

Effect of Copper Sulfate and Salt Stress on Seed Germination and Proline Content of Psyllium (*Plantago psyllium*)

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Abstract: In order to study, the effects of copper sulfate and salt stress on germination indices of *plantago psyllium* an experiment in factorial-laid out complete randomized design with three replications was conducted at Seed Technology Laboratory, Faculty of Agriculture, Mashhad Branch, Islamic Azad University-Mashhad Iran, in 2012. Factors were included of copper sulfate in five levels (0, 15, 30, 45 and 60 mg/lit) and osmotic potential in five levels (0,-0/4,-0/6,-0/8 and -1 M pa) obtained by Na cl. The results showed that, highest germination percentage, germination rate and hypo cotyle length obtained in 45 mg/lit of copper sulfate. But root length decreased when copper sulfate concentration increased. Also, percentage and rate of germination and seed vigor decreased when osmotic potential decreased. The highest proline content were obtained in 60 mg / lit copper sulfate concentration and -1 MPa osmotic potential and the lowest rate was obtained in control treatment. The results of this experiment showed that when salt stress and copper sulfate increased, the rate of proline accumulation increased in seedling.

Key words: Germination percentage • Germination rate • Seed vigor

INTRODUCTION

Adverse environmental conditions, such as high salinity, high alkalinity, the presence of heavy metals and drought limit plant growth and drastically reduced plant productivity [1].

Salt stress unfavorably affected plant growth and productivity during all development stages. For example salinity decreased seed germination, retards plant development and reduces crop yield [2-4].

Seed germination is a crucial stage in the life history of plants and salt tolerance during germination is critical for the stand establishment of plants that grow saline soils [2]. In many plant species, seed germination and early seedling growth are the most sensitive stage to salinity stress. Salt stress negatively affected seed germination; either osmotically through reduced water absorption or ionically through the accumulation of Na⁺ and Cl⁻, causing an imbalance nutrient uptake and toxicity effect [4].

Metal pollution is continuously increasing and root case is the anthropogenic behavior which interferes with the environmental activities and make conditions toxic for living organism. Many studies have shown that under heavy metal stress the water transport in plant decreased and resulted in water deficit in shoots [5, 6].

Copper is widely prevalent in our environment and was considered as an essential element for living organisms including plants. The nutritional studies have demonstrated that copper and other metals are essential for optimal growth of plants and animals [6]. Although copper is essential element for growth and development of plants, but in excessive concentration can cause oxidative stress and affected numerous physiological activities directly and indirectly, such as growth and photosynthesis. Copper reduces plant growth by preventing absorption other essential elements particularly Fe, K and Ca [7].

Proline is a compatible osmolyte and is known to accumulate in response to various kinds of abiotic stress, such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals [8-11]. Proline is accumulated in cytosol and contributes to osmotic adjustment in response to dehydration stress and tends to maintain sufficient cell turgor for growth and avoid dehydration [1, 8]. So, Proline protects folded protein structures against denaturation which may be caused by stress and stabilizes cell membranes [9,12].

Psyllium (*Plantago psyllium*) is one of the medicinal plant belong to *Plantaginacea* family. The importance of this plant is to mucilage seed and seed coat, that in the medical and cosmetic industries have wide applications [13]. Recent research also indicates that psyllium fiber is effective to reduce cholesterol, fat and sugar of blood [14].

Therefore, this research aimed to study the effects of copper sulfate and salt stress on seed germination indices and proline content of seedling was applied.

MATERIALS AND METHODS

This research was conducted in Seed Technology Laboratory, Faculty of Agriculture, Islamic Azad University, Mashhad Branch, in 2012. The experiment arranged in factorial laid out in completely randomized design with three replications. Factors including: Copper sulfate in five levels (0, 15, 30, 45 and 60 mg/l) and osmotic potential in five levels (0, -0.4, -0.6, -0.8 and -1) obtained by NaCl. Psyllium seeds have been provided from seed production and distribution centers. Seeds were disinfected with sodium hypochlorite 10% for 3 min and rinsed with distilled water.

25 seeds placed in each Petri dish on filter paper. Then 5 ml of the prepared solution was added to each Petri dish. All Petri dishes were placed in germinator at 25°C.

Germination was continued for 10 days and germinated seeds were counted on a daily. Seeds were considered germinated when their radical length was 2 mm.

After 10 days, traits such as: percentage and rate of germination, Radical and Hypocotyl length, seed vigor and proline were measured. Proline was measured according to the Bates (1973) guideline [15].

First, the required solutions such as: Phosphoric acid (6M), Sulphosalicylic acid (3%), Ninhydrin solution and Toluene were prepared. All solutions were stored on 4°C until proline measurement.

Then 10 ml of Sulphosalicylic acid was added to 0.04g of dry powder of each sample. And after 24 hours it was filtered with using filter paper. 1 ml of this solution was poured into the test tube and 1 ml of Ninhydrin solution and 1 ml of Acetic acid (glacial) was added. The test tubes were placed in boiling water bath (100°C) for one hour, until color fixed. Then they were placed in cold water bath for 15 minute. After, the test tubes were cooled completely; and 2 ml of Toluene was added. The test tubes were shaken. With this, solution became two phase. The solution of color phase, containing Toluene. This solution was used to measure proline with Spectrophotometer at 520 nm wavelength.

Data were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test at 5% probability (for comparison the treatment means) and using MSTAT-C.

RESULTS

Analysis of variance shown in Table 1.

Percentage and Rate of Germination: Copper sulfate and osmotic potential had significant effect on germination percentage and germination rate at 1% probability.

The means comparison showed that the highest and lowest germination percentage were obtained at 45 mg/l copper sulfate concentration and control (0 mg/l) respectively 78% and 69%. But had no significant effect between 45 and 60 mg/l copper sulfate concentration (Table 2).

Evaluation of these results indicated that increasing copper sulfate concentration to 60 mg/l had not negative effect on germination percentage. Germination percentage significantly reduced at the highest level of salt (Osmotic potential -1 MPa). But the difference was not significant among control and the other osmotic potential (Except -1 MPa) (Table 3).

The highest and lowest germination rate were obtained at 45 mg/l copper sulfate concentration and control respectively 11 and 9 seedling / day (Table 2).

This results indicated that increasing copper sulfate concentration up to 45 mg/l led to increased germination rate. But, with the increasing concentration up to 60 mg/l germination rate was reduced.

Evaluation of germination rate at the various levels of osmotic potential up to -0.8 MPa had not significant effect on this trait. The lowest germination rate (8 seedling / day) was obtained in -1 MPa. The difference was significant between -1 MPa and the other osmotic potentials (Table 3).

Table 1: Analysis of variance of the measured traits

S.O.V	df	M.S					
		Germination percentage	Germination rate	Radicle length	Hypocotyl length	Seed vigor	Proline
Copper sulfate (A)	4	252.05**	9.06**	6.03**	1.41**	4131.4ns	0.044**
Osmotic potential (B)	4	380.4**	16.8**	0.38ns	0.03ns	6058.3*	0.072**
A × B	16	71.98ns	1.9ns	0.42ns	0.21ns	2394.19ns	0.002**
Error	50	48.05	1.9	0.33	0.12	2126.9	0.000

ns,*,** non significant, significant at 5% and 1% probability, respectively.

Table 2: Effect of copper sulfate on germination percentage, germination rate, Radicle and Hypocotyl length and proline

Copper sulfate (mg/lit)	Germination percentage (%)	Germination rate	Radicle length (Cm)	Hypocotyl length (Cm)	Proline (μmol/gdw)
0	69.2c	9.0c	2.58a	2.9c	0.32d
15	72.6bc	9.7bc	1.76b	2.9c	.038c
30	70c	9.4bc	1.58b	3.24b	0.42b
45	78a	11.0a	1.15c	3.52a	0.42b
60	76ab	10.3ab	0.95c	3.5a	0.46a

Means with similar letters in each column are not significant at the 5% level of probability.

Table 3: Effect of osmotic potential on germination percentage, germination rate, seed vigor and proline

Osmotic potential (MPa)	Germination percentage (%)	Germination rate	Seed vigor	Proline (μmol/gdw)
0	75.7a	10.6a	364.6a	0.3e
-0.4	76.5a	10.7a	381.4a	0.37d
-0.6	76.5a	10.3a	357.9ab	0.41c
-0.8	73.7a	9.7a	342.2b	0.43b
-1	64.6b	8.1b	329.4b	0.49a

Means with similar letters in each column are not significant at the 5% level of probability.

Table 4: Effect of copper sulfate and osmotic potential on proline

Copper sulfate (mg/lit)	Osmotic potential (MPa)				
	0	-0.4	-0.6	-0.8	-1
0	0.29kl	0.23m	0.28l	0.34j	0.34j
15	0.37i	0.25m	0.38hi	0.40fg	0.39gh
30	0.41fg	0.30k	0.40fg	0.41def	0.42cde
45	0.43cd	0.35j	0.41efg	0.44bc	0.43cd
60	0.44bc	0.45b	0.44bc	0.48a	0.49a

Means with similar letters are not significant at the 5% level of probability.

Radicle and Hypocotyl: Copper sulfate had significant effect on length of Radicle and Hypocotyl at 1% probability, but the effect of osmotic potential was not significant.

Increasing copper sulfate concentration reduced Radicle length. The highest and lowest Radicle length were obtained at control and 60 mg/l copper sulfate concentration respectively 2.58 and 0.95 Cm (Table 2).

Increasing copper sulfate concentrations had not negative effect on Hypocotyl. So, the highest Hypocotyl length was obtained at 45 mg/l. But difference was not significant between 45 and 60 mg/l (Table 2).

Seed Vigor: Effect of copper sulfate on seed vigor was not significant, but osmotic potential affected seed vigor significantly at 1% probability.

Decreasing osmotic potential reduced seed vigor. The lowest seed vigor (399) was obtained at osmotic potential (-1 MPa) (Table 3).

Proline: The effect of copper sulfate and osmotic potential was significant on proline content at 1% probability.

Increasing copper sulfate concentration and decreasing of osmotic potential increased proline content. The highest proline content was obtained at the 60 mg/l copper sulfate concentration and -1 MPa of osmotic potential respectively 0.46 and 0.49 $\mu\text{mol.g}^{-1}\text{dw}$ (Tables 3 and 4).

Evaluation, the interaction effects of copper sulfate \times osmotic potential indicated that the highest proline content obtained at the 60 mg/l copper sulfate and -1 MPa osmotic potential (Table 4).

DISCUSSION

The results of this study indicated that increasing copper sulfate concentration up to 60 mg/l increased all germination indices and proline content expects Radicle length. These results are consistent with Muler *et al*, [13], These researchers observed seed germination and seedling shoot growth of *Typha latifolia* were not significantly affected by copper concentrations (up to 402 $\mu\text{g/l}$) as compared to control [13]. While, Singh *et al*, [16], observed a significant reducing effect in wheat (*T. aestivum*) germination, plumule length, Radicle length, number of lateral roots, fresh weight and dry weight with increased copper concentration. Only a slight increased was observed in germination percentage in 5 mg/l of copper concentration [16].

The effect of salt stress (Osmotic potential) indicated that increasing of salt stress decreased all germination indices. But this increasing started at -1 MPa osmotic potential. Salt induced inhibition of seed germination could be attributed to osmotic stress or specific ion toxicity (1).

These results showed that, psyllium is relative resistance to salinity in germination stage. The reducing of germination indices observed in many studies. Such as, Kaya and Ozturk, (2003), in the safflower, Shon *et al*, (2005), in the rice and Akbari *et al*, (2007), in the wheat [2-4].

Increasing copper sulfate concentration and salt stress increased proline accumulation. These results consistent with many studies. So, Cha-Um and

Kirdmanee, [16] reported that, the proline content the osmotic-stressed leaves of eucalyptus increased significantly [16]. Ku *et al*, [1], observed that, the excess of Cu increased proline content to drought, salinity and heavy metals stresses, many plant species accumulate high levels of proline, which is thought to function in stress adaptation [16-19].

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