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Research article

Arsenic affects the production of glucosinolate, thiol and phytochemical compounds: A comparison of two *Brassica* cultivars

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ABSTRACT

Arsenic (As), a non-essential metalloid, severely affects the normal functioning of plants, animals and humans. Plants play a crucial role in metabolic, physiological and numerous detoxification mechanisms to cope up with As induced stress. This study aimed to examine the differential response in two *Brassica juncea* cultivars, Varuna and Pusa Jagannath (PJn) exposed to different doses of As (50, 150, 300 μM) for 48 h duration. Change in morphological traits, concentration of individual as well as total GSL, sulfur related thiol proteins, sulfur content, and phytochemicals were analyzed in both cultivars. Accumulation pattern of As showed dose dependent accumulation in both the cultivars, being more in PJn. Our finding revealed that both cultivars were tolerant at low concentrations of As, while at higher concentration Varuna excelled over PJn. The increased tolerance of Varuna cultivar exposed to 150 and 300 μM concentration of As, correlated with its increased thiol related proteins, sulfur content and phytochemicals, which serves as defence strategy in the plant against oxidative stress. Differential pattern of total as well as individual GSLs content was observed in both Varuna and PJn cultivars. Varuna cultivar showed higher level of total and aliphatic GSLs, which serves as defence compound with other detoxification machineries to combat As stress. Our findings provide foundation for developing metalloid tolerant crops by analyzing the role of different genes involved in GSL mechanism and signaling pathways in different organs of plant.

1. Introduction

Arsenic (As) is an ubiquitous element in the earth crust, and non-essential metalloid in the environment, causes toxicity symptoms when taken in excessive amounts by plants, animals and human beings. Arsenate [As (V)] and arsenite [As (III)] are the major forms of inorganic As that exist in nature. As (III) form predominates under anaerobic condition, whereas in aerobic condition As (V) is the dominating form (Ahmad et al., 2012). Depending on the early insight of As induced stress, plants play pivotal role in metabolic, physiological and various detoxification processes to adopt metal tolerance (Pandey and Gupta, 2015). Recently, Kanwar et al. (2015) showed As induced inhibition in plant growth, and chlorophyll content is repressing by brassinosteroids mediated detoxification in *Brassica juncea*. Overall, plants overcome the detrimental effects of metals, through activation of the stress alleviators by regulating antioxidative defence system comprising an enzymatic and non-enzymatic component (Kanwar et al., 2015). The majority of detoxification processes and metal tolerant strategies rely on the synthesis of sulfur containing compounds, such as cysteine, glutathione, phytochelatins and other thiol related enzymes (Van De Mortel et al., 2008). Further, there is an evidence that glutathione act as a sulfur donor in GSL biosynthesis, and that phytochelatin synthase enzyme serve as a peptidase in indole GSL biosynthesis, suggest connection between glutathione, phytochelatin and GSL for metal homeostasis (Clemens and Peršoh, 2009).

Glucosinolates (GSLs), a group of nitrogen (N) and sulfur (S) containing secondary metabolites, belongs to order Brassicale, which includes several important crops such as mustard, cabbage, garden cress (Hanschen et al., 2012). Glucosinolates are generally known to be inactive compounds, however, mechanical injury causes disruption of GSLs through the hydrolytic activity of

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myrosinase, resulting in the production of highly active compounds such as isothiocynates, thiocyanates and nitriles (Angelino and Jeffery, 2013). On the basis of the amino acid precursors, GSLs are primarily divided into aliphatic, aromatic and indolic GSL (Halkier and Gershenzon, 2006). Biosynthesis of GSL is highly complex process involving three distinct steps viz., side-chain elongation, the core structure formation, and side chain modifications, thereby generating a huge repertoire and diversity of GSL across members of Brassicale. Till date around 130 different GSL structures have been reported from plant species. Glucosinolate and their degradation products, also known as mustard-oil-bomb forms a key defence arsenal of plants against herbivores and pathogens (Brader et al., 2006; Gu et al., 2012), and have marked effect on flavour and anti-cancerous activity of *Brassica* vegetables. GSLs and their hydrolysis in plants are known to be regulated across developmental stages by genetic fluctuations, and various environmental factors including biotic and abiotic stresses (Martinez-Ballesta et al., 2013; Rasmann et al., 2015). Generally, elevated levels of GSLs are recorded in response to temperature (Martinez-Ballesta et al., 2013), ultraviolet-B radiation (Mewis et al., 2012), nutrient availability (Variyar et al., 2014), and plant signaling molecules like salicylic acid (SA), jasmonic acid (JA) and methyl jasmonate (MeJA) (Mewis et al., 2005). For example, exogenous applications of SA or its analogues, herbivore damage or JA treatment have been reported to induce increased indole GSLs in *Brassica napus* (Bodnaryk, 1994; Kiddle et al., 1994), *B. campestris* (Ludwig-Muller et al., 1997), and in *B. juncea* (Augustine and Bisht, 2015). Furthermore, GSL biosynthesis is also regulated by various phytochemicals such as terpenoids, phenolics, and sulfur containing compounds (Thiruvengadam and Chung, 2014). Sulfur is an essential ubiquitous element and has strengthened thiol-based antioxidant system in terms of induced level of thiol metabolism (Dixit et al., 2015). Since, GSL are known as S and N containing secondary metabolites, previous studies showed the binary role of GSL as defence compound, and as S storage under stress condition (Pongrac et al., 2010). Relationship of GSL metabolism and thiol compounds, proved the role of glutathione (GSH) as S donor in GSL biosynthesis under Cd stress, and helped the plant to cope up with reactive oxygen species (Ernst et al., 2008; Jakovljevic et al., 2013).

Recent researches came out with the defensive role of GSLs in plants in response to various biotic stresses such as insect and pathogen attack, but not much detail are known about GSL biosynthesis under heavy metal stress (Variyar et al., 2014). It has been reported that cadmium stress showed no change in GSL production, and selenium was found to affect the content in a concentration-dependent manner in *B. rapa* (Jakovljevic et al., 2013; Kim and Juvic, 2011). Crops belonging to the genus *Brassica* are mainly cultivated in the arid and semi-arid regions of India, where it is an important oilseed and plays a great economical importance to mankind. Ample understanding of the physiological and biochemical mechanisms underlying the metal tolerance at sub-cellular levels aid in developing metal-tolerant plants. However, studies related to the effect of exogenous supply of As on GSLs content, and its metabolism in Brassicaceae plant have not been conducted. The adaptive molecular mechanism rendered by plants at different metabolic pathways to cope with environmental stress conditions is highly desirable to maintain crop productivity. To examine the possibility that As exerted these responses, we analyzed changes in morphological traits, concentrations of individual as well as total GSL content, sulfur related thiol protein, and phytochemicals content in two *Brassica juncea* cultivars, Varuna and Pusa Jagannath (PJn) in response to As toxicity. This work will provide new insight into the role of GSL, and other phytochemical compounds in both *Brassica* cultivars under As toxicity.

2. Materials and methods

2.1. Plant material and treatment conditions

Seeds of two cultivars of *Brassica juncea* (Varuna and PJn) were procured from IARI Pusa, New Delhi, sterilized in 30% ethanol for 1 min, washed thoroughly with distilled water to discard remains of ethanol. Equal numbers of seeds (20) of *B. juncea* cultivars were soaked in double distilled water for overnight, then transferred to petri plate containing moist cotton bed, and kept in dark for another 2 day at temperature of 25 ± 2 °C. After germination seedlings were transferred to PVC cups fixed in a tray containing 10% Hoagland medium (Hoagland and Arnon, 1950; Gupta and Gupta, 2015; Pandey et al., 2016). Each cup consisted of 10 germinated *Brassica* seedlings. The trays were kept in 16-h photoperiod culture room with a day/night temperature of 25 ± 2 °C and relative humidity of 70%. The nutrient solution was replaced at an interval of 2 days. Experiments were performed in two sets: (i) 10 germinated seedlings were watered with 10% Hoagland medium with or without As (III), and after seven days preliminary observation were recorded in terms of phenotypic differences in both cultivars; (ii) for measurement of other parameters, germinated seedlings were grown for 14 days, and then subjected to 50 μM, 150 μM and 300 μM of As (III) solution for a period of 48 h. Different concentrations of As (III) were prepared using the salt ($AsNaO_2$), and used for the treatment. Before harvesting, the treated seedlings were washed thoroughly with double distilled water and untreated seedlings served as controls. All parameters were done using leaves of *Brassica* cultivars.

2.2. Arsenic and sulfur accumulation

Harvested plant samples were thoroughly washed with distilled water, weighed and dried at 60 °C for 72 h. For estimation of total As content, equal amount of dried plant material (0.2 g) powdered and acid digested using 2 ml of HNO_3 and 2 ml of H_2O_2. Digestion was performed in microwave at 95 °C. Then the samples were diluted in a solution containing 10% HCl, 5% ascorbic acid and 10% KI. Measurement of total As content was done by hydride generation atomic absorption spectrophotometry (AA 6800, Shimadzu) as described by Ahmad and Gupta (2013).

For measuring total S content, dried plant material (0.1 g) was digested in a diacid mixture (85 HNO_3: 15 $HClO_4$, v/v). A suitable aliquot was taken in a 10 ml test tube, and volume was made up to 0.5 ml with distilled water. To this aliquot, sodium acetate buffer pH 4.8, 50% glycerol and 20% barium chloride were added. The turbidity of the solution was measured at 470 nm using violet filter on spectrophotometer. Total sulfur estimation was done according to Chesnin and Yien (1951).

2.3. Determination of thiol protein and phytochemicals

Activity of GR (EC 1.6.4.2) was evaluated using the method of Foyer and Halliwell (1976) with slight modification. Fresh plant tissue (0.2 g) was homogenized in 0.2 M phosphate buffer (pH 7.0). Reaction mixture consisted of 0.2 M phosphate buffer (pH 7.0) with 2 mM EDTA, 20 mM GSSG, 2 mM NADPH and enzyme extract in a final volume of 1 ml. The decrease in absorbance of NADPH at 340 nm for GSSG dependent oxidation of NADPH was monitored for 2 min at an interval of 1 min. The enzyme activity was computed using the extinction coefficient of 6.22 mM^{-1} cm^{-1}.

Activity of GST (EC 2.5.1.18) was assayed by the method described by Hossain et al. (2010). Fresh plant tissue (0.2 g) was homogenized in 0.1 M phosphate buffer (pH 6.5). Reaction mixture consisted of 100 mM Tris-HCl buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-