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Proteomic Analysis of Cadmium Responsive Proteins in Wheat

Buğdaydaki Kadmiyuma Duyarlı Proteinlerin Proteomik Analizi

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ABSTRACT

The current study aims to assess the changes in protein abundance in wheat roots upon exposure to cadmium, using a proteomic approach. Wheat seeds were cultivated in a nutrient solution comprised specific macro and micronutrients under controlled environmental conditions and treated with 30 µM Cd for three days. In order to comprehend the impact of Cd stress on the protein level in wheat, a differential proteomics investigation was conducted utilizing two-dimensional polyacrylamide gel electrophoresis (2-DE). Fifty two protein spots were clearly identified and exhibited a consistent and significant alteration between the control and stressed samples. Out of the fifty two proteins, twenty seven exhibited changes in abundance following Cd stress, with seventeen proteins being up-regulated and ten proteins being down-regulated. Differentially regulated proteins were selected after image analysis and identified using MALDI-TOF MS. The identified differential proteins were primarily associated with stress response (41%) and metabolism (35%). These proteins were discovered to play a role in various processes, including protein biosynthesis, carbon metabolism, transportation, and stress response. The findings from our proteomics analysis indicate that Cd stress significantly impacts the stress response in wheat. This study provides novel insights that contribute to an enhanced comprehension of the molecular mechanisms implicated in the plant's reaction to cadmium-induced stress.

Key Words

Cadmium, wheat, stress response, proteomic, root.

ÖZ

Bu çalışma, proteomik bir yaklaşım kullanarak, kadmiyuma maruz kaldıktan sonra buğday köklerindeki protein bolluğundaki değişiklikleri değerlendirmeyi amaçlamaktadır. Buğday tohumları kontrollü çevre koşulları altında belirli makro ve mikro besin maddelerinden oluşan bir besin çözeltisinde yetiştirilmiş ve 3 gün boyunca 30 µM Cd ile muamele edilmiştir. Cd stresinin buğdaydaki protein seviyesi üzerindeki etkisini anlamak için, iki boyutlu poliakrilamid jel elektroforezi (2-DE) kullanılarak bir diferansiyel proteomik araştırması yapılmıştır. Elli iki protein beneği açıkça tanımlanmış ve kontrol ve stres altındaki örnekler arasında tutarlı ve önemli bir değişiklik sergilemiştir. Bu elli iki proteinden yirmiyedisi Cd stresini takiben bollukta değişiklik göstermiş, onyedi protein yukarı regüle edilirken 10 protein aşağı regüle edilmiştir. Diferansiyel olarak regüle edilen proteinler görüntü analizi ile seçildikten sonra MALDI-TOF MS kullanılarak tanımlandı. Tanımlanan farklı proteinler öncelikle stres (%41) ve metabolizma (%35) ile ilişkilendirilmiştir. Bu proteinlerin protein biyosentezi, karbon metabolizması, taşıma ve stres tepkisi dahil olmak üzere çeşitli süreçlerde yer aldığı bulunmuştur. Proteomik analizimizden elde edilen bulgular, Cd stresinin buğdaydaki stres tepkisini önemli ölçüde etkilediğini göstermektedir. Bu çalışma, bitkinin kadmiyum stresine verdiği yanıtın altında yatan moleküler mekanizmaların daha iyi anlaşılmasına katkıda bulunan yeni bilgiler sağlamaktadır.

Anahtar Kelimeler

Kadmiyum, buğday, stres tepkisi, proteomik, kök.

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INTRODUCTION

Heavy metals possess a high level of toxicity and are prevalent environment pollutants, posing a significant risk to both ecological balance and human health. Cadmium (Cd), ranked as the sixth most toxic heavy metal as identified by the Agency for Toxic Substances and Disease Registry (ATSDR), has garnered significant scientific interest due to its potential to harm human health [1]. Cd leaks into the environment from several sources, including fertilizers, industrial plants and sewage. After entering the human body, this metal will persist and accumulate over the course of an individual's lifetime. Cd, present in the environment, will endure in soils and sediments for numerous decades. Plants will gradually absorb these metals, leading to their accumulation and concentration along the food chain, ultimately impacting the human body [2]. The majority of chemical contaminants present in soil and water have been found to interfere with the growth and development of crops, leading to a subsequent reduction in crop productivity. Recent studies have highlighted the detrimental impact of heavy metals, particularly Cd, on wheat, with heavy metals being shown to impede both root and shoot growth. [3-5].

Cd, which is highly toxic and serves no biological purpose in plants, can cause a range of issues including decreasing chlorophyll content and photosynthetic activity [6], inhibition in transpiration, ion balance disruption, and producing low-quality products [7]. Cd is a dangerous heavy metal that has the potential to be absorbed by the roots of plants, resulting in adverse impacts on the growth and development of the plants. Roots are the initial point of contact for Cd ions, and research has demonstrated that plants employ a primary defense mechanism to prevent the entry of Cd into root tissues. Plants have the ability to adjust to unfavorable conditions by undergoing various physiological, cellular, and molecular processes, ultimately providing stress tolerance. While numerous research studies have been conducted on the topic of physiological aspects of plant stress responses, there is a few proteomics research, specifically focusing on wheat roots.

Proteomics, a new field within the realm of 'omics', has experienced rapid growth, particularly in the therapeutics sector. It encompasses the study of protein interactions, functions, compositions, structures, as well as their cellular activities [8]. Compared to genomics, proteomics offers a more comprehensive understanding of an organism's structure and function. It serves as a valuable instrument for comprehending the entirety of protein alterations across diverse organisms, and can also facilitate the comparison of variations in protein profiles at various levels, including organs, tissues, cells, and organelles. This includes studying the effects of stress, such as heavy metal stress, on protein expression [9]. Proteomics, a valuable analysis technique, has been employed to examine the alterations in proteins caused by variations in environmental factors, with the aim of elucidating the resistance mechanism of wheat under Cd-induced stress. The primary objective of this research is to ascertain the proteins that exhibit significant regulation under the influence of Cd stress in the roots of wheat plants. Furthermore, the aim of this study aims to enhance our perceptiveof the molecular mechanisms that regulate plant reactions to Cd stress at the protein level.

MATERIALS and METHODS

Plant growth and stress treatment

Wheat seeds (*Triticum turgidum* L. *durum*, cv.Balcali-2000) were subjected to surface sterilization using a 1% (w/v) calcium hypochlorite solution for a duration of 10 minutes, followed by rinsing with distilled water. Subsequently, the seeds were germinated in darkness for a period of five days in perlite that had been moistened with a saturated solution of calcium sulphate ($CaSO_4$) at room temperature. Saturated $CaSO_4$ was used in order to reduce the fragility percentage of the seeds. Following this, the germinated seeds were transplanted into 2.5 L plastic pots containing continuously aerated nutrient solutions. These nutrient solutions comprised specific macro and micronutrients, including 0.88 mM K_2SO_4, 0.2 mM KH_2PO_4, 0.2 µM $CuSO_4$, 2 mM $Ca(NO_3)_2$, 100 µM Fe-EDTA, 1.0 mM $MgSO_4$, 0.1 mM KCl, 1.0 µM $ZnSO_4$, 1.0 µM H_3BO_3, 1.0 µM $MnSO_4$, and 0.02 µM $(NH_4)6Mo_7O_{24}$. Plants were cultivated for a period of seven days in a growth chamber, where environmental conditions such as light/dark cycle (16/8 h), relative humidity (60/70%), temperature (24/22°C), and photon flux density (600-700 µmol m^{-2} s^{-1}) were carefully controlled. Nutrient solutions were replaced every three days, and the growth medium was enriched with 30 µM Cd) exists in the chemical form of cadmium sulfate ($CdSO_4$), with the medium being renewed every three days. Following the application of Cd for three days, plants were harvested after total 15

daysand the roots and shoots were gathered individually. For each concentration of Cd, including the control group, three separate sets of five pots were arranged. The roots were initially rinsed with 2 mM $CaCl_2$ for approximately 15 minutes to eliminate surface-absorbed Cd, followed by a rinse with distilled H_2O. Subsequently, the roots designated for dry matter and Cd concentration determination were dried at 70°C. Furthermore, 0.2 g of roots from each pot were promptly frozen in liquid nitrogen and maintained at a temperature of -80°C for RNA extraction.

Protein isolation

To isolate total protein for two-dimensional electrophoresis (2-DE), 1 gram of plant samples from plants treated with varying concentrations of Cd were pulverized using liquid nitrogen in cold mortars maintained at -80°C. Subsequently, 3 ml of cold extraction buffer comprising 700 mM Sucrose, 2% β-Mercaptoethanol, 500 mM Tris-HCl, 1 mM PMSF, 100 mM KCl and 50 mM EDTA was added to the samples without allowing them to thaw, followed by homogenization for 30 seconds. The resulting homogenates were then transferred to 15 ml falcon tubes, to which 3 ml of Tris-buffered phenol were introduced and agitated for a duration of 10 minutes at ambient temperature. The samples were subsequently centrifuged at 15,000 rcf for 15 minutes at a temperature of +4°C, and the resultant supernatant was then transferred to a separate falcon tube. Subsequently, 12 ml of precipitation solution was added to the supernatant to precipitate the phenol-extracted proteins, and the mixture was left at -20°C overnight. The samples were then centrifuged at +4°C at 15,000 rcf for 30 minutes, and the resulting pellet was washed three times with cold precipitation solution, followed by cold 100% acetone. After each washing step, the pellet was centrifuged at 15,000 rcf at +4°C for 15 minutes. Following this, an equal volume (3 ml) of extraction buffer was added to the aqueous phase, and the mixture was centrifuged for 15 minutes at 15,000 rcf at +4°C, after which the pellet was allowed to dry. The final pellet was resuspended in 0.2-0.5 ml of IEF rehydration buffer (comprising 65 mM DTT, 7 M urea, 4% CHAPS, 2 M thiourea, and 2% pH 3-10 ampholytes) at 25°C through pipetting and vortexing. The sample was then incubated for 1 hour at room temperature with agitation. Following this, the protein concentration was assessed using the Bradford method, and the sample was preserved at -80°C until it was employed for 2-DE.

Two-dimensional electrophoresis

A total of 400 µg of protein samples were combined with a rehydration solution comprising 4% CHAPS, 7 M urea, IPG buffer (2%V/V, pH 3-10), 2 M Thiourea, and 65 mM DTT, resulting in a final volume of 400 µL. The mixture was then applied to immobilized pH gradient (IPG) strips (17 cm, pH 3-10 nonlinear gradient) for isoelectric focusing (IEF) at 20°C. The IEF process involved sequential steps at different voltages and durations: 200 V for 300 Vhours, 500 V for 500 Vhours, 1000 V for 1000 Vhours, 4000 V for 4000 Vhours, 8000 V for 24000 Vhours, and 8000V for 30000 Vhours. Subsequently, the strips were equilibrated in reduction solution (0.375 M Tris pH 8.8, 2% SDS, 20% glycerol, 6 M urea and 2% DTT) for 15 minutes, followed by alkylation solution (0.375 M Tris pH 8.8, 2% SDS, 6 M urea, 20% glycerol, and 125 mM iodoacetamide) for another 15 minutes. The second dimension separation was carried out using 12% polyacrylamide gels, and the resulting 2-DE gels were stained with Coomassie Brilliant Blue (CBB) solution.

Image analysis of 2-D gels

It was employed the PDQuest 8.0.1 software from Bio-Rad, USA for the analysis of 2-DE gels to quantify spots. The spot detection parameters were adjusted to reduce false positive detection and enhance accurate spot detection. Our analysis focused on identifying spots with qualitative (presence/absence) and quantitative changes of ≥1.5-fold increase/decrease.

Protein identification by MALDI-TOF-TOF

The process of identifying proteins was conducted through the extraction of gel spots stained with CBB. The spots were removed from the gel, fragmented, and subjected to two washes with a solution of 5% acetic acid and 50% (v/v) methanol until colorless. The gel fragments, which had been destained, were dehydrated using acetonitrile (ACN), then exposed to 10 mM concentration of dithiothreitol (DTT) in a solution containing 100 mM NH_4HCO_3 for a duration of 30 minutes at ambient temperature. Following this, they were treated with 100 mM iodoacetamide in 100 mM NH_4HCO_3 for 30 minutes in a light-free environment. Following dehydration with ACN and rehydration with NH_4HCO_3, the gel fragments were digested with a trypsin solution (20 µg) in 100 mM NH_4HCO_3 and the sample was placed in an incubator at a temperature of 37°C for the duration of one night. The peptides were extracted from the gel slices twice using a solution consisting of 5% formic acid

in 50% ACN. The peptide solution underwent desalting through ziptip purification. Following this, mass spectrometry (MS) analysis was conducted utilizing a MALDI-TOF-TOF instrument (Bruker Autoflex III Smartbeam), and the resultant spectra were processed and analyzed employing the BioTools software (Bruker Daltonics). Individual database searches were conducted using an in-house MASCOT server provided by Matrix Science.

RESULTS

In previous study, the impact of Cd on the growth of wheat seedlings was assessed through the application of varying concentrations of the element. It was observed a clear dose-dependent root growth inhibition even at low Cd concentration and higher Cd accumulation in roots than that of shoots. The findings suggested that the wheat root exhibited a higher susceptibility to Cd toxicity compared to the shoot [10]. Total proteins were extracted from the roots of both control and cadmium-treated plants and subsequently separated using 2-DE. (Figure 1).

Each gel stained with CBB displayed clearly separated protein spots. The PDQuest software was utilized to compare the protein expression patterns and spot intensities between the control and treated samples, revealing that 52 protein spots exhibited a 1.5-fold difference in abundance compared to the corresponding control spots. The average number of protein spots in the root on the 2-DE gels for the Cd treatment and control was 32 and 20, respectively, with 17 up-regulated and 10 down-regulated protein spots. Subsequently, MALDI-TOF MS analysis was performed on the 27 differentially expressed protein spots. (Figure 1).

In the process of identifying proteins through peptide mass fingerprints (PMF), we employed the MASCOT software to search the protein database. Subsequently, all protein spots were effectively recognized using MALDI-TOF-TOF-MS (Tables 1 and 2, Figure 1). The functions of the seventeen proteins that were significantly up-regulated in the Cd treatment were categorized as stress response (41%), metabolism (35%), energy (18%) and unknown function (6%) (Table 1). Seven proteins were involved in the stress response: heat shock cognate protein (spot1), Heat shock 70 kDa protein 3 (spot2), HSP70 (spot3), ascorbate peroxidase (spot23), Formate dehydrogenase (spot25), peroxidase 10 (spot29), peroxidase 11 (spot30). The other six proteins were involved in metabolism: UTP--glucose-1-phosphate uridylyltransferase (spot12), Eukaryotic initiation factor 4A-1 (spot13), S-adenosylmethionine synthase (spot15), glutamate-ammonia ligase (spot19), Glutamine synthetase cytosolic isozyme (spot20), probable acetoacetyl-CoA synthetase (spot24). Other proteins that were found to be up-regulated and are involved in energy include ATP synthase subunit beta (spot10), ATPase subunit (spot14), and ATPase subunit (spot26).

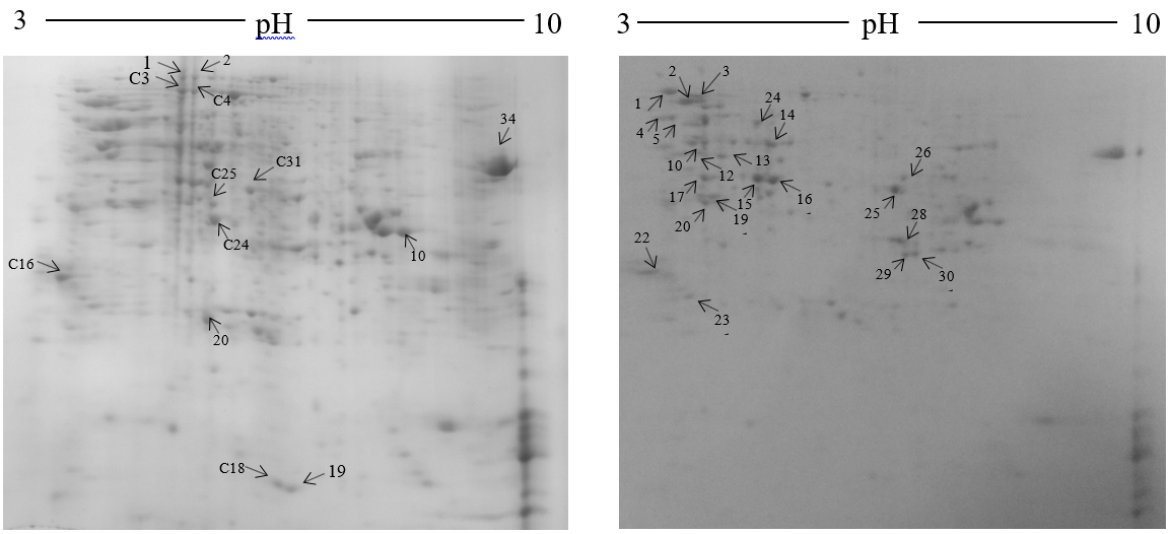

Figure 1. Two-dimensional electrophoresis (2-DE) maps depicting wheat proteins obtained from both cadmium (Cd) treated and control groups are presented. The total protein content of roots was extracted and subsequently separated using 2-DE. During isoelectric focusing (IEF), 400 µg of proteins were applied onto pH 3–10 immobilized pH gradient (IPG) strips measuring 24 cm in a linear configuration. Subsequently, SDS-PAGE was conducted using 12.5% gels. Visualization of protein spots was achieved using Coomassie blue staining. The maps display fifteen spots with elevated expression (A) and eleven spots with reduced expression (B).

Table 1. Proteins of roots whose expression was notably increased in response to cadmium-induced stress.

Spot ID	Protein Name	Parent Ion	Accession #	Mass	Score	Biological Process
Spot 1	Heat Shock Cognate Protein	1527.8	gi|899060	79832	78	Stress Response
Spot 2	Heat Shock 70 Kda Protein 3	1680.8	HSP7C_ARATH	71103	48	Stress Response
Spot 3	HSP70	1509.9	gi|476003	66975	70	Stress Response
Spot 23	Ascorbate Peroxidase	1113.6	gi|555576	33456	62	Stress Response
Spot 25	Formate Dehydrogenase	1209.6	gi|21263612	46332	58	Stress Response
Spot 29	Peroxidase 10	1010.6	gi|57635165	40443	75	Stress Response
Spot 30	Peroxidase 11	1010.6	gi|57635165	40450	82	Stress Response
Spot 12	UTP--Glucose-1-Phosphate Uridylyltransferase	1539	gi|6136111	51612	71	Metabolism
Spot 13	Eukaryotic İnitiation Factor 4A-1	1114.7	IF4A1_ARATH	46675	65	Metabolism
Spot 15	S-Adenosylmethionine Synthase	1775.8	METK_PINBN	43141	99	Metabolism
Spot 19	Glutamate-Ammonia Ligase	1817.8	gi|99698	44678	90	Metabolism
Spot 20	Glutamine Synthetase Cytosolic İsozyme (Glutamate--Ammonia Ligase)	1552.8	gi|8928127	43665	90	Metabolism
Spot 24	Probable Acetoacetyl-Coa Synthetase	1185.6	gi|27379393	56443	52	Metabolism
Spot 10	ATP Synthase Subunit Beta	1399.7	ATPBM_DAUCA	59099	59	Energy
Spot 14	Atpase Subunit	1537.8	gi|417745	54964	90	Energy
Spot 26	Citrate Synthase	1204.7	CISY_CITMA	48776	40	Energy
Spot 28	Hypothetical Protein Noca_1038	2285.2	gi|119715279	42665	48	Unknown

The ten down-regulated proteins (Cd vs. control) belongs to metabolism (%90): Protein disulfide-isomerase (spot4), Actin-105 (spot17), 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (spotC3), methionine synthase (spotC4), Malate dehydrogenase (spotC24), Formate dehydrogenase (spotC31), Nucleoside diphosphate kinase 1 (spotC18), Protein BMH1 (spotC16), Alpha-1,4-glucan-protein synthase [UDP-forming] (spotC25), 14-3-3-Like Protein A (spot22) (Table 2).

Table 2. Root proteins of wheat whose expression were significantly down-regulated under Cd stress

Spot ID	Protein Name	Parent Ion	Accession #	Mass	Score	Biological Process
Spot 4	Protein Disulfide-İsomerase	gi|1709620	1865.8	56498	61	Metabolism
Spot 17	Actin-105	gi|3219770	1747.9	48679	85	Metabolism
Spot C3	5-Methyltetrahydropteroyltriglutamate--Homocysteine Methyltransferase	METE_MESCR	1096.5	70556	80	Metabolism
Spot C4	Methionine Synthase	gi|50897038	1157.7	70662	82	Metabolism
Spot C24	Malate Dehydrogenase	MDHC1_ARATH	1360.8	45776	78	Metabolism
Spot C31	Formate Dehydrogenase	FDH_ARATH	1083.7	40555	70	Metabolism
Spot C18	Nucleoside Diphosphate Kinase 1	NDK1_SPIOL	1609.8	18222	86	Metabolism
Spot C16	Protein Bmh1	BMH1_YEAST	1189.7	35778	70	Metabolism
Spot C25	Alpha-1,4-Glucan-Protein Synthase [UDP-Forming]	UPTG_MAIZE	1240.6	45002	65	Metabolism
Spot 22	14-3-3-Like Protein A	gi|100554	1819	38667	62	Signaling

DISCUSSION

Heavy metals have a detrimental effect on the growth and productivity of crops by negatively influencing the process of photosynthesis and also impeding root development. Cadmium (Cd) is thought to cause harm even at minimal concentrations, impacting various crucial physiological processes and diminishing the productivity and caliber of plants [11]. Proteins play a central role in biological activities and are closely linked to the phenotypic level. Consequently, it is imperative to investigate the mechanism by which wheat responds to Cd stress at the protein level. Proteomic techniques have been extensively employed to comprehensively analyze the expression patterns of proteins in plants under various abiotic stresses, such as heavy metal exposure.

Our prior research revealed that elevated levels of Cd in wheat led to a reduction in the biomass of both roots and shoots, with a more pronounced impact observed in the roots. Additionally, the roots exhibited a greater ability to accumulate Cd[10]. In this study in the root part, 17 and 10 protein spots were up-regulated and downregulated, respectively, in response to Cd stress (Figure 1).

Among the 17 root proteins with elevated expression, 7 are involved in stress response. The heat shock proteins (HSP70s) are a set of molecular chaperones that exhibit a high degree of conservation among different species and play essential roles in diverse cellular processes, particularly in response to abiotic stress. The upregulation of HSP70 expression in response to Cd exposure can be attributed to the accumulation of damaged cellular macromolecules, such as fragmented cellular membranes or misfolded proteins [12-13]. The observed increase in HSP70 levels can therefore be interpreted as an indication of cellular stress induced by Cd.
The plant's defense system against antioxidants consists of various antioxidant molecules and enzymes. One such enzyme is ascorbate peroxidase (APX), which plays a role in increasing antioxidative enzyme activities when exposed to cadmium. When plants are subjected to adverse environmental conditions, there is an elevation in the generation of Reactive Oxygen Species (ROS) , leading to cell damage. Plants have antioxidant defenses that can detoxify ROS. The ascorbate-glutathione cycle is a prominent mechanism within plant cells for the detoxification of hydrogen peroxide, with APX enzymes playing a pivotal role in the conversion of H_2O_2 to H_2O using ascorbate as an electron donor. Different APX isoforms can be found in separate subcellular locations. The regulation of APX gene expression is responsive to both biotic and abiotic stresses, as well as during plant development. The responses of APX directly contribute to the protection of plant cells against unfavorable environmental conditions. In the case of pea plants exposed to cadmium, the antioxidant defenses, both enzymatic and non-enzymatic, were affected, yet no significant differences were observed in APX activity or the accumulation of its transcripts. However, in coffee cells, APX activity increased at lower cadmium concentrations, while it was undetectable in cells exposed to higher cadmium concentrations after 24 hours of treatment [14]. The level of activity of APX were found to significantly increase in roots by exposure of Cd in *Impatiens glandulifera*, a recently identified potential Cd hyperaccumulator [15]. In another study, the expression of APX was upregulated in roots by exposure of Cd in wheat [16].

The levels of formate dehydrogenase (FDH) were also found to be elevated in response to cadmium exposure, indicating its role as an enzyme involved in defense against oxidative stress. Formate dehydrogenase likely plays a crucial role in managing an excessive production of formaldehyde and is implicated in the denitrification process of nitro compounds, which are known to accumulate under conditions of cadmium-induced stress. Under Cd exposure the level of FDH were also found to increase in other plant studies [17-18].

Peroxidases (PODs) are a class of enzymes that aid in the reduction of hydrogen peroxide (H_2O_2) by transferring electrons to various donor molecules. Within the realm of plant biology, PODs are integral to a multitude of physiological processes, including suberization, lignification, crosslinking of cell wall compounds, and defense against a range of environmental stressors, such as heavy metals. [19-20].

Six of the proteins of roots identified as upregulated were involved in metabolism (Table 1). UTP-glucose-1-phosphate uridylyltransferase, also referred to as glucose-1-phosphate uridylyltransferase, is an enzyme that plays a crucial role in carbohydrate metabolism, glycogenesis, and cell wall synthesis. The eIF4A (eukaryotic initiation factor 4A) proteins are members of the DEAD-box RNA helicase family, involved in the unwin-

ding of double-stranded RNA and binding to the 40S ribosomal subunit during the translational initiation process. Additionally, these proteins activate stress-induced pathways that contribute to stress tolerance [21]. The eIF4A genes associated with tolerance to abiotic stress have been identified in wheat. [22].

It was identified cysteine related enzyme that up-regulated in response to cadmium stress, consisting of S-adenosylmethionine synthetase (spot15). S-adenosylmethionine (SAM) is a crucial methyl donor in plants, synthesized from methionine and ATP through the catalysis of S-adenosylmethionine synthetase (SAMS). The interaction between SAMS and DNA methylation plays a significant role in the plant's response to abiotic stresses. SAMS is responsible for the synthesis of SAM, which is involved in regulating plant-environment interactions. Previous research has demonstrated that SAMS can be induced by various stress treatments and is instrumental in enhancing plant tolerance to environmental changes. The markedly induced expression of SAMS involved in cysteine biosynthesis has been observed in Cd-treated wheat roots [15]. Another important enzyme in plants is glutamine synthetase (GS), also known as glutamate-ammonia ligase and glutamine synthetase cytosolic isozyme. GS catalyzes the ATP-dependent biosynthesis of glutamine from glutamate and ammonia and is a key component in the nitrogen metabolic pathway. It is also essential for the production of reduced glutathione and phytochelatins. Rana et al. (2008) observed that the expression of cytosolic GS was induced by exposure to cadmium and salt stress. Acetoacetyl-CoA synthetase (AACS) is a pivotal enzyme involved in the anabolic utilization of ketone bodies (KBs) for de novo lipid synthesis. This process bypasses citrate and ATP citrate lyase [23].

Three of the proteins that exhibited upregulation are associated with energy-related processes (Table 1). For instance, citrate synthase is responsible for catalyzing the initial step in the citric acid cycle, also referred to as the tricarboxylic acid cycle or the Krebs cycle, wherein it facilitates the conversion of oxaloacetate and acetyl-coenzyme A into citrate and coenzyme A. Additionally, the ATP synthase subunit beta plays a role in the generation of ATP from ADP, utilizing a proton gradient across the membrane that is produced by the electron transport complexes of the respiratory chain.

CONCLUSION

The proteomic method is widely acknowledged as a reliable technology for analyzing the protein composition of plants experiencing stress. In this specific investigation, the proteomic approach was utilized to assess the changes in the root protein profile of wheat plants subjected to cadmium (Cd) treatment. Through this approach, novel candidate proteins associated with the response of wheat roots to Cd-induced stress were identified. The subsequent section delves into the potential functions of these proteins, taking into account their distribution and role in the Cd-stress response. This study showed that wheat have evolved sophisticated defense mechanisms as an adaption to Cd stress. The findings of this study enhance our comprehension of the mechanism underlying plant reactions to cadmium-induced stress.

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