Korean J. Crop Sci. 44(2):163~165(1999)

Zinc Chloride Toxicity on Free Proline and Organic Acids in Germinating Rice Seed

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ABSTRACT

The study was conducted to find the critical concentrations of zinc toxicity and to determine the changes of the contents of free proline and organic acids with treatment of different zinc chloride concentrations during rice germination and seedlings grown for seven days. The concentration of zinc chloride, 140 ppm, inhibited root elongation as much as 46 times compared with the control, and the germination rate was also decreased in all treatments of zinc chloride, showing that the germination rate decreased more with increasing conentrations of zinc chloride. Its rate was only 13% with treatment of 140 ppm zinc chloride. The content of free proline with trea tment of zinc chloride, 140 ppm, was highest about 4,873 μM at 3 days compared with the control. Malic acid concentration with treatment of zinc chloride, 140 ppm, increased to approximately 4 times compared to the control. Citric and succinic acid content were also slightly increased in all treatments of zinc chloride.

Keywords : rice seedling, $ZnCl_2$ toxicity, chlorophyll, free proline, organic acid.

The average zinc content of the lithosphere is about 80 ppm (Goldschmidt, 1954). In soil, it is usually present in the range of 10~300 ppm occurring in a number of different minerals. When the zinc supply is large, zinc toxicity can readily be induced in non-tolerant plants, an inhibition of root elongation is a very sensitive parameter (Godbold et al., 1983; Ruano et al., 1988). Quite often, zinc toxicity leads to chlorosis in young leaves. Zinc toxicity may occur in areas particularly in the neighbourhood of zinc ore deposits and spoil heaps. However, some plants are zinc tolerant and are able to grow in soils, which is abnormally high in zinc. For example, Antonovics et al. (1971) reported that zinc level ranged from 600 to 7,800 ppm based on the dry matter of tolerant plants, growing on calamine soils. Generally, concentrations of 150 to 200 $\mu g\ Zn\ g^{-1}$ based on the dry matter of plant tissue are considered as toxic (Sauerbeck, 1982). The objective of this research is to find growth responses, related to free proline content and organic acids with treatments of different concentrations of zinc chloride in germination and early seedling stages of rice.

MATERIALS AND METHODS

One rice variety (*Oryza sativa* L. cv Ilpumbyeo) seeds were sterilized with 0.1% diluted emulsificable chemicals (Spotak) for 24 hr, and then throughly washed with clear distilled water. Fifty rice seeds of each treatment were grown with four different concentrations of zinc chloride, 0, 100, 120 and 140 ppm for seven days under controlled environmental conditions, which are maintained temperature of 30 ± 1℃, relative humidity of 75 ± 1%, and photon flux density of 400 $\mu\ mol\ m^{-2}s^{-1}$. Thirty plants of each treatment harvested at one, three, and five days after germination were used to determine the free proline content. The samples were also collected at three days to determine organic acid content after the treatments of zinc chloride. Statistical data analysis was performed with MYSTAT PC package.

Analysis of total chlorophyll content

Five g of fresh leaves was cut into pieces and the fresh tissue was put into a mortar, and crushed with 80% acetone. The homogenate was filtered through Whatman No. 2 filter paper and the decant was put into a 100 ml volumetric flask. The chlorophyll content was measured through the absorbance read at 663 and 645 nm (Cock et al., 1976).

Analysis of free proline

10 g of fresh weight of plant was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper, 2 ml of homogenate was reacted with 5 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100℃. The reaction was stopped in an ice bath, the reaction mixture was extracted with 8 ml toluene, mixed vigorously with a test tube stirrer for 20 sec., and chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm(Bates, 1973).

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Analysis of organic acid

10g of fresh weight of plants was cut off, rinsed with distilled water throughly, dried with a paper towel, weighed and immediately frozen in liquid nitrogen, and stored at -72℃ before the analysis of organic acids. The frozen whole plants were ground in a mortar with cold 70 % ethanol.

The mixture was centrifuged at 4,000 rpm for 15 min., and pellet was extracted twice with boiling water. The supernatant from each of these extractions was transferred to a rotary evaporator and concentrated at 35℃ under reduced vacuum. The dried residues were dissolved in deionized water and filtered through a membrane filter (0.2 ㎛). Concentrations of malic, citric and succinic acid were measured by ion chrotography (Dionex/SP). Each sample of the treatments was analyzed twice with 3 replicates. The eluant was 2.3 mM heptaflurobutyric acid (pH 2.6 adjusted with 1N NaOH) at a flow rate of 0.3 ml min^{-1} and the reagent solution was 5 mM tetrabutylammonium hydroxide at a flow rate of 10 ml min^{-1} (Yang et al. 1994).

RESULTS AND DISCUSSION

Early growth response and chlorophyll content

Growth characters of plant height, root length, germination rate, and chlorophyll content with treatment of zinc chloride is shown in Table 1. As zinc chloride concentration increased, plant height was significantly decreased compared with the control, and the highest concentration of zinc chloride, 140 ppm, inhibited root elongation as much as 46 times, and the germination rate was also decreased in all treatments of zinc chloride. Particularly, the germination rate was only 13% with treatment of zinc chloride (140 ppm). This result was similar to the result of Kim et al. (1997) reported that zinc chloride treatment of 100 ppm in rice seedlings decreased the root length and germination rate compared with the control.

Changes of free proline content

Extensive research in this area has revealed proline accumulation as a universal response of plants under diverse stress. Generally, proline accumulation due to water, heavy metal and salt stress results from a simulated synthesis in the tissue, an inhibited oxidation or an impaired incorporation of proline into proteins. A tremendous free proline accumulation (up to 100 times of the control) is one of the most dramatic stress characteristics, it has been used as a single parameter to determine physiological stress. Changes of free proline content under different zinc chloride treatment during seed germination are shown in Table 2. Compared with the control, when zinc chloride concentration is increased, free proline content was slightly increased during germination and early rice seedlings. At three days, there was totally higher increments in all zinc chloride treatments including the control. Particularly, compared with the control, the free proline content of zinc chloride treatment (140 ppm) at 3 days was highest as 4,873 μM On the contrary, Nyan et al. (1984) reported that free proline content increased in 5-day-old rice seedlings subjected to salt treatments. According to two experiments, we could suggest that different stress inducers (salts and heavy metals, zinc chloride) could affect the differences in free proline content at early growth stages.

Changes of three organic acid content

In long-distance transport of the xylem, zinc is either bound to organic acids or occurs as the free divalent cation. In this experiment, malic acid, one of the three organic acids, was considerably increased with all zinc chloride treatments (Table 3). Compared with the control, malic acid concentration in zinc chloride (140 ppm) increased by approximately 4 times, and citric and succinic acid content were also slightly increased in all treatments of zinc chloride. We observed that contents of three organic acids and free proline at only 3 days after the treatments of zinc

Table 1. Effects of zinc chloride on plant height, root length, germination rate and chlorophyll content in rice cultivar, Ilpumbyeo.

$ZnCl_2$ concentration (ppm)	Plant height† (cm)	Root length† (cm)	Germination rate† (%)	Chlorophyll content† (mg/g fresh wt.)
Control	3.4a¶	4.6a¶	93a	1.38a
100	2.8b	2.6b	68b	1.06b
120	2.0c	0.3c	32c	0.45c
140	1.1d	0.1c	13d	0.27d

†Measured at the seven days after treatment of zinc chloride solution.
¶Same letters are not significantly different at the 5% level by DMRT.

Table 2. Effects of zinc chloride on free proline content in rice cultivar, Ilpumbyeo.

$ZnCl_2$ concentration (ppm)	proline content(μ M/10g fresh wt.)		
	1 DAT[†]	3 DAT	5 DAT
Control	2,345Free	2,232d[¶]	2,187
100	2,873	3,286c	3,021
120	3,449	4,678b	3,320
140	3,592	4,873a	3,641

[†]Days after treatment of zinc chloride solution, respectively.
[¶]Same letters are not significantly different at the 1% level by DMRT.

Table 3. Effects of zinc chloride on three organic acid content in rice cultivar, Ilpumbyeo.

$ZnCl_2$ concentration (ppm)	Organic acid content(μ M/10g fresh wt.)[†]		
	Citric acid	Malic acid	Succinic acid
Control	3.3	23.8d[¶]	2.3
100	6.8	88.3c	4.6
120	7.1	91.5b	5.1
140	7.5	103.7a	5.3

[†]Measured at the three days after treatment of zinc chloride solution.
[¶]Same letters are not significantly different at the 1% level by DMRT.

chloride simultaneously increased in all treatments of zinc chloride, except for the control. According to the above results, it was suggested that the zinc chloride treatment above 100 ppm would induce the dramatic increment of the both organic acid and free proline contents at 3 days after the treatments of zinc chloride. These biochemical components, organic acid and free proline are also attributed to tolerance of rice seedlings.

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