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Impact of cadmium ions on some antioxidant compounds and their biosynthetic enzymes in *Spergularia marina*

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Abstract : Heavy metals accumulation in plants triggers oxidative stress. Priming *Spergularia marina* L seeds in cadmium chloride ($CdCl_2$) solution for varying time periods resulted in increased amino acid, proline content as an antioxidant compared to the control. The activities of pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), two enzymes involved in proline biosynthesis, were enhanced in response to cadmium exposure. Additionally, anthocyanin content, another antioxidant agent, was elevated in seeds primed with $CdCl_2$. This rise in anthocyanin dye may play a key role in reducing cadmium toxicity by scavenging free radicals or sequestering Cd^{2+} ions in vacuoles. The activity of anthocyanin dye synthase increased after priming the seeds with the seaweeds.

keywords: cadmium, *Spergularia*, antioxidant, proline,anthocyanin.

1.Introduction

Plants are negatively impacted by various environmental factors that restrict their growth and development. Among these, heavy metals stress, is one of the most significant abiotic stresses, having drawn considerable attention over the past 30 years [1]. Heavy metals are defined as elements with a high density that can exert toxic effects even in trace amounts. Specifically, heavy metals fall into a category of metals with an atomic density greater than 5 g cm^{-3}[2]. Because it is easily absorbed by the cells of many plant species, cadmium (Cd) stands out among heavy metals for its important role in food chain pollution. [3]. Cd is highly soluble in water, allowing it to pass through the semi-permeable membranes of root cells via active transport, using a pathway similar to that of zinc transport [4].The movement of Cd ions across these membranes is facilitated by metal transporters, specialized transmembrane proteins that assist in transporting metals across biological membranes [5].

Cadmium (Cd) contamination in the biosphere has toxic effects on plants, inhibiting growth, damaging the root system, and causing chlorosis and leaf necrosis [6]. Cd exposure also hampers seed germination and root elongation [7]. Furthermore, Cd disrupts various physiological processes, including respiration, photosynthesis, water transport, and gas exchange, leading to metabolic impairment in plants [8]. Although Cd is a non-redox metal that cannot directly participate in Fenton-type reactions, ample evidence shows it can indirectly promote the production of reactive oxygen species (ROS) by disrupting electron transport, a major process in photosystem II [9].

Plants, like many aerobic organisms, can adapt to and counteract oxidative stress induced by heavy metals. One such response to heavy metal toxicity is the increased production of both enzymatic and non-enzymatic antioxidants, which play a crucial role in mitigating oxidative stress and neutralizing free radicals [6].

The current study's goal was to investigate the impact of Cd on certain non-enzymatic antioxidants, including proline, anthocyanin, and reduced glutathione (GSH), as well as the activity of their biosynthetic enzymes in *Spergularia* leaves.

2-Materials and methods

Experimental plant

The experimental plant was *Spergularia marina* L. (Family: Caryophyllaceae).

Seed germination and plant growth

The collected seeds were germinated according to [10] and the plants seedlings were treated with cadmium chloride at the various tested concentrations being 0, 50, 100, 150 and 200 µmoL according to [11].

Preparation of plant extract

Fresh plant leaf samples (3 g) were homogenized in distilled water at room temperature then centrifuged at 200 rpm for 20 minutes, and the supernatant was collected for the determination of proline [12],reduced glutathione (GSH) [13] and anthocyanin [14].

Preparation of enzyme extract

Fresh plant leaf samples (2 g) were ground in 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA and 2 mM DTT. After centrifuging at 10 rpm for 10 minutes at 4 ºC to remove debris, the supernatant was collected for enzyme activity analysis [15]. (P5CS) and (P5CR) activities were determined using the method of [16]. While anthocyanin synthase activity was measured according to [17].

3-Results and Discussion

Table 1: Impact of Cd on proline content in plant leaves

Cd concentrations (µmol)	Proline content (mg g^{-1} F. Wt)
0	10.0±0.3
50	12.6±0.4
100	15.6±0.4
150	17.8±0.5
200	16.0±0.7

Table 2: Impact of Cd on P5CS activity in plant leaves.

Cd concentrations (µmol)	P5CS (Umg^{-1} protein)
0	12.5±0.2
50	15.5±0.4
100	18.4±0.4
150	22.3±0.5
200	19.0±0.4

Table 3: Impact of Cd on P5CR activity in plant leaves.

Cd concentrations (µmol)	P5CR (Umg^{-1} protein)
0	15.0±0.4
50	18.9±0.3
100	22.0±0.5
150	26.4±0.6
200	20.0±0.4

Table 4: Impact of Cd on anthocyanin content in plant leaves.

Cd concentrations (µmol)	Anthocyanin (mg g^{-1} F.Wt)
0	7.5±0.2
50	9.5±0.2
100	11.4±0.3
150	13.6±0.4
200	12.0±0.4

Table 5: Impact of Cd on AS activity in plant leaves.

Cd concentrations (µmol)	AS (Umg^{-1} protein)
0	12.5±0.2
50	15.5±0.4
100	18.4±0.4
150	22.3±0.5
200	19.0±0.4

Table 6: Impact of Cd on GSH in plant leaves.

Cd concentrations (µmol)	GSH (mgg^{-1}F.Wt)
0	7.5±0.2
50	9.0±0.4
100	13.6±0.5
150	18.0±0.5
200	15.0±0.4

Since Cadmium (Cd) is a heavy metal with no known beneficial role in plant metabolism [18]. It has been reported to generate reactive oxygen species (ROS), including superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), through indirect pathways [19].

The proline content, acting as an antioxidant (Table 1), increased in *Spergularia* leaves under Cd stress in a concentration-dependent manner. Cd stress also enhanced the activities of two enzymes involved in proline synthesis: pyrroline-5-carboxylate synthetase (P5CS) (Table 2) and pyrroline-5-carboxylate reductase (P5CR) (Table 3). Heavy metal stress impacts water balance in plants and induces a significant increase in proline, which helps in osmotic regulation at the cellular level [20]. Several studies suggest that various abiotic stresses trigger plants to produce and accumulate high levels of proline in their tissues as a mechanism to resist stress.

Prolyl residues in proteins also offer protection against oxidative stress caused by $^1O_2$, and proline may help preserve proteins, DNA, and membranes due to its function as a singlet oxygen ($^1O_2$) quencher (81) [21]. Proline is an essential osmotic protective substance, playing a crucial role in maintaining cell function, maintaining the stability of biological macromolecules and the integrity of cell membranes [22].

In *Spergularia* leaves, anthocyanin content increased under Cd treatment at lower concentrations but decreased at higher concentrations (Table 4). Anthocyanin synthase activity followed a similar trend to that of anthocyanin (Table 5). The cytoplasm produces anthocyanins, which are generally non-photosynthetic pigments that are kept in the vacuolar lumen of epidermal cells. Because of their distinct molecular structure, anthocyanins and anthocyanidins have stronger antioxidant qualities than other flavonoids. These substances' capability to bind metal ions implicated in the generation of free radicals, therefore lowering metal-induced peroxidation, is what gives them their antioxidant potential. [23,24].

Furthermore, because of their positive charge, the quantity and location of hydroxyl and methoxyl groups, and the existence of substituents that donate and remove electrons, anthocyanins are very good at giving hydrogen to free radicals and reactive oxygen species.

This process detoxifies the radicals and prevents further radical formation, thereby protecting essential biomolecules—such as proteins, DNA and lipids from oxidative damage, which can cause different diseases and aging [25].

Different studies, including, animal models, human clinical trials and cell culture experiments have demonstrated that anthocyanidins and anthocyanins possess antioxidative and antimicrobial properties, enhance neurological ,visual health, and offer protection against different non-communicable diseases [26]. Additionally, It has been noted that Cd^{2+} can promote the synthesis of the enzyme glutathione-S-transferase (GST), which in turn increases the synthesis of anthocyanins [27].

The last stage of anthocyanin biosynthesis is catalyzed by the essential enzyme GST.As demonstrated by the elevated GSH levels, exposure to Cd has been demonstrated to stimulate glutathione production (Table.6). Given that increased Cd concentrations can adversely influence biomass, plant height, and the tolerance index, this has important ramifications for plant growth and health. Since GSH is the substrate for phytochelatin production, which forms complexes with Cd and sequesters the metal within the vacuole, an increase in GSH is essential for Cd detoxification. [28].

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