea performed better in terms of growth and development than the *kabuli* chickpea (Table 1 and 2) possibly due to greater trehalose accumulation and better germination metabolism (Farooq *et al.*, 2018b). Application of Zn at higher concentration caused delay and impeded the germination of chickpea (Table 1 and 2 and Fig. 1) possibly due to decrease in gibberellic acid and zeatin in the germinating chickpea seeds (Atici *et al.*, 2005). Zinc seed coating at higher concentrations (35 and 50 mg Zn kg⁻¹) caused decrease in seedling dry weight up to 48%, root length up to 227% and shoot length up to 95% (Table 1 and 2). This reduction in biomass at high Zn level may be attributed to limited cell division and elongation (Jain *et al.*, 2010; Glińska *et al.*, 2016).

et al., 2010; Glińska *et al.*, 2016).

Zinc supply at above optimum level behaves like toxic heavy metals, and suppresses seed germination (Ali *et al.*, 2000; Herrero *et al.*, 2003) and plant growth (Ali *et al.*, 1999, 2000) as metabolic functions linked with proper plant development are interloped with excessive Zn (Wierzbicka and Obidzinska, 1998; Gadallah and El-Enany, 1999). The heavy metals when supplied at high concentrations disturb the physiological and biochemical processes including damage to biological membranes and photosynthetic apparatus, and decrease in the activities of vital enzymes (Talanova *et al.*, 2000; Monni *et al.*, 2001; Atici *et al.*, 2003).

Conclusion

Zinc seed coating at 5 mg kg⁻¹ improved the chickpea germination and seedling growth. However, application of Zn above the 5 mg kg⁻¹ suppressed the chickpea germination/emergence and seedling growth which showed the Zn toxicity. Therefore, recommended seed coating dose of Zn should be investigated in field to observe its impact on yield and grain Zn biofortification in further studies.

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