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Assessment of Morpho-Biochemical and Gene Expression Levels in *Solanum nigrum* L. as Hyperaccumulator Plant at Cadmium Conditions

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ABSTRACT

Physiological, morphological, biochemical responses and genes expression of black nightshade (*Solanum nigrum* L.) seedlings to cadmium (Cd: 0 mg kg^{-1}, 12.5 mg kg^{-1}, 25 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1}) were assessed, as well as their cadmium accumulation potential for phytoremediation. Plant growth, physiological parameters (photosynthesis pigments), biochemical parameters (oxidative stress indicators and osmolyte contents), cadmium accumulations and gene expression were altered as Cd levels increased. Plant growth and chlorophyll content were negatively affected by Cd only at 100 mg kg^{-1}. Cd was found to significantly raise carotenoid and osmolyte contents as well as the expression of *P5CS* and *W36* genes. As a result of Cd treatments, the seedlings exhibited more oxidative stress indicators than control plants, which confirmed their exposure to stress; however, growth parameters and chlorophyll contents did not significantly change. The Cd accumulation in seedlings increased with elevated Cd concentration (the maximum value of Cd accumulation was 174 mg Cd kg^{-1} and 151 mg Cd kg^{-1} DW in shoots and roots, respectively). The value of the Translocation Factor (TF) of Cd enhanced by increasing the levels of Cd in soil with an average value of 1.08. This study indicated that black nightshade seedlings unchanged photosynthesis capacity and growth parameters as well as developed defense mechanisms by regulating carotenoid content, osmolyte accumulation and some genes against Cd stress. Also, this plant was favorable for Cd uptake and we suggest its ability for the remediation of Cd contaminated soils as a hyperaccumulator plant.

Keywords: Black nightshade; Cadmium stress; Hyperaccumulator plant; Physio-biochemical response; *P5CS* and *W36* genes

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INTRODUCTION

Heavy Metal (HM) contamination is currently a huge ecological problem with devastating long-term effects on our planet. The emission of untreated industrial waste effluents is the primary source of harmful contaminants in the environment. The accumulation of heavy metals in soils and watercourses has been caused by ecological unconsciousness mixed with a lack of legislation in many nations, impacting all living species, from bacteria to animals, including humans. Cadmium (Cd), one of the most poisonous heavy metal elements, is one such contaminant that has a severe impact on plant growth and development. Many studies have been conducted to try to understand the mechanism of Cd toxicity in plants. Although most plants are poisonous to Cd, some species can accumulate large quantities of Cd without showing any signs of toxicity which known as hyperaccumulators. In contrast, numerous low Cd-accumulating species have developed diverse strategies to limit Cd entrance into the xylem to prevent Cd accumulation in shoots [1].

Oxidative damage is a common sign of Cd poisoning. Cd toxicity produces Reactive Oxygen Species (ROS) by displacing Fe from proteins and disrupting the electron transport chain in plant chloroplasts and mitochondria. Excess ROS interact with lipids, proteins and pigments, causing membrane damage and enzyme deactivation. Several investigations have found that Cd's suppression of antioxidative mechanisms enhances ROS generation.

Toxic amounts of ROS cause electron leakage, lipid peroxidation and subsequent membrane damage, as well as nucleic acid and protein destruction. Plants have evolved a number of antioxidant mechanisms, both enzymatic (e.g., Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Guaiacol Peroxidase (GPX)) and non-enzymatic (e.g., glutathione, ascorbic acid, tocopherol, osmolyte contents and carotenoids) [2].

In terms of non-enzymatic processes, soluble proline accumulation is acknowledged as having an essential protective effect against heavy metal stress, having been reported to serve as a radical scavenger or to act in metal chelation. Furthermore, carotenoids, which are lipid-soluble antioxidants, serve a variety of roles in plant metabolism, including oxidative stress tolerance. Thus, the quantification of membrane damage, carotenoids and free proline levels in plants exposed to high concentrations of Heavy Metals (HM) serve as indicators of the adverse effects caused by such exposure, allowing us to know how much plants have suffered from this type of environmental stress.

Plants produce proline from two distinct precursors. The first process converts glutamate to proline *via* two sequential decreases catalyzed by P5CS or 1-pyrroline-5-carboxylate synthase and *P5CR* or pyrroline-5-carboxylate reductase respectively and is the most essential mechanism in response to stress. The other precursor for proline biosynthesis is ornithine. Furthermore, ATPases can catalyze the conversion of adenosine triphosphate to adenosine diphosphate and a free phosphate ion and they play critical roles in ion transport. The membrane-bound proton-translocating V0 subcomplex and the cytoplasm ATP-hydrolyzing V1 subcomplex, which has 8 subunits of A, B, C, D, E, F, G and H, are conserved in all eukaryotes. The vacuolar H^+-ATPase, also known as V-Type H^+-ATPase, acts as an electrogenic H^+ pump. Cold, salt and drought stress may increase the expression of the wheat E subunit of the V-type H^+-ATPase gene, *W36*. W36 protein also accumulates in the cytoplasm and contains certain cis-acting regions that respond to abiotic stressors. Furthermore, overexpression of the *W36* gene in arabidopsis transgenic plants improves stress tolerance [3].

International authoritie's acknowledgement of the problem of HM pollution resulted in the establishment of tougher legislation to limit discharges of these metals into the environment, as well as the development of technology to address this major ecological issue. Phytoremediation is a new technology that harnesses plants and their rhizosphere-associated bacteria to clean up the environment. It is possible to use different phytotechnologies to remedy environmental issues using phytoremediation. In terms of metal decontamination of soils, the main treatment pathways are phytostabilization, which uses plants to stabilize pollutants in soil by preventing leaching or erosion or converting pollutants into less bioavailable and toxic forms and phytoextraction, which uses plants to clean up pollutants by extracting and accumulating toxic elements in harvestable tissues. The concept of hyperaccumulators, which are plant species that can accumulate one or more inorganic elements, such as HM, to levels 100-fold higher than other species, has accompanied the development of phytoextraction technologies [4].

Solanum nigrum L., also known as black nightshade, is a plant species that has been reported to hyperaccumulate HM such as cadmium and zinc. It has the advantage of being a fast growing, easily adaptable plant with a higher biomass than most hyperaccumulators, making it a potential candidate for phytoremediation and metal accumulation. The physiological reaction to heavy metal tolerance is known as hyperaccumulation.

Although several studies have looked at the properties of black nightshade's Cd uptake, as well as its growth and physiological responses to Cd stress, such as antioxidative defines and nitrogen metabolism, the mechanisms of metal tolerance and accumulation in *S. nigrum* remain unknown. The basic goals of this study were to look into the effects of Cd on growth, physiological and biochemical parameters linked to stress tolerance in *S. nigrum* and look into probable links between Cd toxicity and alterations in the expression of P5CR and W36 genes in black nightshade [5].

LITERATURE REVIEW

This research was conducted based on four-replication completed randomized design at the graduate university of advanced technology. Black nightshade (*Solanum nigrum* L.) seeds were sterilized by soaking them in a 0.1 percent (w/v) sodium hypochlorite solution for five minutes and then rinsing them thoroughly with distilled water. Black nightshade seeds were planted in sterilised perlite-filled pots (5.17 cm diameter 15.5 cm height) under usual conditions (light intensity: 1500 lux, light period: 16/8 hours in a temperature range of 20°C-25°C). The plants were watered daily with distilled water for a week before being irrigated with the entire hoagland nutritional solution on alternate days for 30 days. To evaluate the effects of cadmium, $CdCl^2$ (0; as a control, 12.5 mg kg^{-1}, 25 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1}; as varying levels of stress) was added to irrigation nutrient solutions and the treatment was continued for 14 days [6].

Plant growth variables such as fresh weight, dry weight, root length and shoot length were measured after the plants were harvested. The physiological and biochemical properties of samples were assessed using the third leaf.

Photosynthetic Pigments

Leaf samples from the examined cultivars were wrapped in aluminium foil to protect the pigments from light degradation. TChl (Total Chlorophyll) and TCar (Total Carotenoid) contents were determined spectrophotometrically using Lichtenthaler and Buschmann methods. The leaf samples (1 g) were homogenised in 10 ml acetone (100%). The absorbance of leaf extract solution (after filtration) was measured at 663 nm (for Chla), 646 nm (for Chlb) and 470 nm (for carotenoids). TChl content was obtained by combining the Chl a and b values. The photosynthetic pigments were provided as mg/g fresh weight [7].

Oxidative Stress Indicators

The amount of hydrogen peroxide was calculated using the method described in Velikova et al. 0.5 g of plant shoots were crushed with 0.1 percent Trichloroacetic Acid (TCA) in an ice bath. The extracted material was centrifuged for 15 minutes at 10,000 g in a centrifuge at 4°C. 0.5 mL of supernatant was added to 0.5 mL of 10 mm potassium phosphate buffer (pH 7) and 1 mL of one M Potassium Iodide (KI), then the absorbance was measured at 390 nm. The hydrogen peroxide level was calculated using an extinction coefficient of 0.28 M^{-1} cm^{-1} and expressed in micromoles per gram of fresh weight.

According to the study, the lipid peroxidation index was calculated using the level of Malondialdehyde (MDA). First, 5 ml of 0.1 percent TCA was mixed with 0.2 g of fresh shoot tissue from each sample in a porcelain mortar. The collected material was centrifuged at 10000 g for 5 minutes in a centrifuge. One milliliter of centrifuge supernatant was combined with 5 milliliters of a 20% TCA solution containing 0.5 percent Thiobarbituric Acid (TBA). The completed mixture was heated in a water bath at 95°C for 30 minutes. The sample was immediately chilled in an ice bath, then centrifuged at 5000 g for 10 minutes. The absorbance intensity of this solution at 523 nm was measured using a spectrophotometer. The absorption of other non-specific pigments was measured at 600 nm and removed from this value. The extinction coefficient of 105 1.55 $M^{-1}cm^{-1}$ was used to quantify MDA concentration and the findings were calculated in micromolar per gram of fresh weight.

Osmolyte Contents

The homogenised leaf samples (0.1 g) were centrifuged at 12000 g for 20 minutes in 3% aqueous sulfosalicylic acid (10 ml). The mixture of supernatant, ninhydrin and glacial acetic acid (in the ratio of 1:1:1 v/v) was heated at 95°C for 2 hours before adding 6 ml toluene. Toluene absorbance was measured at 520 nm and PRO content was calculated using the proline standard curve.

To determine the TSC content, a 500 mg leaf sample was mashed with 5 ml of 95% ethanol. The alcoholic extract was then mixed with 3 ml of anthrone solution (100 ml of sulphuric acid (72%) and 150 mg anthrone). Following that, the samples were heated in a boiling water bath for 15 minutes. TSC was calculated using absorbance at 625 nm and the glucose standard curve.

To extract TSP, 500 mg homogenized samples were centrifuged at 16700 g and 4°C for 26 minutes in 5 ml of potassium phosphate buffer (pH 7.0, 10 mm) containing 4% PVP. The Bradford solution (980 µl) was then added to the supernatant (20 µl) and the absorbance at 595 nm was measured. Finally, TSP content was calculated using a standard curve established from varied amounts of bovine serum albumin [8].

Cadmium Measurements

The dried leaves and roots were ground into powder using a mortar and pestle. Ground samples (0.5 g) were placed in a 10 ml nitric acid solution. This solution was heated for 24 hours to eliminate any acidic gases before being filtered into a 50 ml volumetric flask and filled to the top with deionized water. Ions in these sample solutions were determined using atomic absorption spectroscopy using the flame technique. Calibration curves for Cd were constructed using standard solutions. Throughout, analytical reagent grade chemicals were used.

Translocation Factor

The translocation factor indicates a plant's ability to transfer heavy metals from roots to shoots. The Cd translocation factor was calculated as follows: Translocation factor=Cd concentration in the shoot/Cd concentration in the root.

RNA Extraction, cDNA Synthesis and Real-Time Polymerase Chain Reactions

Total RNA was isolated from leaves as previously described using RNX plus solution (Cinnagen, Tehran, Iran) and the manufacturer's instructions. For DNA removal, DNase (Thermo Fisher Scientific, USA) was utilised. The integrity of extracted RNA was demonstrated using 1% agarose gel electrophoresis. For cDNA synthesis, RNA with OD 260/280 and OD 260/230 values greater than 2 was employed. Following the manufacturer's instructions, first-strand cDNA was generated from 1 g of total RNA using the PrimeScriptTM RT reagent Kit (Takara, Japan). *P5CS* primers (GenBank: KM523670.1) were created for Real-Time Polymerase Chain Reactions with lengths of 212 base pairs and melting temperatures of 60°C, including P5CSF: 5'-CCATGCTGATGGTTTGTC-3' and P5CSR: 5'-CAGGCCCGCAATAAAGATTA-3'. W36 Primers (GenBank: DQ272489.1) with lengths of 210 base pairs and melting temperatures of 60ºC were determined for real-time PCR, including W36F: 5'- TTCGGTTGAAAGAGCCAGCT-3' and W36R: 5'-CCAGCACAACACCTCCATG-3'. This internal control gene was designated as the reference gene based on prior results that GAPDH was the most stable reference gene under stress. At 60°C melting temperature, Forward GAPDH, 5′-GGTGCCAAGAAGGTCATCAT-3′ and reverse GAPDH, 5′-TGGTCATCAAACCCTCAACA-3′ create a piece with 187 bp length. The NCBI blast search engine was used to verify the correctness of all primers. The following settings were used to execute polymerase chain reactions in a rotor-gene 3000 real-time fast thermocycler (Sydney, Australia): 90°C for 35 seconds, 45 cycles of 95°C for 10 seconds, and melting temperature of each primer for 30 seconds. The negative control was employed to ensure that the reactions were accurate. The 2-Ct technique was used to calculate the expression of the *W36* and *P5CS* genes [9].

Statistical Analysis

Significant differences in growth characteristics, physiological and biochemical indicators, gene expression and cadmium measurement were detected using an analysis of variance based on RCBD. Using SAS version 9.1 statistical software, the LSD test was employed to compare the means of the groups. All statistical tests were examined for significant differences at $P \leq 0.05$.

DISCUSSION

The current study investigated the toxicity of cadmium as well as the biochemical reaction of *S. nigrum* as a hyperaccumulator plant. The relationship between plant stress tolerance and ROS scavenging system activity is primarily determined by plant species, genotype, developmental stage of the plant and stress duration.

Cadmium application at 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1} and 50 mg Cd kg^{-1} had no effect on plant growth characteristics such as fresh weight, root and shoot length in our experiment. At all cadmium treatments, there were no obvious signs of cadmium toxicity in black nightshade. However, the drastically lowered growth characteristics were only detected at a cadmium concentration of 100 mg Cd kg^{-1}. Cadmium poisoning is defined as a reduction in chlorosis, inhibition of root growth, plant biomass and necrosis as a result of increased reactive oxygen species generation and harmful cellular interactions; one of the most significant symptoms of cadmium and other heavy metals is suppression of plant root growth. Numerous studies have previously documented that heavy metals have a negative impact on plant growth and have caused oxidative damage in plants by altering metabolism. According to Wei, et al. cadmium can alter cell wall extension, elongation and cell division, resulting in decreased growth. However, no apparent chlorosis or necrosis spots were identified in our experiment; additionally, the loss in growth parameters occurred exclusively at 100 mg Cd kg^{-1}. Cadmium interaction with major metabolic mechanisms such as photosynthesis and nutrient transport in plants explain the loss in growth caused by cadmium exogenous. A decrease in cadmium induced chlorophyll concentration was shown in tomato, tobacco, rocket, *Brassica rapa*. Similarly, a decrease in Chl content was described as a physiological response under stress conditions. Cadmium stress reduces chlorophyll concentration, which is connected to chlorophyll biosynthesis suppression.

Similar to our findings, it is obvious that leaf Chla, Chlb and TChl concentrations had decreasing trend only at 100 mg Cd kg^{-1} condition. The suppression of aminolaevulinic acid production by the chlorophyll reductase protocol in chlorophyll biosynthesis is the most fundamental reason for excessive cadmium doses disrupting chlorophyll biosynthesis. The drop in leaf TChl concentration may also be attributed to an increase in chlorophyllase activity, which leads to TChl breakdown and a decrease in TChl content. The drop in TChl concentration in plants under abiotic stress appears to be an adaptive strategy to minimize oxidative damage *via* a lower amount of ROS in chloroplasts. Overproduction of chloroplast-associated ROS is very likely to result in the degradation of photosynthetic pigments. The concentration of Chl in plant tissues has been proposed as one of the possible physiological indicators of tolerance in different crops. Based on the LSD test, black nightshade was identified as a tolerant plant in this study, with a low and non-significant reduction in chlorophyll content under cadmium stress conditions of 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1} and 50 mg Cd kg^{-1}. Moreover, this plant had just 0.8-fold lower TChl than the control condition in 100 mg Cd kg^{-1}. These results confirmed the outcomes of Sharma et al., who presented that the Chl concentration in tolerant genotypes decreased less than that of sensitive ones [10].

CAR pigments can function as antioxidants and play a variety of roles in stress tolerance *via* light-harvesting and oxidative damage prevention mechanisms. Black nightshade in this study had higher TCar content under different cadmium levels than the control condition (1% and 8% increase was measured for 12.5 mg Cd kg^{-1} and 25 mg Cd kg^{-1} respectively, which correspond to 41% and 71% under 50 mg Cd kg^{-1} and 100 mg Cd kg^{-1}). Refers to the positive link discovered between photosynthesis pigments and growth parameters, it can be stated that selecting for increased photosynthesis pigments may lead to improved growth parameter performance under cadmium circumstances. Nonetheless, the maintenance of Chl content as well as growth parameters under different cadmium levels may be demonstrated black nightshade is a tolerant plant that can grow in cadmium-stress locations. According to the observed correlation between TCar and oxidative stress parameters (*i.e.,* MDA and H_2O_2), this antioxidant may scavenge free radicals in black nightshade at cadmium stress conditions. It seems that the balance between free radicals and their elimination by antioxidant scavenging factors like TCar appears to be strongly linked to cadmium-tolerant black nightshade genotypes.

The amount of MDA was formed as a result of abiotic stress-induced membrane damage. Our results in this study showed that the concentrations of PRO, TSP and TSC increased dramatically under cadmium exposure. Oxidative stress causes plants to produce many non-toxic or organic solutes, which protect the plants by detoxifying ROS, stabilizing proteins, maintaining membrane totality and mostly osmotic control. In stress situations, PRO is one of the most essential organic solutes (osmolytes), which was also seen by our results. Siddique, et al., discovered that higher levels of PRO in tissues at different phonological stages improved plant survival in stressful conditions. Aslam et al., discovered that by increasing the concentration of this compound in cells, the plant will retain its defensive capabilities and tolerance to stress. According to the reviewed literature, PRO content can be a significant index under stress conditions, indicating the plant's struggle with ROS toxification; this attempt may lead to stress tolerance and acceptable plant performance. Among four checked levels of cadmium, 100 mg Cd kg^{-1} showed the highest amounts of PRO and also a significant decrease in growth parameters. However, 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1}, and 50 mg Cd kg^{-1} showed a significant increase in PRO content and a non-significant reduction in growth parameters. Therefore, black nightshade can probably endure cadmium stress levels under 50 mg Cd kg^{-1} and show unchanged performance. PRO increases in response to stress can act as effective ROS scavengers and protect plants from oxidative damage, raising plant tolerance to various abiotic stresses. TSC and TSP levels reported highest amount at 100 mg Cd kg^{-1} in this investigation. A positive correlation was seen between the redox modifications with osmolyte contents and genes expression. In this regard, the oxidative stress parameters in black nightshade increased osmolyte content, gene expression and TCar content; it appears that these parameters assisted to diminish the poisonous effects of ROS resulting in cadmium stress. Sharma, et al., specified that enhancement of proteins in plant leaves is considered as an adaptive response to stress conditions, which has an important role in decreasing the impact of stress damage and frostbite on plant tissues [11].

Wiszniewska, et al., demonstrated that the concentration of soluble carbohydrates was associated to stress tolerance improvement. Carbohydrates including sucrose, sorbitol and raffinose are plant defense system components. Carbohydrates, according to Guangqiu, et al., have an important function in protein structure preservation, providing accessible energy and acting against abiotic stress such as heavy metals. Our results are consistent with Wiszniewska, et al., findings in which they reported that proteins increased in aster tripolium under heavy metal stress.

Delta 1-Pyrroline-5-Carboxylate Synthetase (*P5CS*), is an enzyme that catalyzes the first two steps of proline biosynthesis from glutamate and has been identified from various plants. A previous study found a link between *P5CS* gene expression and proline accumulation in wheat. Furthermore, it has been demonstrated that increasing proline content and *P5CS* gene expression increases osmotic stress tolerance and enhancing osmotic stress increases proline content and P5CS gene expression in plants; these findings are perfectly consistent with ours. Proline content increased with increasing cadmium exposure in this study, but the magnitude of increase varied greatly between cadmium levels. In our work, increasing proline content under 50 mg Cd kg^{-1} and 100 mg Cd kg^{-1} cadmium stress into a normal state resulted in 2.6-fold and 4.0-fold enhancements, which correspond to 4.9-fold and 9.1-fold for the *P5CS* gene, respectively. Based on our findings and previous research, we may conclude that proline content and *P5CS* gene expression are both implicated in abiotic stress, such as cadmium exposure. Furthermore, our findings revealed a link between proline content and *P5CS* gene expression, such that increasing proline concentration increased P5CS gene expression. Changes in proline content and *P5CS* gene expression are also affected by species and abiotic stress levels. Finally, a direct relationship was found in this study between proline concentration and *P5CS* gene expression and black nightshade resistance in the vegetative stage under cadmium stress [12].

Plants have sophisticated signaling networks that can recognize and respond to environmental stressors such as abiotic stress. Vacuolar H$^+$-ATPase controlled proton stimulus power (PMF) and pumped H$^+$ from the cytoplasm into the vacuole using the energy supplied by hydrolyzing cytoplasm Adenosine Triphosphate (ATP). As a result, vacuolar H$^+$-ATPase activity may stimulate solute transfer between the cytoplasm and the vacuole, which is required for life activities. Furthermore, under salt stress, V-type H$^+$-ATPase activity is one of the most critical plant survival mechanisms. The vacuolar-type H$^+$-ATPase is an ATP-driven enzyme that transforms ATP hydrolysis energy to electrochemical potential changes of protons through diverse biologic membranes *via* the primitive energetic transport of H$^+$. The membrane-bound proton-translocating V0 subcomplex and the cytosol ATP-hydrolyzing V1 subcomplex comprise vacuolar-type H$^+$-ATPase. There are eight subunits of the ATP-hydrolyzing V1 sub-complex in the cytosol: A, B, C, D, E, F, G and H. *W36* gene expression was investigated under different cadmium stress conditions in this study to survey the involvement of the *W36* gene (E subunit) under cadmium stress. Cadmium levels specifically up-regulated *W36* gene expression in black nightshade, according to our findings. *W36* gene expression was lower in the control condition than that in the cadmium treatments. Furthermore, more than 2-fold changes in *W36* gene expression were calculated between 0 with 12.5 and 25 mg Cd kg^{-1} treatments, compared to 4-fold changes between 0 with 50 mg Cd kg^{-1} and 100 mg Cd kg^{-1} cadmium doses. One of the most essential plant survival strategies is V-type H$^+$-ATPase activity under abiotic stress. Previous research demonstrated that V-type H$^+$-ATPase activity protects the cytoplasm pH, responsiveness to stress, metabolic stability, water potential and Ca^{2+} concentration. They demonstrated that the *W36* gene is transported into plants in response to osmotic pressure.

The main reason for this gene to enter in response to abiotic challenges is due to several cis-acting elements responding to abiotic stresses that are placed on the *W36* promoter. Cis-acting elements are involved in a variety of secondary actions in plant systems, including morphological repairs, controlling senescence, DNA damage maintenance, developmental organization and response to abiotic stimuli. Plants' molecular equilibrium shifts under stress, causing transcription factors to bind to specific diagnostic sequences upstream of stress-responsive genes (called cis-elements). Based on our findings and other research, we may conclude that raising W36 gene expression can increase V-type H^+-ATPase activity and can be used to predict plant resilience by reducing cellular toxicity. Furthermore, when V-type H^+-ATPase activity increases due to increased *W36* gene expression under abiotic stress and because V-type H^+-ATPase energizes the transport of proline through tonoplast to be accumulated under osmotic stresses and proline accumulation leads to increased osmotic adjustment in plants, it can be justified that these plant reactions under cadmium stress are the major reasons for tolerance in black nightshade in this study [13].

Abiotic stresses can increase oxidative stresses by raising H_2O_2 and signs of membrane damage and plants can improve resistance by controlling ROS such as H_2O_2 under stress conditions. In addition, H_2O_2 can enhance the valence of osmotic adjustment. The increase in H_2O_2 content may serve as a signal for the plant to recuperate from the effects of stress. In turn, a stronger antioxidant system can lower H_2O_2 levels, which can significantly lower H_2O_2 and MDA levels In addition, H_2O_2 can enhance the valence of osmotic adjustment. The increase in H_2O_2 content may serve as a signal for the plant to recuperate from the effects of stress. In turn, a stronger antioxidant system can lower H_2O_2 levels, which can significantly lower H_2O_2 and MDA levels MDA, a lipid peroxidation product, is a well-established biomarker of free radicals in plant tissues. Reduced H_2O_2 and MDA levels as a result of higher TCar, osmolyte content and gene expression can ultimately improve plant tolerance to various stressors. MDA and H_2O_2 (oxidative parameters) improved in this study in response to cadmium stress at all examined levels, which is consistent with the findings of Gill and Tuteja and Yildirim, et al. In this study, 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1} and 50 mg Cd kg^{-1} cadmium caused increased oxidative stress but did not result in a significant decrease in growth parameters; this may be due to the fact that black nightshade is a cadmium-tolerant plant, as evidenced by increased osmolyte contents, total carotenoid, P5CS and W36 expression (**Figures 1 and 2**).

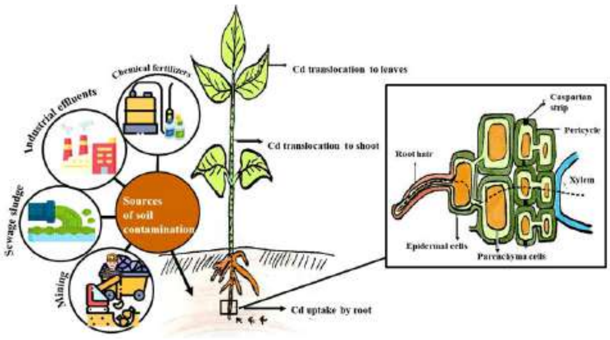

Figure 1: Source, uptake and translocation of cadmium from soil to root, shoot and leaves and mechanism underlying the mobilization of cadmium through root hairs to the xylem vessel.

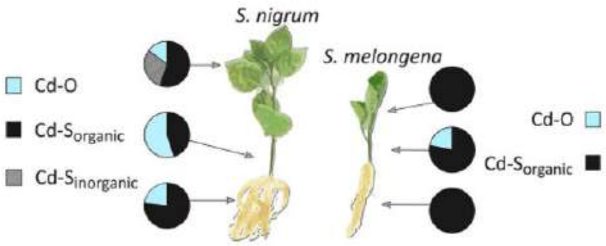

Figure 2: X-ray absorption spectroscopy evidence of sulfur-bound cadmium in the Cd-hyperaccumulator *Solanum nigrum* and *Solanum melongena*.

The success of phytoextraction is dependent on the plant's ability to rapidly acquire biomass and store substantial amounts of heavy metals in the shoot tissue. The biomass of black nightshade, while the amount of cadmium accumulations in shoots and roots. Black nightshade, a putative cadmium hyperaccumulator, only accumulated 18.6 mg/kg, 30.0 mg/kg, 62.2 mg/kg and 174.1 mg/kg DW when exposed to 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1}, 50 mg Cd kg^{-1} and 100 mg Cd kg^{-1}, corresponding to 19.4 mg/kg, 27.8 mg/kg, 70.6 mg/kg and 163.3 mg/kg DW in roots. When the soil Cd concentration achieved 25 mg Cd kg^{-1}, most cadmium accumulators or hyperaccumulators demonstrated a substantial reduction in plant biomass; however, in this study, the shoot, root and total biomass of black nightshade did not decrease considerably under 50 mg Cd kg^{-1} treatment. Cadmium concentrations accumulated in plant shoots over 100 mg Cd kg^{-1} Dry Weight (DW) are frequently employed as one of the criteria for defining cadmium hyperaccumulator. Only a few species, *S. alfredii, V. baoshanensis, S. nigrum* and *B. pilosa*, are identified to be cadmium hyperaccumulators and some of the cadmium hyperaccumulators also demonstrates toxic symptoms and significant biomass loss when their shoot or root faced with cadmium concentration over 100 mg kg^{-1} DW. Under 100 mg Cd kg^{-1} stress, shoot cadmium concentrations in *S. nigrum* exceeded as much as 174.1 mg kg^{-1} DW without toxic symptoms or significant loss of shoot biomass and root cadmium concentrations reached as high as 163.3 mg kg^{-1} DW without significant loss of root biomass [14].

Under a 32 mg Cd kg^{-1} treatment for 120 days, *B. pilosa* L., a suspected cadmium hyperaccumulator, only demonstrated a cadmium accumulation of 26.5 mg m^{-2} in shoot. *Malva sinensis* cavan, was identified as having the highest levels of cadmium accumulation in plant shoots, with only 22.3 mg m^{-2} and 44.7 mg m^{-2} after 60 days of exposure, while soil cadmium treatments approached 125 mg kg^{-1}.

The translocation factor, which is a crucial criterion for categorizing plants for their remediation ability, determines a plant's ability to translocate heavy metals from roots to shoots. Contaminants can be removed by a biological substrate, such as bacteria, fungi, algae or vascular plant surfaces, using biosorption techniques based on the passive binding of cadmium or other contaminants on cell wall surfaces carrying particular active functional groups. The translocation factor in black nightshade was about one in the current investigation for 12.5 mg Cd kg^{-1}, 25 mg Cd kg^{-1}, 50 mg Cd kg^{-1} and 100 mg Cd kg^{-1} treatments. Additionally, black nightshade can be defined as cadmium accumulators, the shoot cadmium concentrations were higher than the root concentrations in all cadmium treatments, indicating a strong capacity for phytoremediation, which is the use of living plants to remove cadmium from the environment or render it less toxic. Plants with TF values less than one show that cadmium transfer to the shoots is limited. In this study, the TF value for black nightshade was approximately one, indicating that cadmium transfer from root to shoot is not limited. Since heavy metals can easily travel in much higher concentrations to shoots from roots, these plants can be used to remediate contaminated area by harvesting their shoots. But so far, the extent and processes of cadmium transfer in the phloem remain unknown. Concluded that the contributions of xylem-derived cadmium to fruit require more investigation; however, in this study, the cadmium accumulation was investigated only in vegetative stage [15].

CONCLUSION

It is possible to estimate a plant's ability to survive in a contaminated site by analyzing a number of growth parameters, such as root growth and toxic visible symptoms; shoot biomass; Growth Rate (RGR) and leaf structural properties; root density; plant biomass. In this study, we found that there were no visible symptoms of Cd toxicity in the leaves of black nightshade. We also observed that black nightshade showed a non-significant decline in plant biomass under cadmium levels ($\leq$ 50 mg Cd kg^{-1} DW). As indicated by the data of shoot, root and total biomass, the black nightshade was tolerant to cadmium stress.

The obtained results showed that black nightshade has the ability to accumulate Cd in its tissues (shoots and roots). The value of TF for Cd was ≥ 1 for the treatments of Cd higher than 25 mg kg^{-1} indicating its ability for Cd uptake. The translocation factor emphasizes the ability of black nightshade for accumulating much amounts of Cd. Additionally, our study showed black nightshade can tolerate and accumulate Cd through an increased non-enzymatic defense system (TCar, PRO, TSC and TSP), some effective genes (*P5CS* and *W36*) which resulted in unchanged growth parameters and photosynthesis capacity.

Moreover, black nightshade has great advantages in their excellent ground cover, wide geographical distribution, easy maintenance and high-shoot biomass, indicating that black nightshade could be potentially used for phytostabilization in cadmium-contaminated soils. This application is particularly useful for barren mining land and garbage dump, where black nightshade can tolerate their harsh soil conditions.

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