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Full Length Article

Exogenous Menadione Sodium Bisulphite Increases Pigments, Osmoprotectants and Alters Metabolism to Attenuate Cadmium Toxicity on Growth and Yield in Summer Squash (*Cucurbita pepo*)

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Abstract

The menadione sodium bisulphite (MSB) is hydrophilic and has been suggested a defensive molecule against different biotic and abiotic stresses. Cadmium (Cd) is a highly mobile element and even its minute amount causes toxicity in different organisms including plants. This 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 m*M*). 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 (H_2O_2) and malondialdehyde (MDA) and secondary metabolites (total phenolics and flavonoids). The Cd stress increased root and shoot Fe (4−18%, respectively) and Ca^{2+} (24−93%, respectively) concentration while decreased root and shoot Mg^{2+} concentration (31−39%, respectively). The summer squash transported Cd to shoot and compartmentalized it in the cells to avoid Cd toxicity. However, the plants raised from seed primed with MSB had higher contents of photosynthetic pigments (17−23% total Chl), secondary metabolites and osmoprotectants when grown under Cd stress. Further, MSB-priming attenuated the toxicity of Cd on nutrients acquisition and increased growth and yield in the summer squash. The MSB-priming reduced Cd uptake (84%) and also altered Cd compartmentalization at sub-cellular level, and mediated its accumulation in the cell wall and soluble fraction (vacuole) rather than in the chloroplasts and cell membranes. Overall, MSB-priming (10 mM) was much more effective and increased growth and yield of summer squash under Cd stress.

Keywords: Cadmium toxicity; Nutrients acquisition; Osmolytes; Sub-cellular compartmentalization; Summer squash; Yield

Introduction

The cadmium (Cd) toxicity in different plants is well documented (Liu *et al.* 2015; Hassan *et al.* 2016; Haider *et al.* 2021). Both geogenic and anthropogenic activities such as smelting of metals, phosphate fertilizers, manufacturing and disposal of Ni-Cd batteries, mining and disposal of urban refuse are some of the major sources of Cd input to the environment (Choppala *et al.* 2014; Haider *et al.* 2021). 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). The tissue Cd concentrations over 5 mg kg^{-1} dry weight are usually toxic for plant growth and development (White and Brown 2010). However, 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 H_2O_2 (Chen *et al.* 2020), and thus disturbs reactive oxygen species (ROS) homeostasis and reduces plant growth (Gallego *et al.* 2012) and yield (Arshad *et al.* 2019). 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 stress altered the synthesis of various osmoprotectants, secondary metabolites and vitamins and thus caused overall toxicity to the metabolism as recently reported in maize (Javaid and Wahid 2019).

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

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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 (*Malus domestica*) rootstocks were able to reduce its mobility and toxicity. The Cd toxicity reduced phosphorus (P) uptake and accumulation both in the root and shoot of maize (*Zea mays* L.) (Rizvi and Khan 2019). Further, Cd interferes with some micronutrients such as zinc (Zn), iron (Fe) and manganese (Mn) and decreases their uptake and reduces growth of plants (Choppala *et al.* 2014).

Of menadione (vitamin K3) 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.* 2009, 2014). Due to its hydrophobic nature, it can easily enter cell organelles mediated by membrane passage, where it produces H_2O_2, OH and O^{-2} radicals (Lehmann *et al.* 2012). The minor oxidative spurt has been shown to induce chilling tolerance in *zea mays* (Prasad *et al.* 1994). Thus, MSB-mediated oxidative spurt could be beneficial under stressed conditions. Further, wide ranges of MSB concentrations exert beneficial effects in plants exposed to both stressed and non-stressed conditions. For instance, the exogenous MSB (10^{-5} M) in the medium 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). 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. The commercial forms of MSB are cheap, and thus its application in agricultural systems could be eco-friendly approach to increase crop yield under both stressed and non-stressed conditions.

Summer squash (*Cucurbita pepo* L.) is morphologically diverse species, and is widely cultivated for both food (blossoms and fruit) and medicinal (fruit and seed) purposes throughout the world. 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 growth (Galal 2016). Despite economic importance of summer squash, a limited work is reported especially when grown under heavy metal stress. Taken together, it was 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 seeds were obtained from Ayub Agricultural Research Institute, Faisalabad. The 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 $CdCl_2$ (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) under natural environmental conditions with average day/night 40/27°C temperature, respectively, relative humidity 44% and 800 mmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) during the growing season.

Growth and photosynthetic pigments

The plants were uprooted from the pots, washed with distilled water to remove sand particles and separated root and shoot carefully to determine root and shoot lengths, and root and shoot fresh weights. After drying in an oven at 70°C for one week, shoot and root dry weights recorded. 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 using formulas 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 (Ainsworth and Gillespie 2007). Briefly, the total phenolics were extracted in 80% methanol and the supernatant was mixed with the 10% reagent, vortex thoroughly and added 20% Na_2CO_3 and incubated at room temperature. The total phenolics were expressed as mg g^{-1} gallic acid equivalent. The total flavonoids were determined as described earlier (Zhishen *et al.* 1999). Briefly, one mL of diluted sample was reacted with 0.6 mL of 5% $NaNO_2$, 0.5 mL of 10% $AlCl_3$ and 2 mL of 1 *M* NaOH, respectively. The absorbance of pink color developed was noted at 510 nm. The sample was homogenized in 10% trichloroacetic acid (TCA) and the AsA concentration was estimated by following the method of Mukherjee and Choudhuri (1983) using DTC reagent (0.5 mL of 9 N sulfuric acid solution containing 2, 4-dinitrophenyl hydrazine, thiourea and copper sulphate at the rate of 2 g, 4 g and 0.08 g per 100 mL, respectively). The fresh leaf sample homogenized in phosphate buffer (pH 7.8) was used for the estimation of anthocyanins (Kubo *et al.* 1999). One absorbance unit was defined as the amount of anthocyanins giving an absorbance of 0.1 at 600 nm.

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

The total soluble sugars were assayed using the method of Riazi *et al*. (1985). Briefly, the methanolic extract was reacted with anthrone reagent followed by heating at 95°C for 10 min. The total soluble sugars were quantified using glucose as a standard (200–1000 mg L^{-1}). The total soluble proteins were assayed as detailed earlier (Bradford 1976) using bovine serum albumin as a standard for quantification. The total free amino acids were extracted in phosphate buffer and the supernatant was reacted with acid ninhydrin and 10% pyridine. The mixture was incubated at 95°C for 30 min and the total free amino acids were determined by following the method of Hamilton and Slyke (1943). The proline was extracted in 3% sulfosalicylic acid and mixed with glacial acetic acid and ninhydrin (2 mL each) and incubated at 100°C. The chromophore was extracted in toluene and assayed as reported earlier (Bates *et al.* 1973).

Oxidants (MDA and H_2O_2) and activities of CAT and POD

The MDA was extracted in 10% TCA and reacted with 0.6% thiobarbituric acid. The mixture was incubated at 100°C for 20 min, quickly cooled and the absorbance of the supernatant was read at 532, 600 and 450 nm (Dhindsa *et al*. 1981). For H_2O_2 concentration, the tissue was extracted in 0.1% TCA and the reaction mixture consisted of 0.5 mL supernatant, 0.5 mL of potassium phosphate buffer (pH 7.0) and 1 mL of 1 *M* potassium iodide. The mixture was incubated at room temperature for 20 min and the Velikova *et al*. (2000) method was used for the estimation of H_2O_2 concentration. The fresh leaf (0.5 g) was homogenized in phosphate buffer (50 m*M*, pH 7.8) and the supernatant was used for the estimation of CAT and POD activities. The CAT activity was estimated as reported earlier (Aebi 1984). The one unit of CAT activity was defined as the amount of enzyme that degrades 1 µmol H_2O_2 in 1 min. The POD activity was assayed by following the method of Chance and Maehly (1955). The change in color of reaction mixture due to the oxidation of guaiacol was read at 470 nm for 1 min and the activity was 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 HNO_3 and H_2O_2 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 m*M* sucrose, 1.0 m*M* dithioerythritol ($C_4H_{10}O_2S_2$), 50 mM tris buffer, 5 m*M* 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 HNO_3 and H_2O_2 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-C_2H_2), 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 HNO_3 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.

Statistical analysis

The data collected for various attributes was subjected to statistical analysis using GLM module of CoStat (CoHort, version 6.204). Two-way ANOVA was used to determine the significant differences among different treatments. When the interaction or individual effects were significant, Duncan's Multiple Range test at 5% probability level was used to compare treatment means.

Results

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

The Cd stress significantly ($P \leq 0.001$) reduced growth attributes *i.e*., root and shoot lengths and fresh and dry weights. The exogenous application of 20 m*M* MSB caused 32.4% increase in root and 33% in shoot lengths under Cd stress. Priming with 10 m*M* MSB caused 66.7% increase in root and 63.6% in shoot dry weights under Cd stress when compared with control (Fig. 1). Further, 10 m*M* of MSB substantially increased (108%) root fresh weight under Cd-stressed conditions. A remarkable reduction in Chl *a* (46.5%), Chl *b* (23.7%), total Chl (38%) and carotenoids (43%) 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). Of different MSB concentrations, 20 m*M* MSB increased Chl *a* (30.8%) and carotenoids (115.7%) under Cd stress. 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 ($P \leq 0.001$) increased phenolics and flavonoids contents. The priming with low concentration of MSB was much more effective in enhancing phenolics under Cd stressed (34%) conditions. The higher concentration of MSB decreased (20%) flavonoids under Cd stress (Fig. 3). In contrast, higher concentration of MSB was much more effective and caused 45% increase in AsA concentration in summer squash exposed to Cd stress. The exposure to Cd stress decreased (39%) anthocyanins contents in summer squash. However, priming with MSB increased (40.5 to 55.9%) anthocyanins under Cd stress. The Cd stress significantly reduced total soluble sugars (19.9%), free amino acids (49.7%) and total soluble proteins (35.7%) in summer squash. In contrast, Cd stress increased 49% proline contents. Plants raised from MSB-primed seed had significantly ($P \leq 0.001$) 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 (40.8%) while low concentration in case of soluble proteins (34.5%) and proline (41%) in summer squash when under Cd stress (Fig. 3).

MSB-priming modulates oxidants and enzymatic antioxidants

The exposure of summer squash to Cd significantly ($P \leq 0.01$) increased oxidative stress indicators such as H_2O_2 and MDA. The priming with MSB increased H_2O_2 contents (15 to 16%) under Cd-stressed conditions. In contrast, priming with low concentration of MSB decreased (3.5%) MDA contents under Cd stress (Fig. 4). The Cd stress caused 14.9% increase in the POD activity while 20.8% decrease in CAT activity. However, the exogenous 10 m*M* MSB enhanced the activity of POD (12%) while MSB treatment decreased CAT activity 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 Ca^{2+} concentrations while decreased Mg^{2+} concentrations (Fig. 5). The exogenous MSB increased (17 to 25%) Mg^{2+} uptake in the roots while decreased (20 to 22%) its transport to the shoots. Thus, shoot Mg^{2+} 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 (14%) shoot Ca^{2+} concentration while higher MSB concentration increased (9.5%) Ca^{2+} accumulation in the roots under Cd stress (Fig. 5). Overall, the exogenous MSB attenuated the effects of Cd on tissue Ca^{2+}, Mg^{2+} and Fe concentrations.

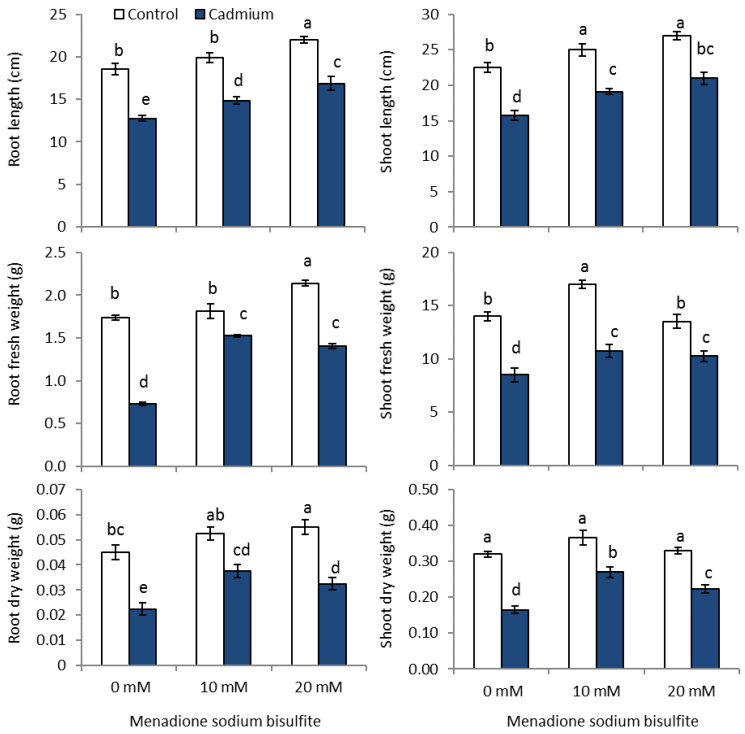

Fig. 1: Influence of seed priming with menadione sodium bisulfite (MSB) on the growth attributes of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*). Data are mean ± SE (*n* = 4); same letters on bars of each parameter show non-significant difference (Duncan's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

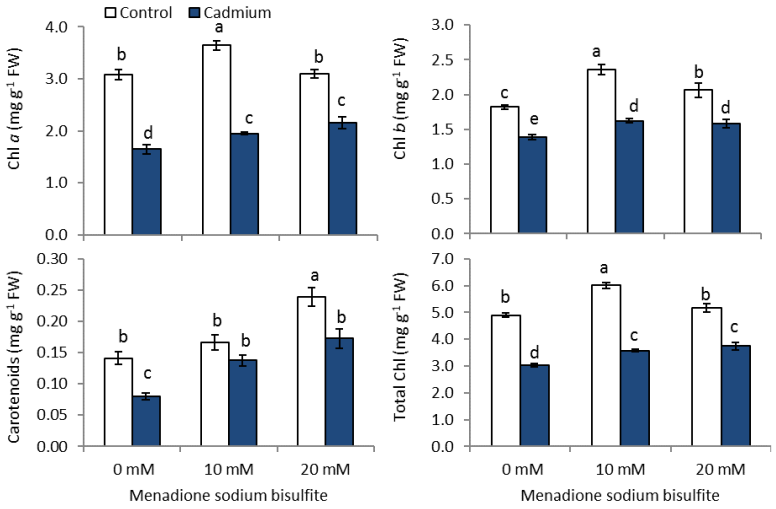

Fig. 2: Influence of seed priming with menadione sodium bisulfite (MSB) on the photosynthetic pigments of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan's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

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 the 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 m*M* concentration of MSB decreased uptake and subcellular accumulation of Cd, the pattern of accumulation was the

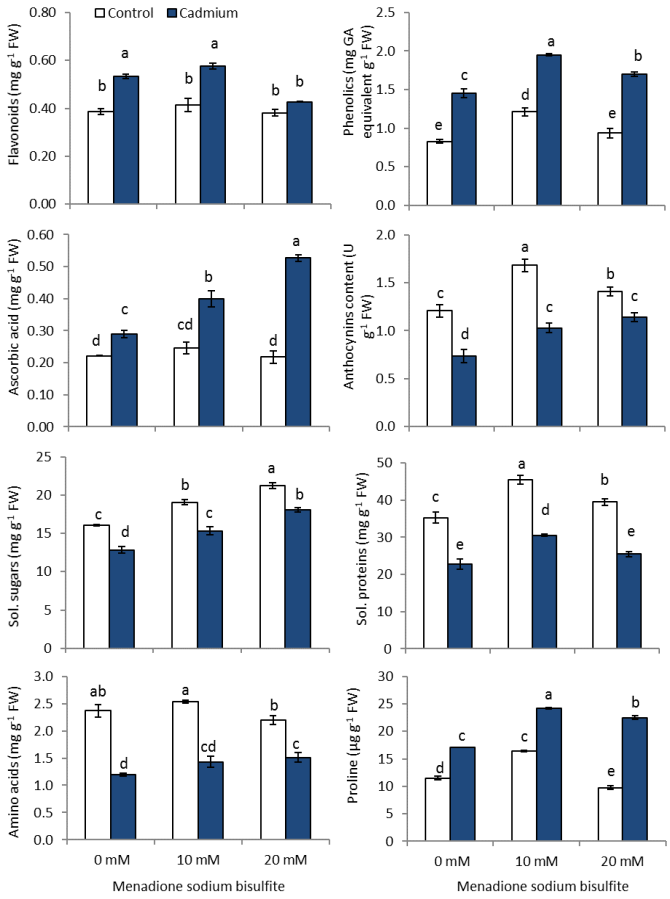

Fig. 3: Influence of seed priming with menadione sodium bisulfite (MSB) on non-enzymatic antioxidants and osmolytes contents of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant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

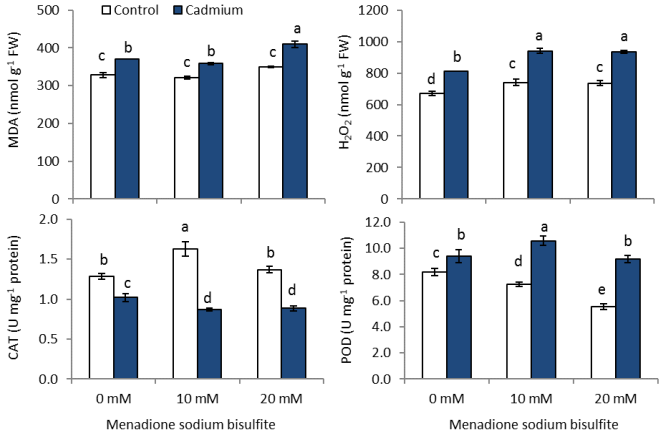

Fig. 4: Influence of seed priming with menadione sodium bisulfite (MSB) on oxidative stress indicators and activities of some enzymatic antioxidants of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*). Data are mean ± SE (n = 4); same letters on bars of each parameter show non-significant difference (Duncan's Multiple Range test at 5% probability level). MDA, malondialdehyde; H_2O_2, hydrogen peroxide; CAT and POD, catalase and peroxidase activities, respectively

same *i.e.*, cell wall > chloroplast > soluble fraction > cell membrane. In contrast, 10 m*M* MSB not only decreased (83.7%) the uptake of Cd but also altered its subcellular accumulation pattern *i.e.*, more Cd accumulated in the cell wall followed by the soluble fraction, chloroplast and cell membrane.

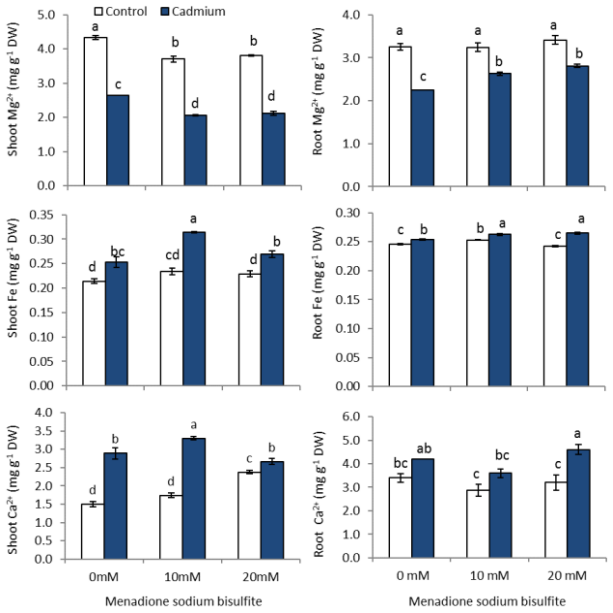

Fig. 5: Influence of seed priming with menadione sodium bisulfite (MSB) on some mineral nutrients of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*)

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

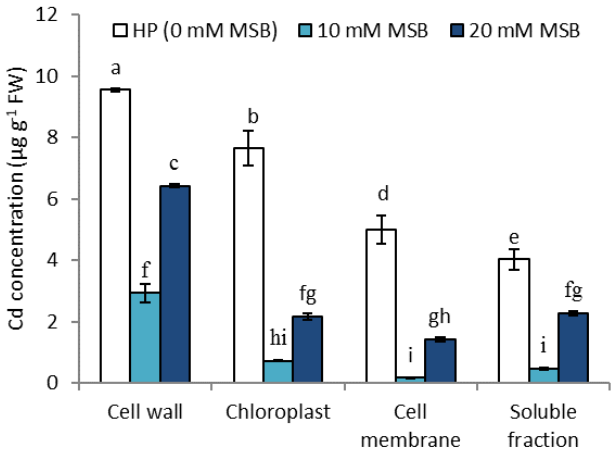

Fig. 6: Influence of seed priming with menadione sodium bisulfite (MSB) on the accumulation of Cd in different organelles of summer squash (*Cucurbita pepo* L.) exposed to Cd stress (0.10 m*M*)

Data are mean ± SE (*n* = 4); same letters on bars show non-significant difference (Duncan's Multiple Range test at 5% probability level)

MSB-priming increases yield attributes irrespective of growth conditions

The Cd stress significantly ($P \leq 0.001$) 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 m*M* concentration increased different yield

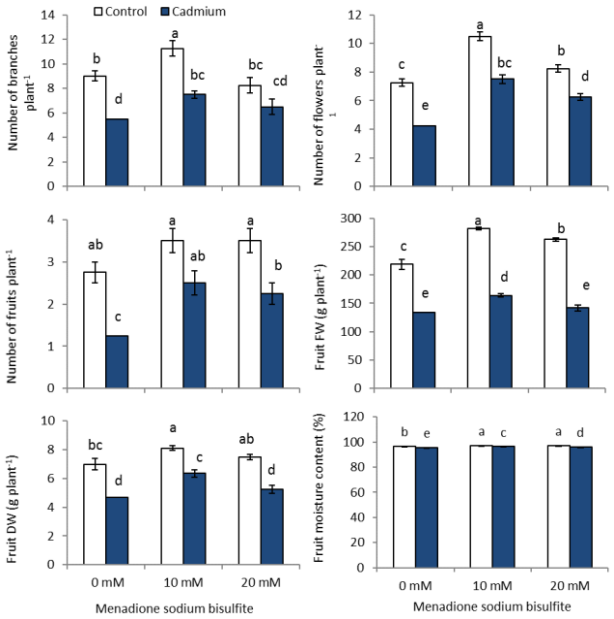

Fig. 7: Influence of seed priming with menadione sodium bisulfite (MSB) on yield characteristics of summer squash (*Cucurbita pepo* L.) grown under control (0 m*M*) and Cd stress (0.10 m*M*)

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)

attributes irrespective of growth medium. The 10 m*M* MSB-mediated increase (100%) in the number of fruits was linked with 36% increase in the number of branches and 76.5% increase in the number of flowers in the summer squash exposed to Cd stress. Overall, the exogenous MSB enhanced the yield of summer squash plants irrespective of growth conditions.

Discussion

In the present study, Cd stress caused significant reduction in the photosynthetic pigments and inhibited growth in the summer squash; the 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 (*Abelmoschus esculentus*) (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* (Perfus-Barbeoch *et al.* 2002). 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 to increase growth under Cd stress.

Plants usually accumulate osmolytes and alter metabolism to cope with different abiotic stresses (Hussain *et al.* 2018; Qin *et al.* 2020; Saleh *et al.* 2020). In the present study, exogenous MSB altered metabolism and caused increase in the concentrations 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 increased Cd tolerance of summer squash. Recently, the beneficial effects of MSB were reported on okra plant metabolism under different stresses (Rasheed *et al.* 2018; Ashraf *et al.* 2019). Overall, our results suggested that MSB-treatment diverted plant primary metabolism and increased osmolytes synthesis and accumulation, and thus modulated growth and yield in the 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 H_2O_2 and MDA contents while reduced CAT and increased POD activities. However, MSB-priming did not lower concentration of H_2O_2 while higher MSB level decreased MDA contents in the summer squash. Such minor raised levels of oxidants could be helpful to initiate the synthesis of antioxidants especially the 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. In the present study, MSB-priming (10 m*M*) increased the activity of POD under Cd-stressed conditions. Nonetheless, the MSB-mediated decrease in Cd toxicity in the summer squash 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). Plants readily uptake Cd and transport to the shoots where it causes toxicity at various levels depending upon crop species. Under Cd stress, plants usually compartmentalize it and/or chelate it to reduce its toxicity. In the present study, Cd increased tissue Fe and Ca^{2+} concentrations while decreased Mg^{2+} 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). 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.

The MSB-priming reduced Cd up take and its accumulation at the 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 Cd in the chloroplast could replace Mg of chlorophyll, and thus affect photosynthesis and growth. Thus, MSB-priming reduced Cd toxicity in the chloroplast through its higher accumulation in the cell wall. 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). Further, 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 thus increased growth and yield in the summer squash.

Conclusion

Cadmium stress altered metabolism and nutrients uptake, and reduced the growth and yield of summer squash; however, MSB-priming mediated increase in the photosynthetic pigments, secondary metabolites, and osmoprotectants coupled with ROS homeostasis to attenuate the Cd toxicity on nutrients acquisition. Further, MSB-priming altered Cd compartmentalization at sub-cellular level and mediated Cd accumulation in the cell wall and soluble fraction (vacuole) rather than in the chloroplasts and cell membranes. Overall, priming with 10 m*M* MSB was much more effective in increasing growth and yield under Cd stress in the summer squash.

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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.

Conflict of Interest

The authors declare that they have no conflicit of interest

Data Availability

Not applicable

Ethics approval

Not applicable

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