



### Full Length Article

## Effects of Different Zn Supply Levels on Physiological Parameters, Seed Zn Concentration and Root Cell Ultrastructure in three Different Genotypes of *Brassica* Species

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### Abstract

Crop Zn content is different between various crop species. The purpose of the present study was to determine Zn response in seed of three species of *Brassica* (*B. napus*, *B. juncea* and *B. campestris*) when exposed to different Zn supply levels (0, 1, 5, 10 and 20 mg kg<sup>-1</sup>) in pot experiment. The results showed that the activities of antioxidant enzymes, net photosynthetic rate (P<sub>n</sub>), stomatal conductance (G<sub>s</sub>), transpiration rate (T<sub>r</sub>) in leaf, and dry weights of plant, straw, leaf and seed increased at CZn<sup>2+</sup> ≤ 5 mg kg<sup>-1</sup>. The highest seed dry weight of three species were observed in 5 mg kg<sup>-1</sup> Zn treatment, increased by 37.7%, 82.6% and 18.0% compared to the controls, respectively. The ultrastructural damage was found in the root tip cells for *B. napus* and YR in the presence of 20 mg kg<sup>-1</sup> Zn, while intact structure of the root tip cells was observed in *B. campestris*. The total amino acid content of root exudates in *B. juncea* and *B. napus* was significantly higher than that of *B. campestris* in the presence of 1 and 20 mg kg<sup>-1</sup> Zn. High supply level of Zn<sup>2+</sup> (20 mg kg<sup>-1</sup>) could promote the secretion of amino acids in root exudates. Dry weight (stem, leaf and seed) of *B. napus* reveal a decreasing trend when Zn supplied at levels above 10 mg kg<sup>-1</sup>. As the increase of Zn supply level, the concentrations of Zn in seed of rape revealed an increasing trend. The best Zn supply level for having more biomass and more Zn in seeds of rape without toxic effects was observed at 10 mg kg<sup>-1</sup>. The highest of Zn concentration in seed were observed in *B. napus* may be due to more root exudates. Zinc concentration in Zn-high-efficiency species is related to the absorption capacity of the plant's roots and the ability to change soil Zn availability © 2017 Friends Science Publishers

**Keywords:** Seed Zn concentration; Physiological characteristics; Root exudates; Rape species; Ultrastructure of root cell

### Introduction

Zinc (Zn) is an essential nutrient in plants, animals and humans, and plays a fundamental role in several critical functions in protein metabolism, gene expression, structural and functional integrity of biomembranes and photosynthetic carbon metabolism of plant as a cofactor for more than 300 enzymes in the human body (Poblaciones and Rengel, 2016). In humans, Zn deficiency is associated with severe health complications, including impairments of physical growth, learning ability an immune system, and increased risk of infections, DNA damage and cancer development (Levenson and Morris, 2011). However, more than 30% of the world's population is Zn deficient, with Zn deficiency being the 11<sup>th</sup> most important factor causing disease or death in the world, and the 5<sup>th</sup> most important factor in developing countries (WHO, 2011). In China, nutrition and food hygiene data reported by Academy of Preventive Medicine Institute show that zinc daily intakes of 60% of China children are less than half of WHO recommended amount, due to seventy-five percent energy

of Chinese come from rice, wheat and corn, whose zinc concentrations are only 10–30 mg kg<sup>-1</sup> (Jiang *et al.*, 2009).

Food consumption provides the principal route of Zn supply in most human populations. A diet consisting of a high proportion of cereal-based food with low Zn contents is considered one of the major reasons for the widespread occurrence of Zn deficiency in humans, especially in developing countries (Alloway, 2008). In Zn-deficient conditions, agronomic biofortification has proved to be an effective and fast solution to increasing Zn concentration in the edible parts of several crops (Ghasemi *et al.*, 2013; Gomez-Coronado *et al.*, 2015). Biofortification via Zn application has been confirmed to be effective in increasing Zn concentration in crops grown in either Zn-sufficient or Zn-deficient soils (Poblaciones and Rengel, 2016).

Zinc content in grain or seed is different between species and species of plant, and soil types (Dong *et al.*, 1995; Erenoglu *et al.*, 2001; Coolong and Randle, 2003; Wang and Jin, 2005). *Brassica* (*Brassica campestris*, *Brassica napu* and *Brassica juncea*) is one of the four main oil crops in China (including rape, soybean, peanut and

sesame), and the main edible plant oil for Chinese. But little investigation was reported about the physiological mechanisms of different seed Zn concentration in different species of *Brassica*. The purpose of this study was to determine Zn response and Zn accumulation in seed of three species (*Brassica campestris*, *Brassica napus* and *Brassica juncea*) when exposed to different Zn supply levels. Understanding the range of genotypic variation in seed Zn accumulation and response to Zn will provide a basis for micronutrient studies.

## Materials and Methods

### Plant Material, Soil and Heavy Metal Treatments

*Brassica campestris* (Nantong, Jiangsu, China), *Brassica napus* (H33, France) and *Brassica juncea* (Luoping, Yunnan, China) seeds were provided by the Oil Crops Research Institute (OCRI) of the Chinese Academy of Agricultural Sciences (CAAS). The soil collected from the town of Jiulongpo, Chongqing, China, was characterized as having a pH of 6.9 (soil: water = 1:2.5), an organic matter and total N content of 33.3 and 1.21 g kg<sup>-1</sup>. Available N, P, K and Zn were 110.8, 10.6, 104.6 and 1.9 mg kg<sup>-1</sup>, respectively. Soil cation exchange capacity (CEC) was 207 mmol kg<sup>-1</sup>.

The pot experiment was conducted with Zn supply levels of 0 (the control), 1, 5, 10 and 20 mg kg<sup>-1</sup> (ZnSO<sub>4</sub>·7H<sub>2</sub>O). Soil (5.0 kg) was mixed with the designated amounts of Zn and fertilizers containing 150 mg kg<sup>-1</sup> N as urea, 100 mg kg<sup>-1</sup> P as KH<sub>2</sub>PO<sub>4</sub> and 150 mg kg<sup>-1</sup> K as KCl. A root-bag technique was used to separate the rhizosphere from bulk soil. The root-bags (12 cm in depth and 16 cm in diameter) made of 500-mesh nylon screen, were filled with 200 g soil as rhizosphere, and then buried in the centre of a plastic pot (30 cm in depth, 28 cm in diameter) containing 4.90 kg bulk soil (Xu *et al.*, 2007).

Two uniform seedlings (3 cm in root length, 10 cm in seedling height) were transplanted into the root-bag of each pot under growth chamber conditions at a day/night cycle: 16 h, 30±2°C/8 h, 25±2°C and 75% relative humidity. The experimental design was completely randomized, and each treatment was replicated four times. Distilled water was added daily to maintain soil moisture to field capacity level. After 90 days.

The youngest and the second youngest fully opened leaves of one pot of each treatment were cut for analysis of photosynthesis and activities of antioxidant enzymes. A replication was used for the determination of root tip cell ultrastructure and characteristics of root exudates. All remaining plants were harvested at maturity, and washed thoroughly with distilled water, separated into roots, shoots and seeds, oven-dried at 65°C for 72 h. Dry weights of roots, shoots and seeds were recorded. The concentrations of Zn in straw, roots and seeds were determined.

### Analyses of Enzyme, Proline and Malondialdehyde

The activity of catalase (CAT) was measured using a microtiter plate assay (Hansen *et al.*, 2006). The activity of peroxidase (POD) was determined according to Nakano and Asada (1981). The activity of superoxide dismutase (SOD) was determined by the method of Minami and Yoshikawa (1979). Proline was extracted and determined by the method of Bates *et al.* (1973). The MDA assay was determined by the method of Bird *et al.* (1983).

### Photosynthesis Characteristics Analyses

Gas exchange was measured between 10.00 and 12.00 local time. Measurements of net photosynthetic rate (P<sub>N</sub>), transpiration rate (T<sub>r</sub>), stomatal conductance (G<sub>s</sub>) and CO<sub>2</sub> concentration (C<sub>i</sub>) were carried out with LiCor-6400 Photosynthetic gas analysis system (Licor Inc. Lincoln, Nebraska, USA).

### Collection and Analyses of Root Exudates

After ninety days of cultivation, the whole root systems of intact plants were removed gently from root-bags. To collect the root exudates, all plants were placed into sterile 500 mL glass container covered with aluminum foil paper to avoid contamination and light of sterile aerated de-ionized water. Each rape was cultured in triplicate and each repetition contained 2 rape seedlings. Plants were maintained in a biochemical incubator at 30°C for 24 h to collect root exudates. The collected root exudates were filtered by a double layer of Whatman no. 1 filter paper, and immediately frozen at -30°C, freeze-dried, and weighed. The lyophilized powder of root exudates was dissolved in deionized water to make concentrated extracts 1 g·L<sup>-1</sup> for the bioassay and the analysis of the composition of organic components. The LMW organic acids were measured by a GBC-HPLC instrument (LC-1140 system organiser, LC-1150 pump, LC-1206 multiwave-length UV/VIS detector) using reverse-phase BST Nucleosil C-18 (250×4 mm, 5 µm particle size) column and a BST Nucleosil C-18 (30×4 mm, 5 µm particle size) guard column. Column effluents were monitored at 210 nm at a flow rate of 0.5 mL min<sup>-1</sup>. The mobile phase was a buffer solution containing 0.01 M KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.2 with H<sub>3</sub>PO<sub>4</sub> solution and was filtered using a Nalgene 0.45-µm nylon membrane filter. The injection volume was 20 µL, and each treatment was analyzed in triplicates. A multilevel calibration method with daily prepared standard solutions was used for quantitative determination of the LMW organic acids (Tolrà *et al.*, 1996). The concentrations of amino acids were determined by a PICO-TAG (Water Ltd, USA).

### Analyses of Root Tip Cell Ultrastructure

For electron-microscopic analyses, five roots per each treatment were fixed in 2% glutaraldehyde in 0.1 M

cacodylate buffer pH 7.5 for 2 h at 4°C, then rinsed with the same buffer and postfixed in 1% osmium tetroxide for 2 h. After dehydration in a graded ethanol series, they were embedded in Epon-Spurr's resin mixture. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined in a Jeol 1010 TEM at 80 kV and documentation was made on a photographic film for electron microscopy (Hitachi-600, Hitachi Ltd, Japan).

The ultrastructure of meristematic cells of Zn-treated plants was observed and the number of altered mitochondria, Golgi apparatus and vacuoles (expressed as the percentage of the total number of those organelles at the analyzed microphotographs) as well the frequency of circular ER and multivesicular bodies (expressed as the percentage of the analyzed cell profiles, in which those structures occurred) were determined on at least 100 micrographs (20 per root) from each series (Glińska and Gabara, 2000).

### Analyses of Plant Zn Concentration and Soil Physico-chemical Properties

Dried plant samples were ground and digested with concentrated HNO<sub>3</sub>-HClO<sub>4</sub>. Available Zn was extracted with diethylene-triaminepentaacetic acid (DTPA). Zinc concentrations in the solution were analyzed using a flame atomic absorption spectrometer (AAC) (Perkin Elmer SIMMA 6000, Norwalk, CT) (Sharma *et al.*, 2004). For quality assurance, the National Institute of Standards and Technology reference plant materials (GBW # 08513) and soils (GBW # 08303) were used to check the efficiency of the digestion/extraction procedures and FAAS measurements. For all plant and soil samples, the recovery rate of Zn was higher than 95%, and the relative standard deviation (RSD) of the precision was within 10%. Soil pH (soil: water = 1:5) was measured with a digital acidometer (pHS-4C, Japan). Cation exchange capacity and extractable P of soil were determined by the method of Rayment and Higginson (1992). Organic matter content was determined using the modified Walkley and Black method (dichromate oxidation and titration; McCleod, 1975).

### Statistical Analysis

The statistical analysis was performed using the SPSS 21.0 software. The effects of Zn supply level on accumulation of Zn, and physiological characterization for different rape species were subjected to a two-way analysis of variance (ANOVA i.e., species and Zn supply levels), followed by the least significant difference test ( $p = 0.05$ ).

## Results

### Biomass

Significant differences of dry weights for roots, stems, leaves and seeds were found among three rape species, as

well as Zn supply levels (Figs. 1–4). Meanwhile, a significant difference of interaction between Zn supply level and species was also observed. As the increase of Zn supply levels, the dry weights of roots, stems, leaves and seeds as well as total dry weight of three rape species firstly increased, and then decreased. The maximum weights of stems, leaves, roots and seeds, as well as total dry weight of *B. napus* and *B. campestris* were determined at Zn supply level of 5.0 mg kg<sup>-1</sup>, which exhibited the enhancement by 14.3%, 75.3%, 79.1%, 37.7% and 1.4% (*B. napus*), and 14.9%, 64.4%, 48.1%, 18.0% and 20.5% (*B. campestris*) for both rape species compared to the control, respectively. The maximum dry weights of stems, leaves, seeds, as well as total dry weight from *B. juncea* were found at Zn supply level of 5.0 mg kg<sup>-1</sup> with the enhancement by 33.9%, 117.2%, 82.5% and 51.5%, respectively except the maximum dry weight of roots from *B. juncea* was determined at Zn supply level of 20.0 mg kg<sup>-1</sup>. The dry weight (stem, leaf and seed) of rape species reveal a decreasing trend when Zn supplied at levels above 10.0 mg kg<sup>-1</sup>. Under the same Zn supply level, seed dry weight revealed an order as *B. campestris* > *B. napus* > *B. juncea*.

### Enzyme Activity

As shown in Table 1, significant differences of SOD, CAT and POD activities in leaf of rape were observed among Zn supply levels and various rape species ( $p < 0.05$ ), and significant interaction were also found between rape species and Zn supply level ( $p < 0.05$ ). The activities of SOD, CAT and POD in three rape species revealed two major trends as the increase of Zn supply levels. The first changing trend was the decrease in activities of SOD, CAT and POD in *B. napus* with the increase of Zn supply level, and the lowest activities of these enzymes were observed at Zn supply level of 5.0 mg kg<sup>-1</sup>; in contrast, as the further increase in Zn supply level, the activities of these enzymes revealed a gradually increasing trend. However, when Zn supply level was increased up to 20.0 mg kg<sup>-1</sup>, the activities of these enzymes exhibited the reduction again. The second changing trend was the increasing activities of SOD, CAT and POD in *B. juncea* and *B. campestris* with the increase of Zn supply level. Under the conditions with various Zn supply levels, the activities of SOD, CAT and POD in *B. campestris* revealed higher level among three rape species.

### Proline and Malondialdehyde Contents

As shown in Table 1, significant difference of transpiration rate (Tr) in leaf was found among rape species and Zn supply levels ( $p < 0.05$ ). The maximum Tr in rape species was observed at Zn supply level of 5.0 mg kg<sup>-1</sup> with the increase by 22.2%, 54.2% and 20.4% when compared with the control, respectively. Tr in leaf of

**Table 1:** Effects of different Zn supply levels on enzyme activity proline and malondialdehyde contents in rape

Species	Zn supply levels (mg kg <sup>-1</sup> )	SOD activity (U min <sup>-1</sup> g <sup>-1</sup> FW)	CAT activity (U min <sup>-1</sup> g <sup>-1</sup> FW)	POD activity (U min <sup>-1</sup> g <sup>-1</sup> FW)	Proline content (μg g <sup>-1</sup> FW)	malondialdehyde content (μmol g <sup>-1</sup> FW)
<i>B. napus</i>	0	508.65±17.59a	4.33±0.52b	473.40±13.53a	44.34±1.67a	7.13±0.64b
	1	488.57±15.11b	4.07±0.47c	430.26±7.65b	24.91±1.21c	6.53±0.51c
	5	445.33±11.27c	3.71±0.32d	405.89±10.27c	20.37±0.79d	5.06±0.73d
	10	456.10±13.64c	4.51±0.51a	483.54±14.63a	31.34±1.11b	6.48±0.41c
	20	400.50±9.35d	4.44±0.44ab	319.44±5.79d	46.82±1.57a	8.48±0.42a
<i>B. juncea</i>	0	482.76±14.23b	4.21±0.37a	501.90±14.19a	30.11±1.23a	6.08±0.26b
	1	449.74±8.11c	3.77±0.29c	470.23±9.09b	23.87±0.83c	5.17±0.33d
	5	418.91±6.98d	3.22±0.25d	341.63±7.76d	11.26±0.62d	5.05±0.21e
	10	465.13±12.45bc	4.07±0.43b	427.62±9.53c	25.72±1.24b	5.99±0.49c
	20	570.50±19.21a	4.22±0.23a	493.55±12.34a	31.28±1.77a	6.50±0.56a
<i>B. campestris</i>	0	533.23±16.72b	4.47±0.41ab	562.85±23.65ab	73.23±4.32b	4.86±0.43b
	1	519.11±15.44c	4.16±0.57c	366.77±6.29c	42.22±2.65c	4.74±0.32c
	5	503.18±9.54d	3.97±0.35d	256.36±5.43d	23.85±1.79e	4.04±0.21d
	10	527.23±16.47bc	4.36±0.46b	559.98±21.12b	37.01±2.46d	5.58±0.43ab
	20	557.73±21.14a	4.59±0.59a	572.48±27.62a	81.43±3.73a	5.66±0.23a
Probability(P)						
Species		<0.001	<0.001	<0.001	<0.001	<0.001
Zn levels		<0.001	<0.001	<0.001	<0.001	<0.001
Species×Zn levels		<0.001	<0.001	<0.001	<0.001	<0.001

Note: Different letters (a, b, c) indicate significant difference at  $P \leq 0.05$  among different Zn supply level at the same species

**Table 2:** Effects of different Zn supply levels on photosynthetic characteristics of rape

Species	Zn supply levels (mg kg <sup>-1</sup> )	Net photosynthetic rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	Stomatal conductance (mol m <sup>-2</sup> s <sup>-1</sup> )	Transpiration rate/ (mol m <sup>-2</sup> s <sup>-1</sup> )	Intercellular CO <sub>2</sub> concentration/ (μmol mol <sup>-1</sup> )
<i>B. napus</i>	0	9.01±0.73e	0.20±0.03bc	4.77±0.31e	275.89±5.03b
	1	9.88±0.69d	0.22±0.05b	5.49±0.47b	272.32±7.03c
	5	11.69±0.59c	0.25±0.02a	5.83±0.21a	235.28±5.03d
	10	12.76±0.83b	0.19±0.03c	4.95±0.30d	276.42±5.03b
	20	14.65±1.21a	0.26±0.05a	5.06±0.59c	284.69±5.03a
<i>B. juncea</i>	0	7.99±0.57d	0.21±0.03cd	3.12±0.15d	329.33±5.03c
	1	9.64±0.62b	0.34±0.04b	4.48±0.40b	318.87±5.03d
	5	11.53±0.47a	0.39±0.07a	4.81±0.43a	304.12±5.03e
	10	9.16±0.52c	0.24±0.02c	3.96±0.23c	342.09±5.03b
	20	7.21±0.34e	0.16±0.03d	2.69±0.17e	398.93±5.03a
<i>B. campestris</i>	0	13.17±0.79d	0.28±0.06b	5.79±0.61e	308.15±5.03b
	1	14.72±0.53c	0.30±0.07ab	6.21±0.50c	297.37±5.03c
	5	16.64±1.32a	0.35±0.04a	6.97±0.73a	285.25±5.03e
	10	15.05±0.95b	0.30±0.03ab	5.50±0.43e	295.14±5.03d
	20	14.62±1.47cd	0.33±0.05a	6.69±0.65b	314.76±5.03a
Probability(P)					
Species		<0.001	0.002	<0.001	<0.001
Zn levels		<0.001	<0.001	<0.001	<0.001
Species×Zn levels		<0.001	<0.001	<0.001	<0.001

Note: Different letters (a, b, c) indicate significant difference at  $P \leq 0.05$  among different Zn supply level at the same species

*B. campestris* and *B. napus* was higher than that in *B. juncea* at the same Zn supply level. Significant difference of Ci in leaf of rape was observed among different species and Zn supply levels ( $p < 0.05$ ). The maximum Ci of leaf in three species was found at Zn supply level of 20.0 mg kg<sup>-1</sup> with the enhancement by 3.2%, 21.3% and 2.3% when compared with the controls, respectively.

### Photosynthetic Characteristics

As shown in Table 2, significant difference of transpiration rate (Tr) in leaf was found among rape species and Zn supply levels ( $p < 0.05$ ). The maximum Tr in rape species was observed at Zn supply level of 5.0 mg kg<sup>-1</sup> with the increase by 22.2%, 54.2% and 20.4% when

compared with the control, respectively. Tr in leaf of *B. campestris* and *B. napus* was higher than that in *B. juncea* at the same Zn supply level. Significant difference of Ci in leaf of rape was observed among different species and Zn supply levels ( $p < 0.05$ ). The maximum Ci of leaf in three species was found at Zn supply level of 20.0 mg kg<sup>-1</sup> with the enhancement by 3.2%, 21.3% and 2.3% when compared with the controls, respectively.

### Root Exudates

The contents and species qualitative and quantitative analyses of amino acids in root exudates of rape revealed an obvious difference among species and between Zn supply levels (Table 3). The contents of 14 kinds of amino acids in

**Table 3:** Effect of zinc supply levels on amino acid contents in roots of rape (ug 100 mL<sup>-1</sup>)

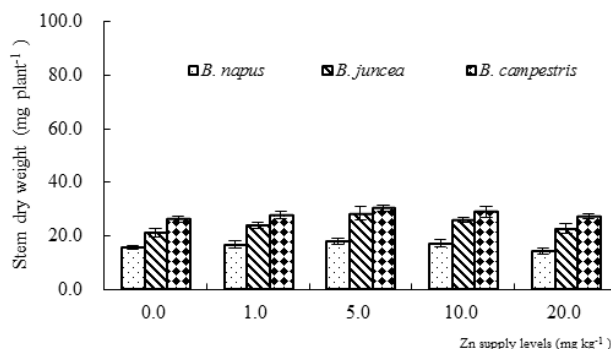
Treatment	1.0 mg kg <sup>-1</sup> Zn			20.0 mg kg <sup>-1</sup> Zn		
Amino acids	<i>B. napus</i>	<i>B. juncea</i>	<i>B. campestris</i>	<i>B. napus</i>	<i>B. juncea</i>	<i>B. campestris</i>
Aspartic	129±9a	149±12a	Nd±0b	98±5b	142±11a	100±5c
Threonine	107±3b	137±9a	Nd±0c	82±5c	155±17a	106±8b
Serine	Nd±00b	Nd±0b	32±2a	96±2a	110±5a	90±1a
Glutamic	572±15ab	699±25a	48±1b	219±9b	501±23a	492±15a
Glycine	92±6a	Nd±0b	Nd±0b	81±5a	71±9a	12±1b
Alanine	151±11a	121±14a	50±3b	106±8b	181±15a	122±9ab
Cysteine	Nd±0a	Nd±0a	Nd±0a	Nd±0a	467±21a	Nd±0a
Valine	Nd±0a	Nd±0a	Nd±0a	Nd±0a	357±15a	Nd±0b
Methionine	710±33a	670±29ab	506±18b	1034±34a	616±12b	664±9b
Isoleucine	209±4a	238±11a	240±9a	215±6b	353±0a	Nd±0c
Leucine	216±15a	199±3a	73±2b	235±9a	263±15a	Nd±0b
Tyrosine	Nd±0a	Nd±0a	Nd±00a	Nd±0a	Nd±0a	Nd±0a
Phenylalanine	679±34ab	712±25a	582±13b	771±23a	765±17a	Nd±0b
Lysine	1451±42a	1470±36a	257±9b	1783±36a	735±14b	701±11b
Histidine	Nd±0a	Nd±0a	Nd±0a	Nd±0a	Nd±0a	Nd±0a
Arginine	54±3b	139±9a	Nd±0c	Nd±0b	56±3a	Nd±0b
Proline	Nd±0a	Nd±0a	Nd±0a	Nd±0a	Nd±0a	Nd±0a
Total	4370±67a	4534±54a	1788±13b	4720±44a	4772±39a	2287±17b

Note: Nd represents not detected (<0.001, mg•100 mL<sup>-1</sup>); Different letters (a, b, c) indicate significant difference at  $P \leq 0.05$  among different species at the same Zn supply level

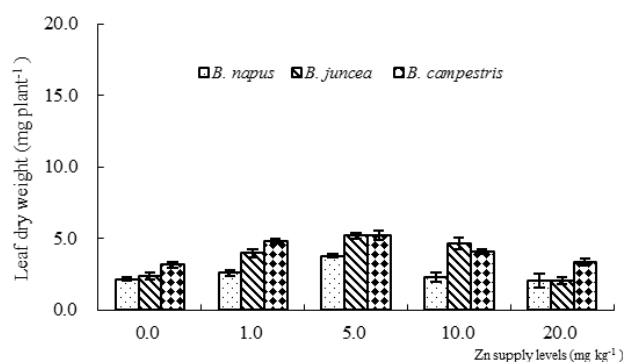
root exudates of rape were in the range of 0.012–1.783 mg 100 mL<sup>-1</sup> FW in the presence of 1.0 and 20.0 mg kg<sup>-1</sup> Zn. Moreover, glutamic acid, methionine, leucine, phenylalanine and lysine were the major amino acids in root exudates of rape with 4.6–21.5%, 12.9–29.0%, 4.1–5.5%, 15.5–32.6% and 14.4–37.8% of total amount of amino acids, respectively. Whether in the presence of 1.0 or 20.0 mg kg<sup>-1</sup> Zn, 11 kinds of amino acids were detected in root exudates of *B. napus*, but only 8 kinds of amino acids were detected in root exudates of *B. campestris*. In root exudates of *B. juncea*, 11 kinds of amino acids were detected in the presence of 1.0 mg kg<sup>-1</sup> Zn, while 14 kinds of amino acids were detected in the presence of 20.0 mg kg<sup>-1</sup> Zn. Whether in the presence of 1.0 or 20.0 mg kg<sup>-1</sup> Zn, the total amino acid content in root exudates of *B. juncea* and *B. napus* species was significantly higher than that in root exudates of *B. campestris*. As seen in the Table 3, the application of Zn could improve the total amount of amino acids in root exudates of rape with the enhancement by 8.01, 5.25 and 27.9% when compared with the Zn treatments of 1.0 mg kg<sup>-1</sup>, respectively. However, Zn (20 mg kg<sup>-1</sup>) resulted in two changes including the increase of serine, methionine, isoleucine, leucine, phenylalanine and lysine content, and the reduction of aspartic acid, threonine, glutamic acid and glycine content.

### Ultrastructure of Root Tip Cells

Fig. 5 shows the root tip cell ultrastructure of rape species in the presence of 1.0 and 20.0 mg kg<sup>-1</sup> Zn during the period of flowering. Obvious differences of root tip cell ultrastructure were observed in the presence of 1.0 and 20.0 mg kg<sup>-1</sup> Zn. At the Zn treatments of 1.0 mg kg<sup>-1</sup>, root tip cells of all species revealed the integral structure, small gap, and normal differentiation of organelles; meanwhile, rich cell

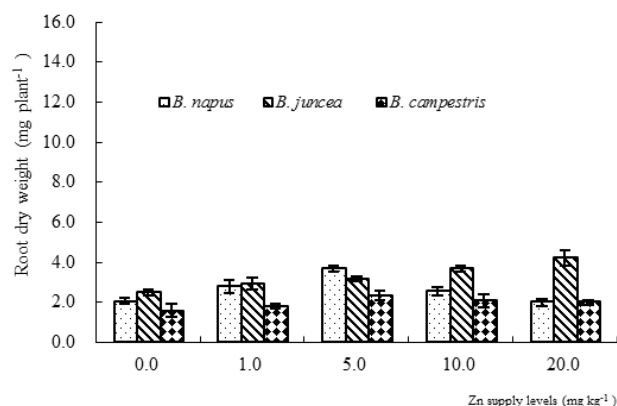


**Fig. 1:** Effects of different Zn supply levels on stem dry weights of rape

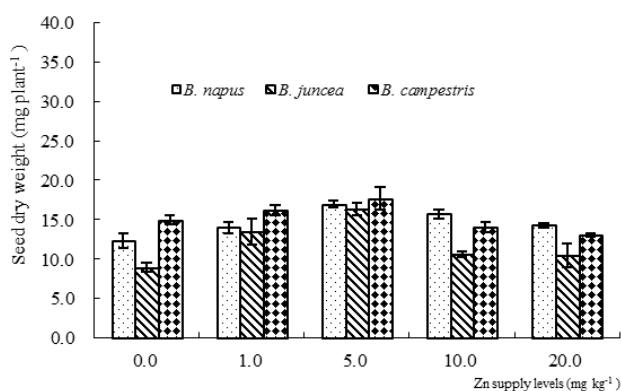


**Fig. 2:** Effects of different Zn supply levels on leaf dry weights of rape

contents, and obvious differentiation of cell wall between primary cells and secondary cells, typical ultrastructure of mitochondria with normal shape and matrix were also observed (Fig. 5A, B and C). At the Zn treatments of



**Fig. 3:** Effects of different Zn supply levels on root dry weights of rape

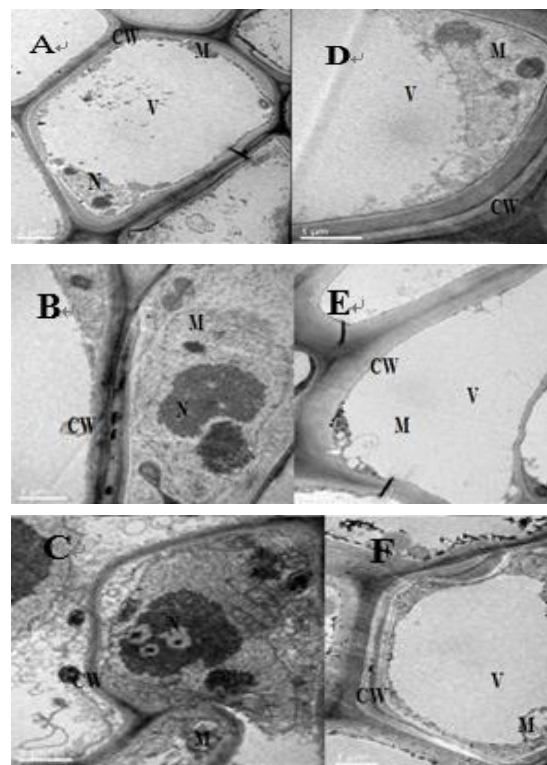


**Fig. 4:** Effects of different Zn supply levels on seed dry weights of rape

20.0 mg kg<sup>-1</sup>, mitochondrial swelling, cell wall thickening and cell content lessening in root tip cell ultrastructure were observed in *B. napus* (Fig. 5D); meanwhile, the obvious lack of cell contents, uneven thickening of cell wall, and the shrinking of nuclei in root tip cell ultrastructure were observed in *B. juncea* (Fig. 5E); however, no obvious differences of root tip cell structure were found in *B. campestris* between 1.0 and 20.0 mg kg<sup>-1</sup> Zn treatments, except slight mitochondrial swelling in root tip cells at the Zn treatments of 20.0 mg kg<sup>-1</sup> (Fig. 5F).

### Concentration of Zn

Significant differences of Zn concentration in seeds, leaves, stems and roots of rape were observed among different species ( $p < 0.05$ ), Zn supply levels and significant interaction of Zn concentration in seeds, leaves, stems and roots between species and Zn levels ( $p < 0.05$ ) (Figs. 6–9). The Zn concentration revealed an order as seeds > leaves or stems > roots. As the increase of Zn supply level, the concentrations of Zn in different tissues or parts of rape revealed an increasing trend. At the present Zn treatments, Zn concentrations in seeds, leaves, stems



**Fig. 5:** Ultrastructure of root tip cells in rape species subjected to different Zn supply levels

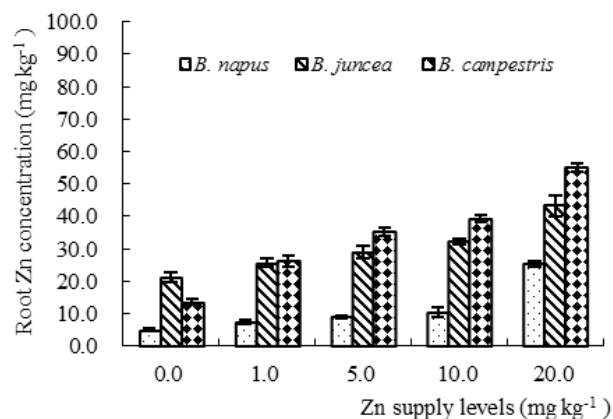
Note: A, B and C are the ultrastructure of root tip cells in *B. napus*, *B. juncea* and *B. campestris* with zinc treatment at the concentration of 1.0 mg kg<sup>-1</sup>; D, E and F are the ultrastructure of root tip cells in *B. napus*, *YR* and *B. campestris* with zinc treatment at the concentration of 20.0 mg kg<sup>-1</sup>, respectively. N, nuclei; M, mitochondria; V, bubble; CW, cell wall

and roots of rape species increased by 8.0–18.6%, 14.2–103.8%, 11.0–135.8% and 21.2–423.2% compared to the control, respectively. Compared with the three rape species, seed Zn concentrations in *B. napus* were higher than that in *B. campestris* and *B. juncea* species.

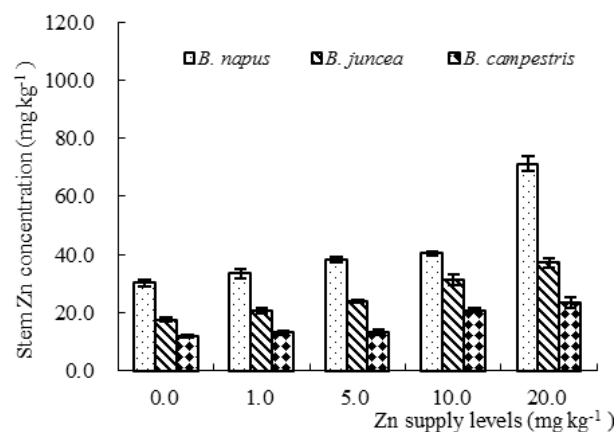
### Discussion

Zinc is an essential element for plant growth which plays a fundamental role in several critical functions. The recent experiments have been found that the dry weight of the three rape species revealed a gradual increasing trend as the increase of Zn supply level, when Zn supply level was less than 5.0 mg kg<sup>-1</sup>. This result is consistent with the results of Xu *et al.* (2005) and Rehman *et al.* (2012). Zinc is not only an essential element for the growth and development of plants, but also a heavy metal (Kösesakal and Ünal, 2009). Stoyanova and Doncheva's (2002) reported that Zn supplied at levels above 70 mM produced toxic effects typical of metal stress in pea (*Pisum sativum* L., cv. *Citrine*) and led to a reduction in the root, stem and leaf growth. In our study, the dry weight (stem, leaf and seed) of rape





**Fig. 6:** Effects of different Zn supply levels on root Zn concentration of rape

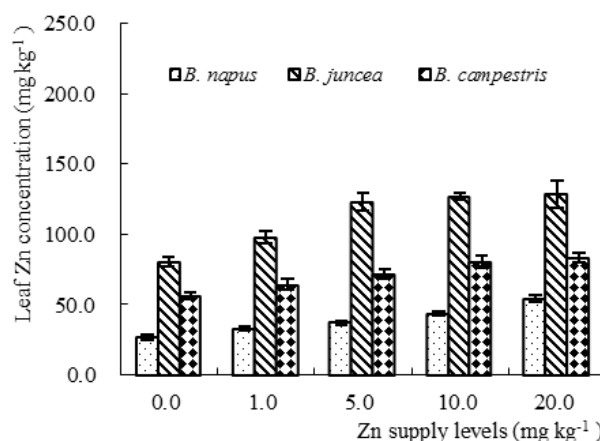


**Fig. 7:** Effects of different Zn supply levels on stem Zn

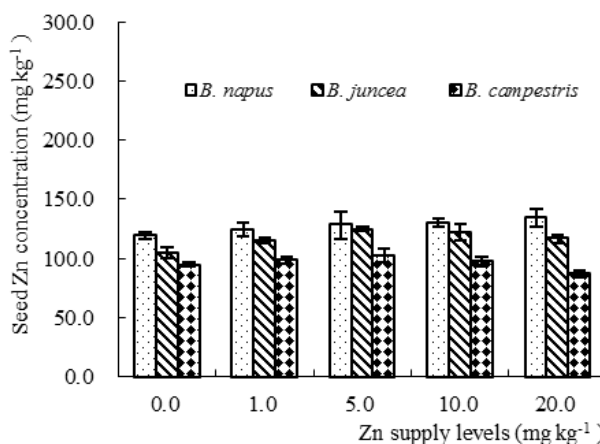
species reveal a decreasing trend when Zn supplied at levels above 10.0 mg kg<sup>-1</sup> due to high level of Zn inhibiting the absorption and utilization of nutrients.

The antioxidant enzymes such as SOD, POD and CAT can scavenge stress-induced active oxygen. As the scavengers of active oxygen in plants, these antioxidant enzymes can effectively eliminate free radicals and peroxides in plants. In the present study, the activities of SOD, CAT and POD in leaf of *B. napus* are lower at low Zn supply level due to the normal growth of plants; in contrast, the excessive Zn increased activities of three antioxidant enzymes for reducing Zn stress-induced cell damage. When Zn supply level is up to 20.0 mg kg<sup>-1</sup>, the activities of SOD, CAT and POD could not continue to increase for relieving stress environment, which is consistent with the similar results from the studies of Xu *et al.* (2005) and Jiang *et al.* (2009). On the other hand, compared to *B. napus*, *B. juncea* and *B. campestris* still maintain high activities of SOD and CAT in response to the ultra-high stress from excessive Zn (20.0 mg kg<sup>-1</sup>), indicating that the two rape species have strong tolerance to Zn.

Free proline content in plants is the biomarker for



**Fig. 8:** Effects of different Zn supply levels on leaf Zn concentration of rape



**Fig. 9:** Effects of different Zn supply levels on seed Zn concentration of rape

physiological and biochemical responses to the stress from environments (Smirnoff, 1993). The alteration of proline content can reflect the adaptation degree of plants to environments. In the present study, proline contents in leaf of rape species revealed an initial decrease and a final increase with the increase of Zn supply level, and reached to the lowest at Zn supply level of 5.0 mg kg<sup>-1</sup>. Proline content in leaf of rape species reached to the peak at Zn supply level of 20.0 mg kg<sup>-1</sup>, suggesting excessive Zn (20.0 mg kg<sup>-1</sup>) induced the increase of proline content in leaf of rape in response to Zn stress. This result is consistent with the conclusion from the studies of Xu *et al.* (2005) and Ramakrishna and Rao (2012).

MDA is the final product of lipid peroxidation, and can interfere with normal physiological metabolism of plant cells through relaxing bridge bonds between cellulose molecules and inhibiting protein synthesis during the interaction between proteins and nucleic acids. The increased MDA content can reflect the stress-induced damage of plants (Sun *et al.*, 2011). In our study, MDA

content gradually decreased with the increase of Zn supply level. When Zn level is higher than 10.0 mg kg<sup>-1</sup>, MDA content shows an upward trend and reached up to the peak level at Zn supply level of 20.0 mg kg<sup>-1</sup>. These results suggest that low Zn is benefit for the growth of plants; in contrast, excessive Zn can induce Zn stress and result in increasing MDA content and influencing the normal growth of plants and same trend was reported by Panda and Choudhury (2005).

The deficiency of Zn affects plant growth and inhibit photosynthesis intensity. In the present study, Zn ( $\leq 5.0$  mg kg<sup>-1</sup>) increased Pn, Gs and Tr in leaf of rape species, suggesting that Zn at low level can improve photosynthetic capacity in plants and productivity and normal energy utilization, and promote normal growth of plants. However, when Zn supply level was higher than 10.0 mg kg<sup>-1</sup>, Pn, Gs and Tr in leaf of rape species showed a downward trend due to Zn stress. This result is consistent with the results from the research of Nguyen-Deroche *et al.* (2012). Meanwhile, the experiments have found that Pn, Gs and Tr in leaf of *B. napus* and *B. campestris* are higher than those in *B. juncea* at Zn supply level of 20.0 mg kg<sup>-1</sup>, maybe due to their high resistance to Zn stress. Many studies have demonstrated that Zn can reduce Ci and Gs, and inhibit the activity of carbonic anhydrase, thus affecting photosynthetic capacity. In the present study, under the condition with Zn at high supply level, both Pn and Gs in leaf of rape reveal an obvious decline, indicating that Gs in leaf of rape is the factor for limiting photosynthetic capacity. According to the theoretical analysis, Ci should be reduced to decrease the photosynthetic rate during the decrease of Gs. However, low photosynthetic rate is accompanied by increased Ci, indicating that the effect of Zn supply level on photosynthetic rate in rape species is due to the non-stomatal factors such as reduced photosynthetic activity in cells, inhibited RUBP carboxylase, or suppressed Calvin cycle as well as other factors.

A large number of experimental studies show that root exudates can change the soil environment by regulating pH value, precipitation, stability and chelating, so as to affect the plant's absorption and utilization of nutrients (Yang *et al.*, 2011). In the present study, high supply level of Zn (20.0 mg kg<sup>-1</sup>) promote the secretion of more amino acids. This result is similar to the reports of Yang *et al.* (2011). Different plant species or varieties have different absorption capacity of Zn. The differences of plant Zn gene genotypes were different not only in root morphology, but also in root exudates (Hajiboland *et al.*, 2003). In our study, the total amino acid content of root exudates in *B. juncea* and *B. napus* was significantly higher than that of *B. campestris* at the Zn treatments of 1.0 and 20.0 mg kg<sup>-1</sup>, and 11 kinds of amino acids were detected in root exudates of *B. napus* and *B. juncea*, and only 8 kinds of amino acids were detected in root exudates of *B. campestris*. High Zn concentration in *B. napus* may be due to more root exudates in *B. napus*, which could increase rhizosphere Zn mobility and

effectiveness. Zinc concentration in Zn-high-efficiency species or varieties is related to the absorption capacity of the plant's roots and the ability to change soil Zn availability (Dong *et al.*, 1995).

## Conclusion

Zinc ( $CZn^{2+} \leq 5.0$  mg kg<sup>-1</sup>) increased the activities of antioxidant enzymes, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) in leaf, and dry weights of plant. The dry weight (stem, leaf and seed) of rape species reveal a decreasing trend when Zn supplied at levels above 10.0 mg kg<sup>-1</sup>. As the increase of Zn supply level, the concentrations of Zn in seed of rape revealed an increasing trend. The best Zn supply level for having more biomass and more Zn in seeds of rape without toxic effects was observed at 10.0 mg kg<sup>-1</sup>. The content of amino acid of root exudates in YR and *B. napus* was significantly higher than that of *B. campestris* in the presence of Zn. The highest of Zn concentration in seed were observed in *B. napus* may be due to more root exudates.

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