**Exogenous menadione sodium bisulphite increases pigments, osmoprotectants and alters metabolism to attenuate cadmium toxicity on growth and yield in summer squash (*Cucurbita pepo* L.)**

**Wajeeha Yaseen, Muhammad Iqbal, Iqbal Hussain, Samira Khaliq and Muhammad Arslan Ashraf**

Department of Botany, Government College University, Faisalabad (38030), Pakistan

\*For correspondence: iqbaluaf@yahoo.com

**Running Title:** MSB-priming reduces subcellular Cd to increase productivity

**Novelty Statement:**

* The ability of summer squash to compartmentalize Cd at subcellular level exhibited its tolerance capacity and adaptability
* Cd uptake and transport interacted with uptake and transport of Ca, Mg and Fe and decreased plant productivity
* MSB-priming altered subcellular Cd accumulation pattern, and thus reduced its toxicity in chloroplasts and cell membranes
* Further, MSB-priming altered primary metabolism and increased growth and yield in summer squash

**Abstract**

The menadione sodium bisulphite (MSB) is hydrophilic, and has been suggested a defensive molecule against different biotic and abiotic stresses. Cadmium (Cd) is highly mobile and even its minute amount induces toxicity in different organisms including plants. The experiment was conducted to elucidate whether seed priming with MSB could induce Cd tolerance in summer squash. The seed were primed with 0, 10 and 20 mM MSB and sown in pots filled with clean and dried sand saturated with Hoagland’s nutrients solution supplemented with different Cd concentrations (0 and 0.1 mM). The Cd stress reduced growth and contents of chlorophyll (Chl), osmoprotectants (soluble sugars, free amino acids, soluble proteins) and yield while increased oxidants such as hydrogen peroxide (H2O2) and malondialdehyde (MDA) and secondary metabolites (total phenolics and flavonoids). The Cd stress increase shoot and root Fe and Ca2+ concentration while decreased shoot and root Mg2+ concentration. The summer squash transported Cd to shoot and compartmentalized in the cells to avoid Cd toxicity. However, the plants raised from seed primed with MSB had higher contents of photosynthetic pigments, secondary metabolites, and osmoprotectants while low contents of oxidants when under Cd stress. Further, MSB-priming attenuated the toxicity of Cd on nutrients acquisition and increased growth and yield in summer squash. The MSB-primed altered Cd compartmentalization at sub-cellular level and mediated accumulation in the cell wall and soluble fraction rather than in chloroplasts and cell membranes. Overall, MSB-priming (10 mM) was much more effective and increased growth and yield under Cd stress in summer squash.

**Keywords:** Sub-cellular compartmentalization; Cd toxicity; osmolytes, nutrients acquisition; growth; yield

**Introduction**

The Cd toxicity in different plants is well documented (Liu et al. 2015; Haider et al. 2021). Different crop species vary in their Cd content that mainly depends on translocation of Cd from root to shoot (Sun et al. 2019; Hussain et al. 2021). Once up taken by plants, Cd increases the tissue contents of oxidants such as MDA and H2O2 (Chen et al. 2020), and reduces leaf and root growth (Zanella et al. 2016). Its accumulation increases the contents of osmoprotectants such as total soluble proteins, and total phenolics as well as the activities of enzymatic antioxidants (Kolahi et al. 2020). Further, Cd reduced the photosynthesis that was associated with Cd-mediated disrupted chloroplast structure (Song et al. 2019; Chen et al. 2020).

The Cd compartmentalization at the subcellular level is very important for overall Cd accumulation and tolerance in plants (Xin et al. 2013). Subcellular distribution of Cd mainly occurs in four different fractions such as cell wall fraction, organelle-rich fraction, membrane-containing fraction, and soluble fraction (Liu et al. 2014). Major sites for Cd compartmentalization in the cell are cell wall or soluble fractions (Wang et al. 2008). Plants can avoid Cd toxicity through decreasing free Cd concentration in the cytosol. Zhou et al. (2017) found that Cd accumulation significantly decreased biomass in four apple rootstocks. They suggested that through Cd immobilization in the cell wall and soluble fraction (most likely in vacuole) and converting it into pectate- or protein-integrated forms as well as undissolved Cd phosphate forms, the apple rootstocks were able to reduce its mobility and toxicity. Further, Cd interferes with some micronutrients and decreases their uptake and reduces growth of plants (Choppala et al. 2014).

Of menadione derivatives, MSB is hydrophilic (Rao et al., 1985) that exists in both natural and synthetic forms. The MSB could play vital role against oxidative stresses in bacteria, mammals, fungi and plants (Mongkolsuk et al., 1998; Sun et al., 1999). Its defensive role against several plant pathogens in different plant species has been widely demonstrated (Borges et al., 2014, 2009). Due to its hydrophobic nature, it can easily enter cell organelles mediated by membrane passage, where it produces H2O2, OH and O˗2 radicals (Lehmann et al., 2012). However, wide ranges of MSB concentrations exert beneficial effects in plants exposed to both stressed and non-stressed conditions. For instance, the exogenous MSB enhanced development of alfalfa callus and tomato plants, and stimulated rooting of mung bean cuttings. Further, its application increased the effect of IAA three to four times on tomato, cucumber, capsicum and corn plants (Rao et al., 1985). The exogenous MSB under minor oxidative spurt induced chilling tolerance in *zea mays* (Prasad et al., 1994). Seed priming with MSB induced resistance in *Arabidopsis* against a pathogenic strain (Borges et al., 2009). Foliar treatment of MSB (100 µM) increased Cd tolerance that was linked with the higher contents of secondary metabolites and higher activities of enzymatic antioxidants in okra at early growth stage (Rasheed et al., 2018). Recently, Ashraf et al. (2019) reported that 100 mM foliar treatment of MSB mitigated the effects of salinity by increasing the contents of free amino acids and proline in two okra cultivars.

Summer squash (*Cucurbita pepo* L.) is morphologically diverse species, and is widely cultivated throughout the world. Different vegetables and plants have different capacity of heavy metal uptake (Mourato et al., 2015). Most of the studies using MSB as exogenous treatment studied its effects under biotic or salt stress at early growth stages of plants. The literature about the long lasting effects of MSB on yield attributes of crop species exposed to heavy metals is very limited. The effects of MSB on different osmolytes, photosynthetic pigments, and yield characteristics of plants exposed to heavy metals need to be explored. Further, the heavy metal bioavailability and the type of crop species primarily determine the metal up take. For instance, the heavy metals accumulation in pumpkin biomass were not linked with the concentrations in the soil (Danilcenko et al., 2015). Exposure of summer squash to Cd caused reduction in Chl contents, and thus in growth (Galal, 2016). In this context, we hypothesized that exogenous MSB might reverse the Cd-induced perturbations in physio-biochemical attributes and decrease subcellular Cd accumulation in summer squash. Thus, the main purpose of the current work was to evaluate whether seed priming with MSB could increase osmolytes, photosynthetic pigments and uptake of some nutrients and alter subcellular Cd compartmentalization to attenuate Cd-induced toxicity on growth and yield in summer squash.

**Materials and Methods**

The summer squash (*Cucurbita pepo* L.) seeds were surface sterilized with 0.1% sodium hypochlorite for 5 min and then washed twice with double distilled water. The seeds were primed with different concentrations (0, 10, 20 mM) of MSB for 24 h. The five seeds were sown in sand-filled pots (8 L) supplemented with Hoagland’s nutrient solution with or without addition of CdCl2 (0 and 0.1 mM Cd, respectively). After germination, three equal size plants per pot were retained. The data for various growth attributes, photosynthetic pigments, oxidative stress indicators, osmoprotectants and **e**nzymatic and non-enzymatic antioxidants was collected after 35 d of germination at the vegetative stage whereas data for yield attributes were collected after 70 days of germination. The experiment was performed with four replicates using a completely randomized design (CRD).

**Growth and photosynthetic pigments**

The plants were uprooted and separated carefully to determine root and shoot lengths and root and shoot fresh weights. The Chl contents were determined using fresh leaf tissues extracted in 80% acetone and the absorbance was taken at 663, 645 and 480 nm. The Chl and carotenoids contents were calculated as described earlier (Arnon, 1949; Kirk and Allen, 1965).

**Determination of total phenolics, flavonoids, AsA and anthocyanins**

Total phenolics were assayed by using the Folin-Ciocalteu reagent (Wolfe et al., 2003). The total flavonoids were determined as described earlier (Zhishen et al., 1999). The AsA concentration was estimated by following the method of Mukherjee and Choudhuri (1983). The fresh leaf sample was used for the estimation of anthocyanins (Kubo et al., 1999).

**Estimation of total sugars, proteins, amino acids and proline contents**

The total soluble sugars were assayed using the method of Riazi et al. (1985). The total soluble proteins were assayed as detailed earlier (Bradford, 1976). The total free amino acids were determined by following the method of Hamilton and Slyke (1943). The proline contents were assayed as reported earlier (Bates et al., 1973).

**Oxidants (MDA and H2O2) and activities of CAT and POD**

The Dhindsa et al. (1981) method was used to assay the contents of MDA in fresh leaf material. The Velikova et al. (2000) method was used for the estimation of H2O2 contents in fresh leaf sample. The fresh leaf was homogenized in phosphate buffer and the extract was taken for the estimation of CAT and POD activities. The CAT activity was estimated as reported earlier (Aebi, 1984). The POD activity was assayed by following the method of Chance and Maehly (1955). The activities were expressed as U/mg protein.

**Mineral nutrients**

Dry material (0.1 g) of shoot and root was finely ground and digested on hot plate using HNO3 and H2O2 until the solution became clear (Wolf, 1982). The concentrations of minerals (Mg, Fe and Ca) were determined by using an atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990).

**Plant tissue Cd fractionation**

Fresh leaves were homogenized and separated into four different fractions (cell wall and cell wall debris, chloroplasts, cell membranes and other organelles and soluble fraction) by following the method of Wu et al*.* (2005) with slight modifications. Fresh leaf (5 g) was homogenized in 14 ml pre-cold buffer solution (250 mM sucrose, 1.0 mM dithioerythritol (C4H10O2S2), 50 mM tris, 5 mM ascorbic acid, pH 7.5 and 10 drops of triton X100/1 liter). The homogenized solution was passed through nylon cloth (240 µM), liquid was squeezed from the residue. Residue on the nylon cloth was washed twice with buffer and remarked as fraction 1 (cell wall and cell wall debris). Remaining filtrate was centrifuged at 1500 *g* for 10 min and the pellet was designated as fraction 2 (chloroplasts). The supernatant was centrifuged at 15,000 *g* for 35 min and the pellet was designated as fraction 3 (cell membranes and other organelles), while the supernatant as fraction 4 (soluble fractions, vacuoles and cytoplasm). All the four fractions were transferred to crucibles and oven dried for one to two weeks. All the four fractions were digested separately using HNO3 and H2O2 on the hot plate (Wolf, 1982). The QA/QC procedures were followed and the Cd concentration was determined by using the atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990). The operating conditions of the instrument for the determination of Cd were; wavelength (228.8 nm), silt width (1.3 nm), lamp current (7.5 mA), burner head (standard type), flame (air-C2H2), burner height (5 mm), oxidant gas pressure (160 kPa), and fuel gas pressure (6 kPa). The standards were prepared using commercially available stock solution (Applichem 1000 ppm) after diluting with milli-Q water. All the working glass apparatus were dipped in the 8 N HNO3 overnight following the washings with milli-Q water before using them for analytical process.

**Yield attributes**

Number of branches, flowers and fruits were counted manually per plant. Fruits were separated carefully for the determination of fresh weight and after drying in an oven for one week, dry weight recorded. The fruit moisture contents (%) were determined by using the following formula; [(Fresh weight – Dry weight) / Fresh weight] × 100.

**Results**

**MSB-priming increases pigments and growth in Cd-stressed summer squash**

The Cd stress significantly (*P* ≤ 0.001) reduced growth attributes i.e., root and shoot lengths and fresh and dry weights. The exogenous application of 20 mM MSB increased root and shoot lengths while 10 mM MSB increased root and shoot dry weights under Cd stress (Fig. 1). Further, 10 mM of MSB increased shoot fresh weight under both control and Cd-stressed conditions. A remarkable reduction in Chl *a*, Chl *b*, total Chl and carotenoids contents was observed under Cd stress. Exogenous application of MSB significantly increased Chl *a*, Chl *b* and total chlorophylls as well as carotenoids under different Cd regimes (Fig. 2). Overall, seed priming with MSB improved contents of photosynthetic pigments in summer squash.

**MSB-priming increases osmolytes and alters metabolism irrespective of growth conditions**

The exposure of summer squash to Cd significantly (*P* ≤ 0.001) increased phenolics and flavonoids contents. The priming with low concentration of MSB was much more effective in enhancing phenolics under both control and stressed conditions. The higher concentration of MSB decreased flavonoids under Cd stress (Fig. 3). In contrast, higher concentration of MSB was much more effective in increasing AsA concentration in summer squash exposed to Cd stress. The exposure to Cd stress decreased anthocyanins contents in summer squash. However, priming with MSB increased anthocyanins under irrespective of growth conditions. The Cd stress significantly reduced total soluble sugars, free amino acids and total soluble proteins in summer squash. In contrast, Cd stress increased proline contents. Plants raised from MSB-primed seed had significantly (*P* ≤ 0.001) higher total soluble proteins, soluble sugars as well as total free amino acids contents. In this context, the higher concentration of MSB was much more effective in increasing soluble sugars while low concentration in case of soluble proteins and proline in summer squash when under Cd stress (Fig. 3).

**MSB-priming modulates oxidants and enzymatic antioxidants**

The exposure of summer squash to Cd significantly (*P* ≤ 0.01) increased oxidative stress indicators such as H2O2 and MDA. The priming with MSB increased H2O2 contents under both control and Cd-stressed conditions. In contrast, priming with higher concentration of MSB decreased MDA contents under Cd stress (Fig. 4). The Cd stress significantly increased the POD activity while decreased CAT activity. However, the exogenous 10 mM MSB enhanced the activity of POD while MSB treatment decreased CAT under Cd-stressed conditions (Fig. 4).

**MSB-priming alters tissue ionic concentrations to attenuate Cd stress**

Exposure to Cd significantly altered nutrients uptake and transport to the shoot in summer squash. For instance, Cd increased tissue Fe and Ca2+ concentrations while decreased Mg2+ concentrations (Fig. 5). The exogenous MSB increased Mg2+ uptake in the roots while decreased its transport to the shoots. Thus, shoot Mg2+ concentration decreased under both control and Cd-stressed conditions in MSB-treated plants. The exogenous MSB, especially its low concentration increased tissue (shoot and root) Fe concentrations irrespective of growth conditions. The low concentration of MSB increased shoot Ca2+ concentration while higher MSB concentration increased Ca2+ accumulation in the roots under Cd stress (Fig. 5). Overall, the exogenous MSB attenuated the effects of Cd on tissue Ca2+, Mg2+ and Fe concentrations.

**MSB-priming alters subcellular tissue compartmentalization to attenuate Cd toxicity**

The subcellular compartmentalization of Cd in the fresh shoot samples of summer squash was investigated. The results showed that Cd mainly compartmentalized in the cell wall fraction followed by in chloroplast, soluble fraction and cell membranes (Fig. 6). Under Cd stress, the Cd accumulation pattern was as follows: cell wall > chloroplast > soluble fraction > cell membrane. Although the 20 mM concentration of MSB decreased uptake and subcellular accumulation of Cd, the pattern of accumulation was same i.e., cell wall > chloroplast > soluble fraction > cell membrane. In contrast, 10 mM MSB not only decreased the uptake of Cd but also altered its subcellular accumulation pattern i.e., more Cd accumulated in cell wall followed by soluble fraction, chloroplast and cell membrane.

**MSB-priming increases yield attributes irrespective of growth conditions**

Cd stress significantly (*P* ≤ 0.001) reduced different yield parameters i.e., number of flowers, number of branches per plant, number of fruits, fruit fresh and dry weights and fruit moisture contents (Fig. 7). The exogenous MSB especially 10 mM concentration increased different yield attributes irrespective of growth medium. Overall, the exogenous MSB enhanced the yield of summer squash plants irrespective of growth conditions.

**Discussion**

The Cd stress not only affects plant development but also threatens human health because directly or indirectly the human nutrition depends on plants (Zhou et al., 2016; Romero-Puertas et al., 2019). In the present study, Cd stress caused significant reduction in photosynthetic pigments and inhibited growth in summer squash. It is earlier reported that Cd accumulates in root (Khaliq et al., 2016), and thus reduces growth in different plants. For instance, exposure to Cd reduced biomass in maize (Qutab et al., 2017), and cotton (Liu et al., 2015). Further, Cd has been shown to bind into the Chl by substituting Mg, and thus reduces Chl contents (Rydzyński et al., 2019). However, in the present study, MSB-priming increased photosynthetic pigments (Chl and carotenoids) and enhanced plant growth under Cd stress. The MSB acts like plant growth regulators (Rao et al., 1985), and plays important defensive role against both abiotic and biotic stresses (Jiménez-Arias et al., 2015). For instance, under salinity stress, foliar application of MSB increased Chl contents and fresh and dry weights in *Arabidopsis thaliana* (Jiménez-Arias et al., 2015) and in okra (Ashraf et al., 2019). Further, foliar application of MSB induced Cd resistance in okra (Rasheed et al., 2018). The MSB-priming mediated beneficial effects on growth of summer squash exposed to Cd stress could be explained in terms of Cd influences on plant water relations and stomatal regulation. For instance, the 5-day Cd treatment (50 µM) did not affect relative water contents in *Arabidopsis thaliana*, *Vicia faba* and *Commelina communis* possibly by regulating stomatal opening in ABA-independent manner (Perfus-Barbeoch et al., 2002). They further suggested that Cd entered the cytosol through Ca2+ channels and ultimately regulated the guard cell activity. In contrast, (Poschenrieder et al., 1989) found less relative water contents and more stomatal resistance in Cd-treated bush bean (*Phaseolus vulgaris* L. cv. Contender) plants. Taken together, our results suggested that MSB treatment not only increased cell turgidity but also increased cell number that was evident from higher shoot and root fresh and dry weights.

Plants usually accumulate osmolytes and alter metabolism to cope with different abiotic stresses (Benjamin et al., 2019; Qin et al., 2020; Saleh et al., 2020). In the present study, exposure to Cd altered plant primary metabolism and caused increase in total flavonoids, phenolics, proline and AsA contents while decreased anthocyanins, total soluble proteins, soluble sugars and free amino acids in the summer squash. The effects of Cd on osmolytes and secondary metabolites could vary depending on crop species, and the exposure time to different stresses. For instance, the Cd stress altered wheat metabolism by changing the contents of major biochemical constituents such as proteins, soluble sugars and free amino acids (Shukla et al., 2003). In white lupin (*Lupinusalbus* L., cv. Multolupa), soluble proteins and N-amino compounds significantly decreased in the nodules under Cd stress (Carpena et al., 2003). In contrast, Cd stress significantly decreased flavonoids, total free amino acids and total soluble sugars while increased total phenolics and free proline contents in different wheat (*Triticum aestivum* L.) cultivars (Perveen et al., 2016). Similarly, in response to Cd treatment, free amino acids accumulation varied in *Tillandsia* species (Kováčik et al., 2014). The exogenous MSB increased contents of phenolics, flavonoids, anthocyanins, proline, AsA, total free amino acids, soluble proteins and soluble sugars in the summer squash when under Cd stress. Thus MSB-priming exerted beneficial effects and increase Cd tolerance of summer squash. Recently, the beneficial effects of MSB were reported on okra plant metabolism under different stresses (Ashraf et al., 2019; Rasheed et al., 2018). Overall, our results suggested that MSB-treatment diverted plant primary metabolism and increased osmolytes synthesis and accumulation, and thus modulated growth and yield in summer squash.

The Cd toxicity inhibits growth mainly through oxidative damage, nutrients imbalance and altering primary metabolism (Hussain et al., 2017). Our study indicated that Cd stress increased H2O2 and MDA contents while reduced CAT and increased POD activities. However, MSB-priming did not lower concentration of H2O2 while higher MSB level decreased MDA contents in the summer squash plants. Such minor raised levels of oxidants could be helpful to initiate the synthesis of antioxidants especially non-enzymatic antioxidants to regulate growth under stressed conditions. Nonetheless, MSB-mediated reductions in oxidative stress were reported in okra under Cd (Rasheed et al., 2018) and salt stress (Ashraf et al., 2019). However, in okra, the lower oxidative stress was linked with higher activities of antioxidant enzymes. However, the MSB-priming did not increase the activity CAT in Cd-stressed summer squash plants. In contrast, 10 mM MSB increased the activity of POD under Cd-stressed conditions. Thus, the MSB-mediated decrease in Cd toxicity in summer squash plants was largely due to the higher levels of non-enzymatic antioxidants, accumulation of osmoprotectants and secondary metabolites.

The exposure to Cd may alter nutrients uptake and translocation thereby reducing growth and development in different crop species (Qin et al., 2020). Further, The Cd stress could alter different minerals on concentration dependent and tissue dependent manner. For instance, 20 μM Cd increased Ca concentration in the roots, shoots and developing fruits while the reverse was true for 100 μM Cd concentration (Hédiji et al., 2015). In the present study, Cd increased tissue Fe and Ca2+ concentrations while decreased Mg2+ concentrations in the summer squash. Earlier some studies have shown the interaction of Cd with the uptake of Ca and Mg such as in okra seedlings (Rasheed et al., 2018). Further, the exposure of potato, tomato and lettuce to Cd and Pb resulted in higher Fe, Ca and Mg accumulation in dietary parts (Khan et al., 2016). However, the exogenous MSB attenuated the toxic effects of Cd on minerals uptake and transport, and thus summer squash plants showed better growth and yield under Cd stress.

Plants readily uptake Cd and transport to the shoots where it causes toxicity at various levels depending upon crop species. Under Cd stress, plants compartmentalize it and/or chelate it to reduce its toxicity. However, the Cd compartmentalization plays an important role for Cd storage and tolerance in plants at subcellular level (Xin et al., 2013). In the present study, summer squash plants compartmentalized Cd mainly in the cell wall fraction and chloroplast to reduce its toxicity in the shoots. The Cd in chloroplast could replace Mg of chlorophyll, and thus affect photosynthesis and growth. Cell wall act as barrier for the Cd uptake, therefore it bind with Cd and confined its entrance into the cytoplasm (Gallego et al., 2012). In our recent study, most of the Cd transported was deposited in the cell wall. The higher Cd accumulation in the cell wall is already reported in apple rootstock (Zhou et al., 2017) and *Bechmeria nivea* (L.) Gaud. (Wang et al., 2008). The MSB-priming reduced Cd up take and its accumulation at subcellular level. Further, MSB-priming (10 mM) altered its subcellular accumulation pattern i.e., more Cd accumulated in the cell wall followed by soluble fraction (possibly vacuole), chloroplast and cell membrane. The compartmentalization of metal in the vacuole is a good strategy to inhibit its accumulation in other organelles of cells, and induces metal tolerance (Bhatia et al., 2005). Our results are supported by some earlier studies using *Brassica napus* (Mwamba et al., 2016), and cucumber (Yan et al., 2019) where higher accumulation of Cd was observed in the cell wall. Thus, MSB-priming effectively attenuated the Cd toxicity and increased growth and yield in the summer squash. The MSB-mediated less transport of Cd to the shoot and altered Cd accumulation at the subcellular level could be due to the beneficial effects of MSB on synthesis of osmolytes and chelates that reduced its uptake and facilitated its compartmentalization at subcellular level.

**Conclusion**

The Cd stress altered metabolism, nutrients acquisition and reduced growth and yield in summer squash. However, MSB-priming mediated increase in photosynthetic pigments, secondary metabolites, and osmoprotectants while reduced oxidants and attenuated the toxicity of Cd on nutrients acquisition. Further, MSB-priming altered Cd compartmentalization at sub-cellular level and mediated accumulation in the cell wall and soluble fraction (vacuole) rather than in the chloroplasts and cell membranes. Overall, priming with 10 mM MSB was much more effective in increasing growth and yield under Cd stress in summer squash.

**Author Contributions**

WY and MI planned the whole work, write-up and interpreted results. WY performed the experiments and collected data. IH, SK and MAS helped in data analyses and write-up. All the authors have read and approved the final manuscript.

**References**

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121–126.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta Vulgaris*. Plant Physiol., 24: 1–15.

Ashraf, M.A., H.F. Asma and M. Iqbal, 2019. Exogenous menadione sodium bisulfite mitigates specific ion toxicity and oxidative damage in salinity-stressed okra (*Abelmoschus esculentus* Moench). Acta Physiol. Plant., 41: 187.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205–207.

Benjamin, J.J., L. Lucini, S. Jothiramshekar and A. Parida, 2019. Metabolomic insights into the mechanisms underlying tolerance to salinity in different halophytes. Plant Physiol. Biochem., 135: 528–545.

Bhatia, N.P., K.B. Walsh and A.J.M. Baker, 2005. Detection and quantification of ligands involved in nickel detoxification in a herbaceous Ni hyperaccumulator *Stackhousia tryonii* Bailey. J. Exp. Bot., 56: 1343–1349.

Borges, A.A., A. Dobon, M. Expósito-Rodríguez, D. Jiménez-Arias, A. Borges-Pérez, V. Casañas-Sánchez, J.A. Pérez, J.C. Luis and P. Tornero, 2009. Molecular analysis of menadione-induced resistance against biotic stress in *Arabidopsis*. Plant biotechnol. J., 7: 744–762.

Borges, A.A., D. Jiménez-Arias, M. Expósito-Rodríguez, L.M. Sandalio and J.A. Pérez, 2014. Priming crops against biotic and abiotic stresses: MSB as a tool for studying mechanisms. Front. Plant Sci., 5: 642.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248–254.

Carpena, R.O., S. Vázquez, E. Esteban, M. Fernández-Pascual, M.R. De Felipe and P. Zornoza, 2003. Cadmium-stress in white lupin: Effects on nodule structure and functioning. Plant Physiol. Biochem., 41: 911–919.

Chance, B., A.C. Maehly, 1955. Assay of catalases and peroxidases. Methods Enzymol., 2: 764–775.

Chen, L., C. Long, D. Wang and J. Yang, 2020. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by *Brassica juncea* L. enhanced with exogenous application of plant growth regulators. Chemosphere, 242: 125112.

Choppala, G., Saifullah, N. Bolan, S. Bibi, M. Iqbal, Z. Rengel, A. Kunhikrishnan, N. Ashwath, Y.S. Ok, 2014. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. Crit. Rev. Plant Sci., 33, 374–391.

Danilcenko, H., M. Gajewski, E. Jariene, V. Paulauskas and R. Mažeika, 2015. Effect of compost on the accumulation of heavy metals in fruit of oilseed pumpkin (*Cucurbita pepo* L. var. Styriaca). J. Elementol., 21: 21–31.

Dhindsa, R.S., P. Plumb-dhindsa and T.A. Thorpe, 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32: 93–101.

Fu, S., Y. Lu, X. Zhang, G. Yang, D. Chao, Z. Wang, M. Shi, J. Chen, D.Y. Chao, R. Li, J.F. Ma, J. Xia and H. Küpper, 2019. The ABC transporter ABCG36 is required for cadmium tolerance in rice. J. Exp. Bot., 70: 5909–5918.

Galal, T.M., 2016. Health hazards and heavy metals accumulation by summer squash (*Cucurbita pepo* L.) cultivated in contaminated soils. Environ. Monit. Assess., 188: 1–12.

Gallego, S.M., L.B. Pena, R.A. Barcia, C.E. Azpilicueta, M.F. Iannone, E.P. Rosales, M,S. Zawoznik, M.D., Groppa and M.P. Benavides, 2012. Unravelling cadmium toxicity and tolerance in plants : Insight into regulatory mechanisms. Environ. Exp. Bot., 83: 33–46.

Haider, F.U., C. Liqun, J.A. Coulter, S.A. Cheema, J. Wu, R. Zhang, M. Wenjun, M. Farooq, 2021. Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol. Environ. Saf., https://doi.org/10.1016/j.ecoenv.2020.111887.

Hamilton, P.B., D.D.V. Slyke, 1943. The gasometric determination of amino acids in mine by the ninhydrin-carbon dioxide method. J. Biol. Chem., 150: 251–258.

Hédiji, H., W. Djebali, A. Belkadhi, C. Cabasson, A. Moing, D. Rolin, R. Brouquisse, P. Gallusci and W, Chaïbi, 2015. Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: Consequences on fruit production. S. Afr. J. Bot., 97: 176–181.

Hussain, B., M.J. Umer, J. Li, Y. Ma, Y. Abbas, M.N. Ashraf, N. Tahir, A. Ullah, N. Gogoi, M. Farooq, 2021. Strategies for reducing cadmium accumulation in rice grains. J. Clean. Prod., https://doi.org/10.1016/j.jclepro.2020.125557.

Hussain, I., M.A. Ashraf, R. Rasheed, M. Iqbal, M. Ibrahim, T. Zahid, S. Thind and F. Saeed, 2017. Cadmium-induced perturbations in growth, oxidative defense system, catalase gene expression and fruit quality in tomato. Int. J. Agric. Biol., 19: 61–68.

Jiménez-Arias, D., J.A. Pérez, J.C. Luis, V. Martín-Rodríguez, F. Valdés-González and A.A. Borges, 2015. Treating seeds in menadione sodium bisulphite primes salt tolerance in *Arabidopsis* by inducing an earlier plant adaptation. Environ. Exp. Bot., 109: 23–30

Khaliq, A., S. Ali, A. Hameed, M.A. Farooq, M. Farid, M.B. Shakoor, K. Mahmood, W. Ishaque and M. Rizwan, 2016. Silicon alleviates nickel toxicity in cotton seedlings through enhancing growth, photosynthesis, and suppressing Ni uptake and oxidative stress. Arch. Agron. Soil Sci., 62: 633–647.

Khan, A., S. Khan, M. Alam, M.A. Khan, M. Aamir, Z. Qamar, Z.U. Rehman and S. Perveen, 2016. Toxic metal interactions affect the bioaccumulation and dietary intake of macro- and micro-nutrients. Chemosphere, 146: 121–128.

Kirk, J.T.O., R.L, Allen, 1965. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. Biochem. Biophys. Res. Commun., 21: 523–530.

Kolahi, M., E. Mohajel Kazemi, M. Yazdi and A. Goldson-Barnaby, 2020. Oxidative stress induced by cadmium in lettuce (*Lactuca sativa* Linn.): Oxidative stress indicators and prediction of their genes. Plant Physiol. Biochem., 146: 71–89.

Kováčik, J., P. Babula, B. Klejdus and J. Hedbavny, 2014. Comparison of oxidative stress in four *Tillandsia* species exposed to cadmium. Plant Physiol. Biochem., 80: 33–40.

Kubo, H., A.J.M. Peeters, M.G.M. Aarts, A. Pereira and M. Koornneef, 1999. *ANTHOCYANINLESS2*, a homeobox gene affecting anthocyanin distribution and root development in *Arabidopsis*. Plant Cell., 11: 1217–1226.

Lehmann, D., N. Radomski and T. Lütke-Eversloh, 2012. New insights into the butyric acid metabolism of *Clostridium acetobutylicum*. Appl. Microbiol. Biotechnol., 96: 1325–1339.

Liu, J.G., P. Qu, W. Zhang, Y. Dong, L. Li and M.X. Wang, 2014. Variations among rice cultivars in subcellular distribution of Cd: The relationship between translocation and grain accumulation. Environ. Exp. Bot., 107: 25–31.

Liu, Y., T. Xiao, P.C Baveye, J. Zhu, Z. Ning and H. Li, 2015. Potential health risk in areas with high naturally-occurring cadmium background in southwestern China. Ecotoxicol. Environ. Saf., 112: 122–131.

Mongkolsuk, S., R. Sukchawalit, S. Loprasert, W. Praituan and A. Upaichit, 1998. Construction and physiological analysis of a Xanthomonas mutant to examine the role of the *oxyR* gene in oxidant-induced protection against peroxide killing. J. Bacteriol., 180: 3988–91.

Mourato, M.P., I.N. Moreira, I. Leitão, F.R. Pinto, J.R. Sales and L.L. Martins, 2015. Effect of heavy metals in plants of the genus *Brassica*. Int. J. Mol. Sci., 16: 17975–17998.

Mukherjee, S.P., M.A. Choudhuri, 1983. Implications of water stress‐induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant., 58: 166–170.

Mwamba, T.M., L. Li, R.A. Gill, F. Islam, A. Nawaz, B. Ali, M.A. Farooq, J.L. Lwalaba and W. Zhou, 2016. Differential subcellular distribution and chemical forms of cadmium and copper in *Brassica napus*. Ecotoxicol. Environ. Saf., 134: 239–249.

Perfus-Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier, 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J., 32: 539–548.

Perveen, S., M. Shahbaz, M. Iqbal, M.S. Akram, A.Parveen and H.M.M. Ali, 2016. Induction of cadmium stress tolerance in *Triticum aestivum* L. by alfalfa leaf extract. Appl. Ecol. Environ. Res., 14: 121–136.

Poschenrieder, C., B. Gunsé and J. Barceló, 1989. Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiol., 90: 1365–71.

Prasad, T.K., M.D. Anderson, B.A. Martin and C.R. Stewart, 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell, 6: 65–74.

Qin, S., H. Liu, Z. Nie, Z. Rengel, W. Gao, C. Li and P. Zhao, 2020. Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: A review. Pedosphere, 30: 168–180.

Qutab, S., M. Iqbal, R. Rasheed, M.A. Ashraf, I. Hussain and N.A. Akram, 2017. Root zone selenium reduces cadmium toxicity by modulating tissue-specific growth and metabolism in maize (*Zea mays* L.). Arch. Agron. Soil. Sci., 63: 1900–1911

Rama Rao, A.V., K. Ravichandran, S.B. David and S. Ranade, 1985. Menadione sodium bisulphite: A promising plant growth regulator. Plant Growth Regul., 3: 111–118.

Rasheed, R., M. Arslan Ashraf S. Kamran, M. Iqbal and I. Hussain, 2018. Menadione sodium bisulphite mediated growth, secondary metabolism, nutrient uptake and oxidative defense in okra (*Abelmoschus esculentus* Moench) under cadmium stress. J. Hazard. Mater., 360: 604–614.

Riazi, A., K. Matsuda and A. Arslan, 1985. Water-stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. J. Exp. Bot., 36: 1716–1725.

Romero-Puertas, M.C., L.C. Terrón-Camero, M.A. Peláez-Vico, A. Olmedilla and L.M. Sandalio, 2019. Reactive oxygen and nitrogen species as key indicators of plant responses to Cd stress. Environ. Exp. Bot., 161: 107–119.

Rydzyński, D., A.I. Piotrowicz-Cieślak, H. Grajek and D.J. Michalczyk, 2019. Chlorophyll degradation by tetracycline and cadmium in spinach (*Spinacia oleracea* L.) leaves. Int. J. Environ. Sci. Technol., 16: 6301–6314.

Saleh, S.R., M.M. Kandeel, D. Ghareeb, T.M. Ghoneim, N.I. Talha, B. Alaoui-Sossé, L. Aleya andM.M Abdel-Daim, 2020. Wheat biological responses to stress caused by cadmium, nickel and lead. Sci. Total Environ., 706: 136013.

Shukla, U.C., J. Singh, P.C. Joshi and P. Kakkar, 2003. Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat seedlings. Biol. Trace Elem. Res., 92: 257–273.

Song, X., X. Yue, W. Chen, H. Jiang, Y. Han and X. Li, 2019. Detection of cadmium risk to the photosynthetic performance of hybrid *Pennisetum*. Front. Plant Sci., 10: 798.

Sun, C., M. Yang, Y. Li, J. Tian, Y. Zhang, L. Liang, Z. Liu, K. Chen, Y. Li, K. Lv and X. Lian, 2019. Comprehensive analysis of variation of cadmium accumulation in rice and detection of a new weak allele of *OsHMA3*. J. Exp. Bot., 70: 6389–6400.

Sun, Y.L., Y, Zhao, X. Hong and Z.H. Zhai, 1999. Cytochrome c release and caspase activation during menadione-induced apoptosis in plants. FEBS Lett., 462, 317–321.

Velikova, V., I. Yordanov and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. Plant Sci., 151: 59–66.

Wang, X., Y. Liu, G. Zeng, L. Chai, X. Song, Z. Min and X. Xiao, 2008. Subcellular distribution and chemical forms of cadmium in *Bechmeria nivea* (L.) Gaud. Environ. Exp. Bot., 62: 389–395.

Wolf, B., 1982. An improved universal extracting solution and its use for diagnosing soil fertility. Commun. Soil Sci. Plant Anal., 13: 1005–1033.

Wolfe, K., X. Wu and R.H. Liu, 2003. Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609–614.

Wu, F. B., J. Dong, Q.Q. Qian and G.P. Zhang, 2005. Subcellular distribution and chemical form of Cd and Cd–Zn interaction in different barley genotypes. Chemosphere, 60: 1437–1446.

Xin, J., B. Huang, Z. Yang, J. Yuan and Y. Zhang, 2013. Comparison of cadmium subcellular distribution in different organs of two water spinach (*Ipomoea aquatica* Forsk.) cultivars. Plant Soil, 372: 431–444.

Yan, L., Y.C. Zhu, C. Chen, S.J. Zhang, G.Y. Ding, Y.P. La and J.J. Qu, 2019. Subcellular distribution and chemical forms of cadmium in cucumber seedlings. J. Agro-Environ. Sci., 38: 1864–1871.

Zanella, L., L. Fattorini, P. Brunetti, E. Roccotiello, L. Cornara, S. D. Angeli, F.D. Rovere, M. Cardarelli, M. Barbieri, L.S. di Toppi, F. Degola, S. Lindberg, M.H. Altamura and G. Falasca, 2016. Overexpression of *AtPCS1* in tobacco increases arsenic and arsenic plus cadmium accumulation and detoxification. Planta, 243: 605–622.

Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555–559.

Zhou, H., W.T. Yang, X. Zhou, L. Liu, J.F. Gu, W.L. Wang, J.L. Zou, T. Tian, P.Q. Peng and B.H. Liao, 2016. Accumulation of heavy metals in vegetable species planted in contaminated soils and the health risk assessment. Int. J. Environ. R. Public health, 13: 289.

Zhou, J., H. Wan, J. He, D. Lyu and H. Li, 2017. Integration of cadmium accumulation, subcellular distribution, and physiological responses to understand cadmium tolerance in apple rootstocks. Front. Plant. Sci., 8: 966.

Fig. 1. Influence of seed priming with menadione sodium bisulfite (MSB) on the growth attributes of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (*n* = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level).

Fig. 2. Influence of seed priming with menadione sodium bisulfite (MSB) on the photosynthetic pigments of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level).

Fig. 3. Influence of seed priming with menadione sodium bisulfite (MSB) on non-enzymatic antioxidants and osmolytes contents of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level). Sol., soluble.

Fig. 4. Influence of seed priming with menadione sodium bisulfite (MSB) on oxidative stress indicators and activities of some enzymatic antioxidants of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level). MDA, malondialdehyde; H2O2, hydrogen peroxide; CAT and POD, catalase and peroxidase activities, respectively.

Fig. 5. Influence of seed priming with menadione sodium bisulfite (MSB) on some mineral nutrients of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level). FW, fresh weight; DW, dry weight.

Fig. 6. Influence of seed priming with menadione sodium bisulfite (MSB) on the accumulation of Cd in different organelles of summer squash (*Cucurbita pepo* L.) exposed to Cd stress (0.10 mM). Data are mean ± SE (*n* = 4); same letters on bars show non-significant difference (Duncan’s Multiple Range test at 5% probability level).

Fig. 7. Influence of seed priming with menadione sodium bisulfite (MSB) on yield characteristics of summer squash (*Cucurbita pepo* L.) grown under control (0 mM) and Cd stress (0.10 mM). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan’s Multiple Range test at 5% probability level).