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Comparative orchestrating response of four oilseed rape (*Brassica napus*) cultivars against the selenium stress as revealed by physio-chemical, ultrastructural and molecular profiling

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ABSTRACT

Selenium (Se) is an essential micro-element for human and animals. In higher plants, Se essentiality or phyto-toxicity is less explored. Therefore, we aimed to examine the effects of Se (0, 25, 50, and 100 μM) as sodium selenite on the physio-chemical, cell ultra-structural and genomic alterations in hydroponically grown seedlings of four cultivars of *B. napus* (cvs. Zheda 619, Zheda 622, ZS 758, and ZY 50). Results showed that excessive (100 μM) Se (IV) exhibited significant reduction in plant growth parameters, declined pigment contents, lower water-soluble protein levels, and overproduction of H_2O_2 and MDA contents. A significant increase in anti-oxidant enzyme activities and transcript levels of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR), except catalase (CAT) were noticed in the leaves and roots. Non-enzymatic antioxidants including glutathione (GSH) and oxidized glutathione (GSSG), except GSSG in roots were enhanced under higher Se (IV) levels. Transmission electron microscopy analysis revealed the ultra-structural damages in leaf mesophyll and root tip cells induced by excessive Se (IV). Less-significant phytotoxic effects were observed in above-mentioned parameters at 50 μM Se (IV). Overall, Se (IV) supplementation at 25 μM displayed marginal beneficial effect by enhancing plant growth, pigment contents, protein levels and restrict H_2O_2 and MDA overproduction. A marginal increase/decrease in ROS-detoxifying enzymes (except CAT activity) and elevated GSH and GSSG levels were noticed. The accumulation of Se (IV) was much higher in roots as compared to leaves. This accumulation was maximum in Zheda 622 and minimum in ZS 758, followed by Zheda 619 and ZY 50. Overall findings showed that Zheda 622 was the most sensitive and ZS 758 as most tolerant to Se (IV) phyto-toxicity. In addition, Se (IV) was found beneficial until 25 μM Se (IV) but phytotoxic at higher Se levels especially at 100 μM Se (IV).

1. Introduction

Selenium (Se) is one of the extensively dispersed metalloid world-wide (Gupta and Gupta, 2017). The major sources of Se pollution are the anthropogenic activities including mining, industrial discharge, fossil fuel burning, irrigation and petroleum refining (Winkel et al., 2015). The global average total of Se in soil is low i.e. 0.01–2 mg/kg (Johnson et al., 2010) and its bioavailability in soils is directly correlated with the Se content in agricultural crops (Joy et al., 2015). However, some areas of the world such as Hubei province of China, Western USA and North-West India contain seleniferous soils with > 10 mg/kg Se content (Fordyce, 2013). In China, Se deficiency in human diet is a common problem which is being fulfilled by biofortification techniques (Pezzarossa et al., 2012). Se is considered as an essential micronutrient for human and animals at lower dose but toxic at higher amounts (El-Ramady et al., 2015). However, Se essentiality or phytoxicity is still debatable (Gupta and Gupta, 2017). Recent findings suggested that Se is not essential element, but considered as beneficial for plants (Iqbal et al., 2015). There are clear evidences that Se is favorable to plants at lower concentrations, acts as an antioxidant mediator against oxidative stress (Han et al., 2015), decrease the lipid peroxidation (Qing et al., 2015), delay senescence and acts as a growth-promoting agent (Lin et al., 2012). Further, it enhances the chlorophyll contents, maintains cell membrane integrity (Feng et al., 2013). Thus,

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Se is usually included in "beneficial" elements (Co, Si, Na and Al) group (Smoleń et al., 2016). Foliar application of Se has been carried out to enhance Se content in edible portion of cereal crops and in parallel neutralize the damaging effects of abiotic stresses such as salinity (Hawrylak-Nowak, 2009), drought (Hasanuzzaman and Fujita, 2011), and heavy metals (Gupta and Gupta, 2017).

Se exists in soils mostly in the forms of selenite (Se IV) and selenate (Se VI). In plants, it is present in the forms of seleno-cysteine (Se-Cys), and seleno-methionine (Se-Met). After absorption by plant roots (either Se IV or Se VI) inside plasma membrane, it is converted to other organic forms such as SeCys and SeMet (Zhu et al., 2009). Due to the chemical similarity with sulphur, Se (VI) directly competes with sulphate ions for plants to uptake and transported via sulphate transporters. While, Se (IV) is transported by phosphate transporters (White and Broadley, 2009) and easily assimilated by plants (De Souza et al., 1998). Both, Se (IV and VI) have same metabolic pathways as that of corresponding sulphur (S) leads to the absorption of Se into S metabolites that induce Se-phytotoxicity (Terry et al., 2000). Previous reports suggested that elevated Se levels imposed the phytotoxic effects as revealed by leaf chlorosis, stunted plant growth, membrane damage, lower crop yield, antioxidant depletion (Mostofa et al., 2017), trigger the oxidative stress and membrane damages by lipid peroxidation (Yildiztugay et al., 2017). To encounter the toxic effects, plants improve their antioxidant defense system to scavenge the oxidative damages induced by reactive oxygen species (Naz et al., 2015). The boundaries between essentiality and toxicity of Se is dependent upon its chemical forms, level of dose and target plant species (Feng et al., 2013). Therefore, it is necessary to understand the mechanism of specific plants that how it deals with the excessive amount of Se that can impose the beneficial/toxic effects. As we know the hyperaccumulator plants can tolerate excessive metal or metalloids such as Se (Zhu et al., 2009).

Oilseed rape (*Brassica napus* L.) is well unspoken worldwide as a major source of edible oil production. Brassica species are renowned as heavy metals/metalloids tolerant due to their profligate growth, higher biomass production and metal uptake ability (Meng et al., 2009). Previously, most of the researchers investigated the beneficial role of Se against metal tolerance ability in several plants generally at lower Se dose. Se-induced toxicity in Brassica species at higher levels have not been discussed earlier in details. Keeping in view the importance of *B. napus* as an essential crop and area covered by Se-pollutant (specifically in China), current investigation was directed to elucidate the physio-biochemical, molecular and ultrastructural alterations induced by various levels of Se (IV) (0, 25, 50 and 100 μM) to underlie the beneficial or toxic effects of Se on four *B. napus* L. cultivars.

2. Materials and methods

2.1. Plant materials and experimental conditions

For this study, seeds of four oilseed rape (*Brassica napus* L.) cultivars (cvs. Zheda 619, Zheda 622, ZS 758, and ZY 50), which exhibited different tolerance against heavy metals (Gill et al., 2014, 2015a, b) were taken from the College of Agriculture and Biotechnology, Zhejiang University. Mature seeds were treated with 70% (*v/v*) ethanol for 3 min, 0.1% (m/v) $HgCl_2$ for 8 min, and then rinsed with deionized water until make sure that $HgCl_2$ was completely washed out. In every petri dish, 60 seeds were placed on wet filter paper. After germination, 30 seedlings were selected randomly for each treatment and transformed to Petri dishes filled with two pieces of filter paper to which selenium (Se) solutions (0, 25, 50 and 100 μM) had been added. After 24 h, the excess solutions were discarded, and then seedlings were treated with half-strength Hoagland's nutrient solution. Sodium selenite (Na_2SO_3) was used to maintain different Se levels and treatments were replicated four times. The nutrient solution was renewed after every 3 days. Seeds were germinated in a growth chamber under day/night temperature of 25/20 °C, 16-h photoperiod, an irradiance of 3000 $\mu molm^{-2}s^{-1}$, relative humidity 60–70%. Treatment levels were based on the findings from pre-experimental studies for 7 days after seed germination, in which a series of Se (sodium selenite) were used i.e. 0, 25, 50, 100, 20 and 400 μM. Toxic symptoms such as leaf chlorosis and rolling of leaves were considered as Se-stress markers. At 25 μM Se (IV), enhancement in overall plant growth was observed and 100 μM Se (IV) showed significant phenotypical damages. While, higher levels were too toxic i.e. above 100 μM Se (IV). The purpose of our short-term study was to assess the early response from *B. napus* plants because younger plant organs contain higher Se-levels as compared with the older growth stages (Cappa et al., 2014). After 10 days, seedlings were harvested to calculate the parameters related to physiology and for cell structural observations. Samples for biochemical and qRT-PCR of related genes were stored at − 80 °C.

2.2. Morphological parameters

Plants were harvested immediately separated into leaves, stem, and roots to calculate the fresh biomass. The length of leaves, stems, and roots of randomly selected six plants per treatment were measured manually.

2.3. Determination of pigment contents and total soluble protein

Chlorophyll (Chl) a, Chl b, and carotenoids were carried out spectrometrically (Metzner et al., 1965). After 10-days of Se treatment, the topmost fully expanded fresh leaves were weighted and dipped overnight in 85% (v/v) aqueous acetone for the extraction of Chl pigments. The supernatant was taken and then centrifuged at 4000 g for 10 min and diluted with 85% aqueous acetone to the suitable concentration of spectrophotometric measurements. The disappearance was calculated at the absorbance of 452.5, 644 and 663 nm alongside blank of obsolete 85% aqueous acetone. The Chl a, b, total chlorophyll, and carotenoids were assayed by using the following equations.

$$\text{Chlorophyll } a\ (\mu g/\text{ml}) = 10.3{*}E_{663} - 0.98{*}E_{644}$$

$$\text{Chlorophyll } b\ (\mu g/\text{ml}) = 19.7{*}E_{644} - 3.87{*}_{E663}$$

$$\text{Total chlorophyll}\ (\mu g/\text{ml}) = \text{Chlorophyll } a + \text{Chlorophyll } b$$

$$\text{Carotenoids}\ (\mu g/\text{ml}) = 4.2{*}E_{452.5} - \{(0.0264{*}\text{Chl } a) + (0.426{*}\text{Chl } b)\}$$

Finally, these pigments were calculated as mg/g fresh weight. Total soluble protein (TSP) content was measured by following the estimation of Bradford (1976). Fresh leaves (0.5 g) were homogenized in phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5000 × *g* for 8–10 min. A 0.5 mL of freshly prepared trichloroacetic acid (TCA) was added and again centrifuged at 8000 × *g* for 15 min. The precipitate was dissolved in 1 mL of 0.1 N NaOH and 5 mL Bradford reagent was added. Absorbance was recorded by spectrometer at 595 nm using bovine serum albumin as blank.

2.4. Analysis of Se content in leaf and root blades

For the determination of Se contents in leaf and root blades, plant samples were air-dried at 65 °C for 72 h. The samples were ashed at 500 °C for 24 h and then digested in nitric-perchloric acids mineralization (HNO_3-$HClO_4$; 4:1; v/v) for 48 h at room temperature. After digestion, the solution was diluted in distilled water. The Se concentration was determined by using an inductively coupled plasma-optical spectrometer (ICP-OES; Optima 8000DV; PekinElmer). The translocation factor of Se was calculated by adopting the formula used by Aziz et al. (2015) as presented below:

Translocation Factor (TF) = [(Shoot Se/Root Se) × 100]

Shoot Se = accumulation of Se in shoot

Root Se = accumulation of Se in Root