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Growth, photosynthesis and oxidative responses of *Solanum melongena* L. seedlings to cadmium stress: Mechanism of toxicity amelioration by kinetin

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ABSTRACT

The sand culture experiments were conducted to investigate whether the foliar application of kinetin (KN), a kind of cytokinins alleviates cadmium (Cd) toxicity in *Solanum melongena* L. seedlings. The Cd_1 (3 mg Cd kg^{-1} soil) and Cd_2 (9 mg Cd kg^{-1} soil) doses of Cd declined growth of *S. melongena* seedlings in concentration dependent manner which was due to accumulation of Cd in roots and shoots. Kinetin at 10 μM alleviated Cd toxicity which was accompanied by appreciable decrease in Cd accumulation in seedlings. The photosynthetic pigments: Chlorophyll (Chl) *a*, *b* and carotenoids (Car) contents, and chlorophyll fluorescence parameters: F_m/F_0, F_v/F_0 (except F_v/F_m and qP) were decreased while NPQ was raised by Cd, however, with exogenous KN application the damaging effects on photosynthetic pigments and fluorescence parameters were significantly ameliorated. The oxidative stress markers such as superoxide radical, hydrogen peroxide and malondialdehyde (lipid peroxidation) contents were enhanced by both the doses of Cd; however, together with KN these oxidative indices were diminished significantly. Cadmium treatment increased antioxidative enzymes: superoxide dismutase, peroxidase, catalase and glutathione-*S*-transferase activity and the contents of non-enzymatic antioxidants: non protein thiol and proline as compared to control, and KN application further enhanced the antioxidant capacity in Cd treated and even untreated seedlings. The overall results suggest that foliar application of KN improved the growth performance of *S. melongena* seedlings even in presence of toxic level of Cd by strengthening the antioxidant system and photosynthetic capacity.

1. Introduction

Contamination of water and soil due to heavy metals is a global concern and causes significant loss to crop yield (Singh and Prasad, 2014). It may lead to hazardous effects on human health when the toxic metals enter the food chain (WHO, 2007; Singh and Prasad, 2014). In natural system heavy metals are largely found in dispersed form in rock formations. Cadmium (Cd), a non essential heavy metal contaminant mainly released into soil, water and air by natural and unnatural sources such as metal containing rocks, metal industries, leather tanning and dyeing industries etc. Cadmium is a toxic metal because of its relatively high mobility in the soil–plant system (Groppa et al., 2012). It inhibits photosynthetic pigment biosynthesis, interrupts the photosynthetic and respiratory electron transport flow and also interacts with enzymes of Calvin cycle (Qian et al., 2009; Groppa et al., 2012). It accumulates in edible parts of plants during growth, thereby severely affecting yield and quality of crops, hence causes a potential hazard to human health. In plant cell mitochondrial and photosynthetic electron transfer systems (ETS) are the major targets of Cd toxicity and that consequently resulted into rapid production of reactive oxygen species (ROS) as results of leakage of electrons to O_2 (Heyno et al., 2008). Though, Cd is a non-redox active metal, but it induces the generation of ROS; superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (Gill and Tuteja, 2010), which has to be kept under control. Cadmium at toxic level leads to excessive production of ROS causing cell death due to oxidative stress such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acid (Gill and Tuteja, 2010). In order to persist through harsh stresses and to mitigate the oxidative damage initiated by ROS,

Abbreviations: CAT, catalase; F_v/F_m, maximum photochemical efficiency of PS II; F_v/F_0, the activity of PS II; F_m/F_0, electron transport rate through PS II; GST, glutathione-*S*-transferase; KN, kinetin; MDA, malondialdehyde; NPQ, non-photochemical quenching; POD, peroxidase; qP, photochemical quenching; ROS, reactive oxygen species; SOD, superoxide dismutase.

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plants have developed an antioxidant defense systems comprised of non-enzymatic antioxidants; non protein thiol, proline, cystein, ascorbic acid (AsA) and reduced glutathione (GSH), and enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione-*S*-transferase (GST) (Gill and Tuteja, 2010; Ahammed et al., 2013). These antioxidants remove, neutralize, and scavenge ROS from the vicinity of cell. Besides this, Cd was reported to enhance the activity of cytokinin oxidase hence; a significant reduction in the level of cytokinin in wheat seedlings disturbed the hormonal balance and finally declined the growth of the plant (Veselov et al., 2003).

Plant growth regulators may counteract the deleterious effects of adverse environmental stress on plants. It has been shown that application of plant hormone such as cytokinins in agricultural fields can improve crop yields (Al-Hakimi, 2007; Gangwar et al., 2010). Cytokinins are a class of phytohormones that stimulate water uptake, cell division, chlorophyll synthesis and promote organ development thus, lead to the regeneration and proliferation of shoots (Sabater and Rodrguez, 1978; Letham and Palni, 1983). Kinetin (KN) is one of the artificial cytokinins which is reported to improve the growth of crop plants grown under soil acidity (Gadallah and Sayed, 2001), water logged (Younis et al., 2003) and salinity (Wu et al., 2012) conditions. Exogenous KN application can modify toxic effect induced by Cd on growth, pigments and photosynthesis (Al-Hakimi, 2007) and by manganese on nitrogen metabolism (Gangwar et al., 2010) in pea seedlings, however its mechanism is least explored. Cadmium tolerance in plants may also include metal exclusion, restricted metal entry in roots and distribution in tissues, metal binding to the cell wall, chelation by organic molecules and compartmentalization in vacuoles (Wang et al., 2010).

The vegetables are one of the important components of human diet and are good source of mineral nutrients; vitamins and antioxidants hence provide positive effects on human health. Because of these reasons they play crucial role in preventing a number of chronic diseases. *Solanum melongena* L. (brinjal, eggplant) of Solanaceae family is one of the widely used vegetable crops of subtropics and tropics, and it is much popular in Asia, some part of Africa and central America. Brinjal fruit is primarily consumed as cooked vegetable in various ways. In recent years due to heavy industrialization and urbanization the soil of catchment area of populated cities is frequently contaminated with heavy metals following the irrigation practices with polluted water (Singh and Agrawal, 2013). The quality and productivity of vegetables grown in these areas get affected due to metal contamination of soil (Singh and Agrawal, 2013; Singh and Prasad, 2014). Considering these facts the experiments were conducted in sand culture to explore (i) the physiological and biochemical responses of *S. melongena* L. seedlings exposed to Cd stress and (ii) possible mechanism to alleviate cadmium toxicity by KN application. The outcome of the present finding revealed that foliar application of KN improved the photosynthesis and antioxidant potential of *S. melongena* seedlings hence, ameliorated the Cd toxicity. Besides this, a significant reduction in Cd accumulation in roots and shoots due to KN application may significantly decline the metal load in food crops frequently cultivated in catchment area of the cities.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of brinjal (*S. melongena* L., cv. Neelam) were obtained from Ankur Seeds Pvt. Ltd., Nagpur, India. The healthy seeds were surface sterilized with 2% (v/v) sodium hypochlorite solution for 15 min followed by repeated washing with sterilized distilled water, and thereafter the seeds were soaked for 4 h in distilled water. Further, wet seeds were wrapped in sterilized cotton cloth and kept overnight for germination at 26 ± 1 °C. According to Hayat et al. (2010), seeds were sown in plastic pots (5 cm in diameter and 10 cm in depth) containing 150 g acid washed sterilized sand, already mixed with two levels of cadmium in the form of $CdCl_2$. On the basis of screening experiments with varying concentrations (1.5–15 mg Cd kg^{-1} soil) of cadmium the two doses Cd_1 (3 mg Cd kg^{-1} soil) and Cd_2 (9 mg Cd kg^{-1} soil) were selected for present study where Cd_1 dose is under the safe limit (WHO, 2007). The germinated seedlings were placed in growth chamber (CDR model GRW-300 DGe, Athens) under photosynthetically active radiation (PAR) of 150 μmol photons m^{-2} s^{-1} with 16:8 h day–night regime and 86–90% relative humidity at 26 ± 1 °C and were irrigated with Hoagland and Arnon's (1950) half strength nutrient medium and also with distilled water on alternate day.

2.2. Kinetin (6-furfuyl aminopurine) treatment

At 19th day after sowing the seedlings were sprayed with 10 μM kinetin at every 4th day for three consecutive treatments (19th, 23rd and 27th day). For the preparation of stock solution (10 μM) the required quantity of KN was dissolved in 2 ml of acetone and the final volume was maintained 100 ml by the addition of sterilized double distilled water. The stock solution was also added with Tween-20 (0.1%, v/v) as leaf surfactant. The experimental set up included six combinations: control (without Cd as well as KN treatment), Cd_1, Cd_2, KN, Cd_1 + KN and Cd_2+ KN. There were three replicates for each treatment and each pot contained six seedlings. The control plants were also sprayed with distilled water containing same amount of acetone and Tween-20 without KN. At 4th day of last KN treatment i.e. 30 days old seedlings were sampled to record various observations.

2.3. Determination of growth and photosynthetic pigments

Growth was measured as fresh and dry weight of plants and length of roots and shoots. The seedlings were removed from the pots gently and dipped in a beaker filled with tap water to remove the adhering sand particles, ensuring the safety of roots. The plants were further blotted to measure the length of roots and shoots by using a meter scale and subsequently weighed to record fresh weight of plants by using single pan digital balance (Model-CA 223, Contech, India). Meanwhile, the leaf area of each set was recorded by using leaf area meter (Model-211, Systronics, India). The plant samples were then dried in an oven maintained at 70 °C for 48 h before recording dry weight.

Fresh leaves (50 mg) from treated and untreated seedlings were cut into small pieces and photosynthetic pigments were extracted in 80% (v/v) acetone. The extracts were centrifuged and pellets were further used to extract the pigments till they became colourless. The absorbance of the resulting solutions was recorded at 663.2, 646.5 and 470 nm spectrophotometrically (Shimadzu double beam UV–visible spectrophotometer-1700). The amount of Chl *a*, Chl *b* and carotenoids was calculated by using the equations of Lichtenthaler (1987).

2.4. Estimation of Cd content

For the determination of Cd content in roots and shoots, samples of each set were digested in tri acid mixture (HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1 ratio, v/v) at 80 °C until a transparent solution was obtained (Allen et al., 1986). After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally maintained up to 15 ml with double distilled water. The content of Cd in digested samples was estimated by atomic absorption