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Response of Wheat Seedling Root Proteome to Salt Stress

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Abstract **– In order to reveal the molecular mechanism of wheat treated with NaCl concentration increase will reduce the activity of antioxidant enzymes and the dwarf plant and charcoal to resist high concentrations of NaCl. The application of protein electrophoresis and mass spectrometry to increase the concentration of NaCl and charcoal treated wheat roots differentially expressed proteins were analyzed. The results showed: treated with charcoal and the increase of the concentration of the NaCl, wheat roots were detected the difference in expression of 24 protein spots in Abundance changes in more than 2 times, through MALDI-TOF-TOF analysis and database searching, identified 24 protein spots can be roughly classified into 6 categories according to its function. Class I; proteins related to energy metabolism, including ATP synthase and ATP. Class II: sugar metabolism related proteins, including triosephosphate isomerase, triosephosphate isomerase, 6- phosphogluconate dehydrogenase, malate dehydrogenase and aconitate hydratase. Class III: proteins related to the metabolism of amino acids, such as cysteine synthetase. Class IV: proteins related to genetic material, such as structural proteins of the chromosomes. Class V: the pathogenesis related protein. Class VI: protein with unknown function. These findings provide a clue for the further research on the damage and recovery mechanism of wheat to resist salt stress.**

Keywords **– Salt Stress, Charcoal, Total Protein of Wheat Root, Differential Protein, Two-Dimensional Electrophoresis, Mass Spectrometry.**

I. INTRODUCTION

In recent years, due to the growth of population, industrial pollution and improper use of agricultural fertilizers and other factors, so that the increasing soil salinization, the sustainable development of agricultural production has been seriously threatened. The soil salinization, High concentration of salt ions in soil result in poor plant growth [1]. Damage to crops is shown in the structure, antioxidant enzyme system, ion channel, protein content and species, and charcoal with loose porous structure, strong adsorption, can absorb water and gas. To a certain extent, reduce the mineralization of nitrogen element in the soil, increase content of P, Ca and Mg and other elements in soil, no pollution to the environment. There is no relevant reports about the effect of charcoal on the antioxidant enzyme activities, protein content and expression of the plant body.

In the process of plant growth and development, it is often subjected to some abiotic stress, which leads to the change of protein expression and species. The response mechanism of plants to abiotic stresses, such as drought, low temperature, salt, ozone and heavy metals, was revealed by the molecular level of proteomics, which was marked by two-dimensional electrophoresis and mass spectrometry [2]. Wheat, the most widely grown crop in the world, providing nearly 20% of the protein and food nutrients to nearly 4.5 billion people a day. And wheat root system, as the first sense organ of the soil, not only first contact with the soil salt carbon, and other components, but also the first to respond to its response [3-4]. In this study, we use proteomic techniques to analyzed the differential expression of protein in Wheat Seedling Roots under NaCl stress with the increase of concentration. The aim was to reveal the damage mechanism of salt stress on wheat roots and the molecular mechanism of charcoal resistance to salt stress.

II. MATERIALS AND METHODS

2.1 Test Material

Plant materials for 6339 winter wheat (Triticum aestivum Linn), winter wheat seeds by Shanxi Academy of Agricultural Sciences Institute of Wheat.

2.2 Test Design

6339 winter wheat seeds with full grain size and uniform size were selected. MilliQ water in soaking 24~48 hours culture dish, the white, ventral canal down, evenly placed in same scale culture dish with two layers of filter paper. During the period according to the wheat light cycle in the incubator to set up (light treatment case cycle is 10 h/d, 7:00-17:00, dark incubation period is 14 h/d, 17:00- 7:00) [5].

When the wheat grow to two leavesed into the flower pot, Select the appropriate proportion of charcoal (4%) and Nutrient soil to mixed together to cultivate. The Hoagl and nutrient solution was irrigated with equal volume of NaCl (0%, 0.5%, 1%, 1.5% respectively), Wheat was removed from the pot in nine days, cut the root and weigh it.

2.3 Research Methods

2.3.1 Extraction and Preparation of Total Protein from Root of Wheat Seedlings

Urea / thiourea method is a common method for the extraction of proteins in the laboratory. Referring to the extraction method of Liu Weixia [6]. A slight improvement in the method — DTT in the extract component was omitted.

2.3.2 Two Dimensional Electrophoresis of Protein in Root of Wheat Seedling

First phase solid phase pH gradient isoelectric focusing electrophoresis: Take out the IPG prefabricated (7cm, 3-10, pH). Sample protein amount of 200 -500 ug (volume of 125-250 L). Isoelectric focusing procedure: 50V hydration 16h, 100V linear remove salt 1.5h, 300V remove salt boost 1.5h, 1000V linear boost 30 min, 3000V fast boost 30min, 6000V focus 20000Vh, 500V to maintain.

Second direction SDS-PAGE electrophoresis: Remove the adhesive strip after the isoelectric focusing is done. In the swollen disk containing 10ml buffer I (6 mol/L Urea,

2% SDS 1, 1.5 mol/L Tris-HCl (pH 8.8), 20% Glycerinum, 2% DTT) and 10 ml buffer II (6 mol/L buffer Urea, 2% SDS, 1.5mol/L Tris-HCl, 20% Glycerinum., 2.5% Iodoacetamide) shaking 15min. The 100ml agarose gel was added to the upper surface of SDS-PAGE gel（0.5% DEAE-Sepharose Fast Flow25mm Tris、192mM Glycine、10% SDS1% Bromophenol blue, Put the adhesive strip on the top surface of the second to the SDS-PAGE gel, Make the gel strip fully contact with the surface of SDS-PAGE. The gel was transferred to the electrophoresis tank when the agarose was completely solidified, When the voltage is used to start with 80V, to be observed in the protein sample run into a more smooth straight line, the voltage will be replaced by 120V continue to electrophoresis. Until the bromophenol blue indicator in rubber soled department to stop electrophoresis.

2.3.3 Coomassie Brilliant Blue Staining

After electrophoresis, remove the tape into the 500ml to fixed 30mim (95% Alcohol, 10.0% Glacial, acetic, acid.). using Coomassie brilliant blue staining (0.1% Coomassie brilliant blue R-250. 95% Alcohol 10.0% Glacial acetic acid volume to 1000 ml) In table 2h, then go to the 1000ml (95% Alcohol 5.0% Glacial acetic, destainer acid). During electrophoresis, constantly changing the bleaching solution, until the strip is clearly visible.

2.3.4 Analysis of two Dimensional Electrophoresis

The stained gels were scanned with a scanner, and the images were analyzed using 8.0.1 PDQuest software. The images of each experimental group were automatically detected and matched by cutting. Identification of differentially expressed protein spots was identified by 2 times of expression difference as st and ard (such as 2 fold increase in expression and 2 fold decrease in expression)

2.3.5 Mass Spectrometry and Database Retrieval

Cutting the gel point, using the mass spectrometer MicrOTOF-QII (Bruker Daltonics) to identify the protein. Using Data Analysis Software and Mascot search engine version 2.3.01 to analysis and search for mass spectrometry data. Generally use the software MASCOT in the TAIR database search, the search results for analysis and identification scoring less than 60 were positioned as a false positive protein should be discarded.

2.3.6 Bioinformatics Analysis

Functional classification of proteins based on protein annotation information provided by UniProtKB database (http://www.uniprot.org/)

III. Results

3.1 2-DE Map of Wheat Root Protein under Salt Stress and Charcoal Treatment

Through the use of 2-DE technology to obtain the total protein of wheat seedling roots were divided into CK group (normal treatment group), C group (charcoal treatment group), Y group (NaCl stress group), D.F group (NaCl and charcoal common treatment group) as shown in Figure 1 and Figure 2. It can be seen that the distribution of the root protein in the 2D gel is basically the same in each experimental group.

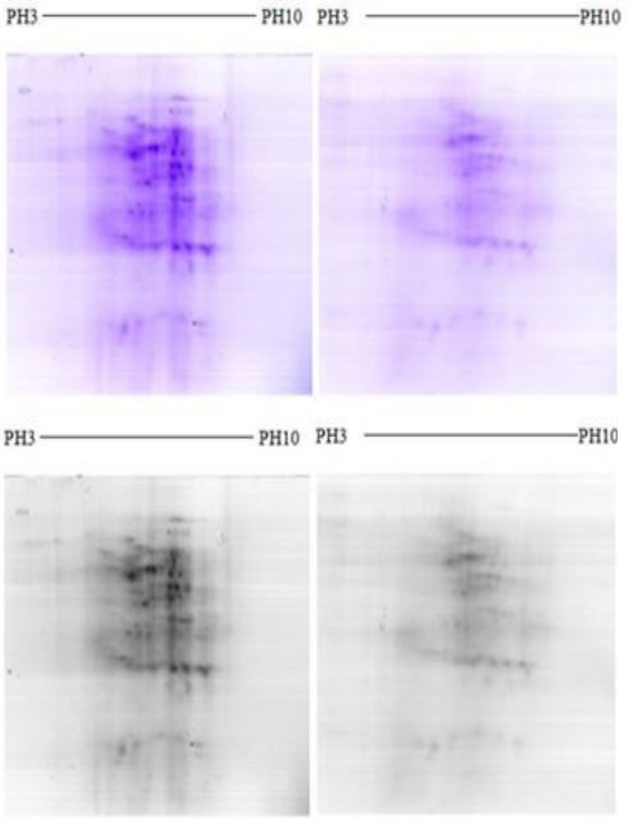

Fig.1 2-DE maps of proteins from Triticum aestivum root of CK group and C group.

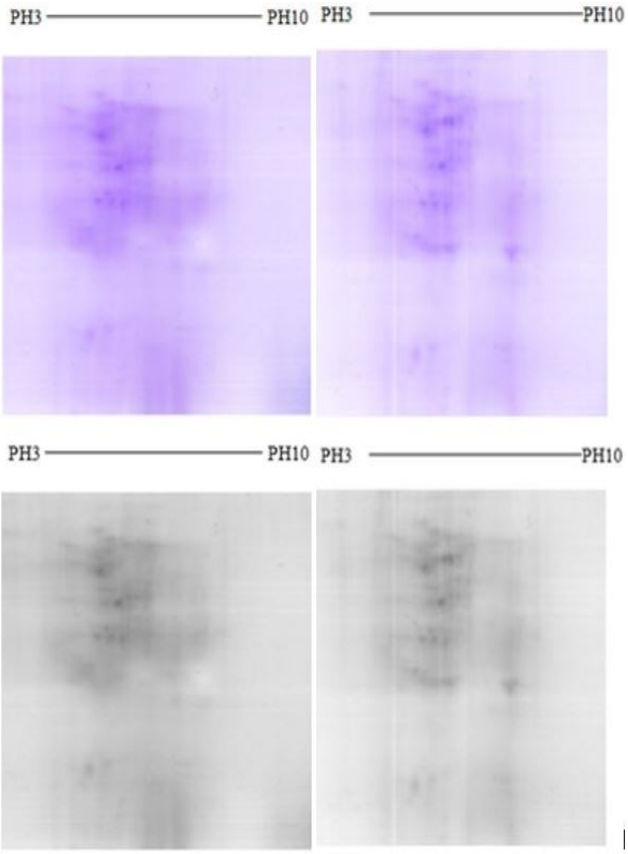

Fig. 2 2-DE maps of proteins from Triticum aestivum root of Y group and D.F group.

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3.2 Comparison of Protein Spots in Wheat Roots after Salt Stress and Charcoal Treatment

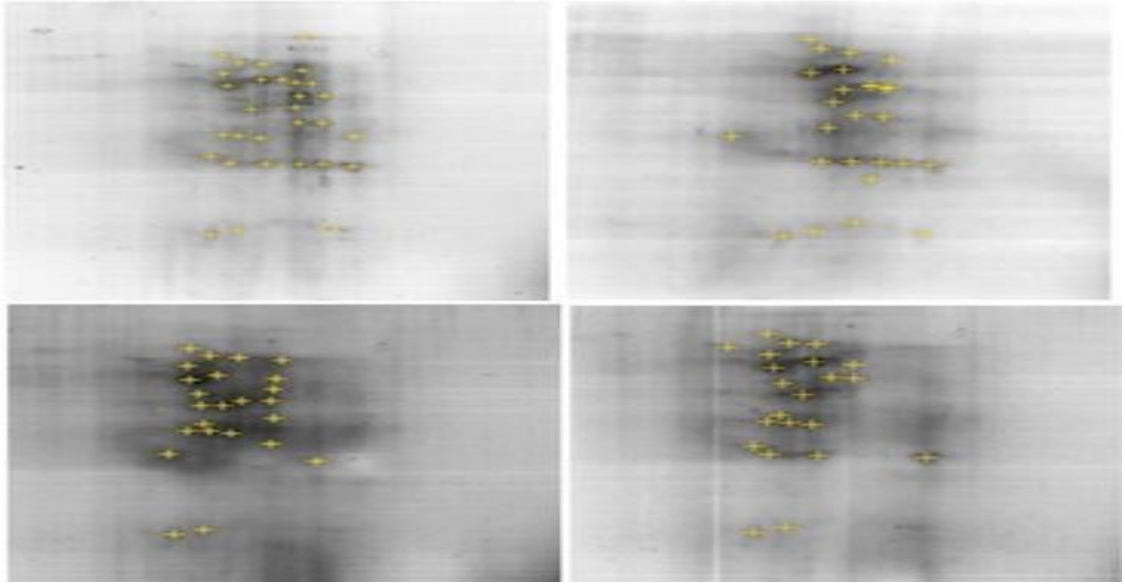

Fig. 3. The map of marking protein spots in each group
(followed by CK group, group C, group Y, group D.F.)

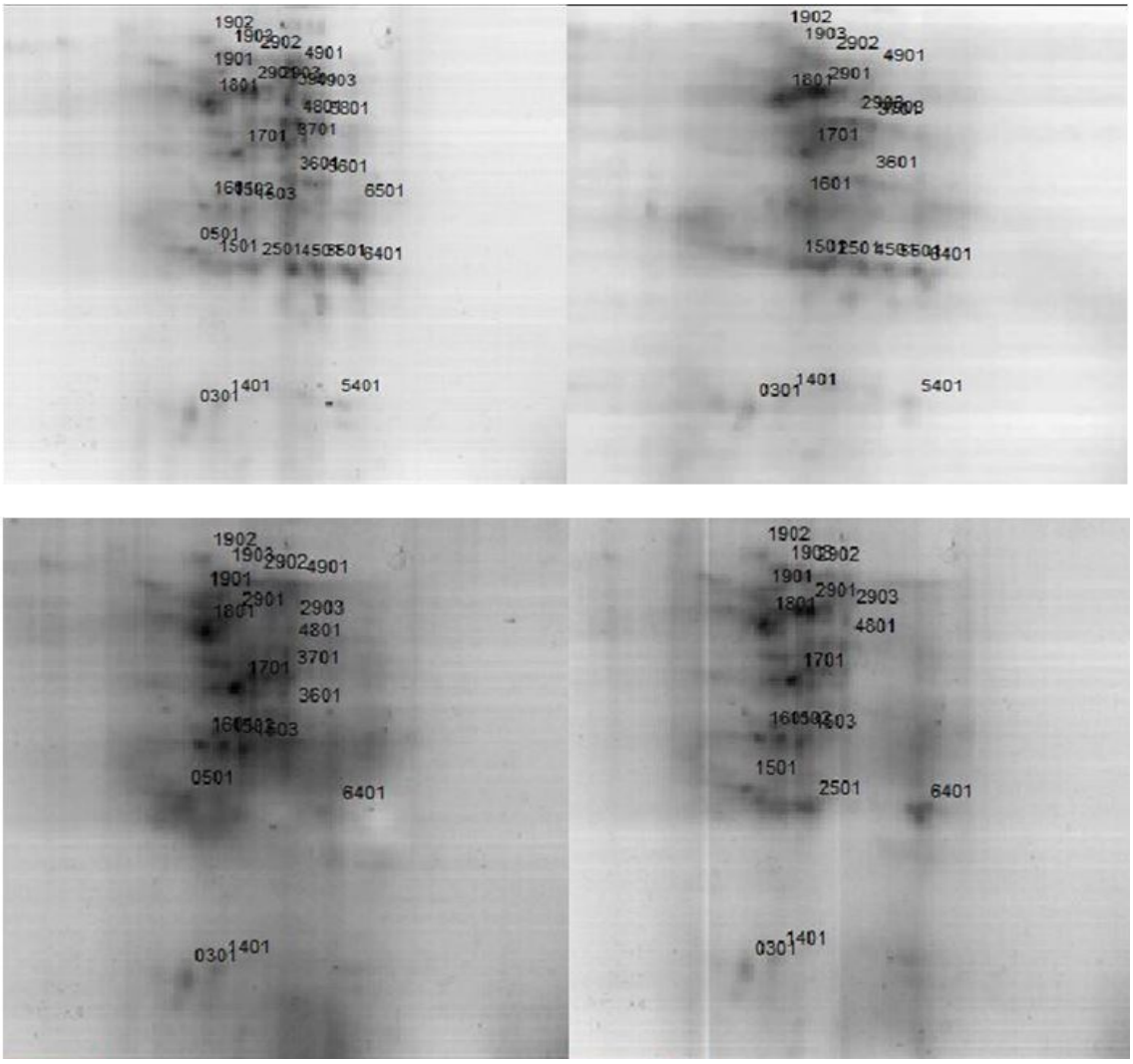

Fig. 4. The named map of spots in each group
(followed by CK group, group C, group Y, group D.F.

Analysis and comparison of 2-DE spectra with 8.0.1 PDQuest software showed that there were differences in protein expression between control group and treatment group (as Figure 3 and Figure 4). The range of molecular weight KD 14.4-97.4, PH3-10, more than 100 protein spots were detected mainly in PH3.5-7.5. The protein molecular weight is mainly concentrated in KD 30-85. Among them, compared with the CK group. The protein expression of C group was up to 8 (1902, 2901, 1401, 1801, 1701, 1601, 2501, 5401), down to10 (5501, 3601, 1501, 4901, 3701, 4501, 2903, 6401, 2902, 0301), and no expression was 10 (4903, 4801, 1901, 4902, 5601, 5801, 6501, 3901, 1503, 0501). Compared with the CK group. The protein expression of Y group was up to 1 (0501), down to18 (3601 4901, 4801, 1901, 3701, 1902, 2903, 2901, 6401, 1401, 2902, 0301, 1801, 1701, 1601, 1502, 1903, 1503), and no expression was 11 (4903, 5501, 1501, 4501, 4902, 5601, 5801, 6501, 3901, 2501, 5401). Compared with the CK group. The protein expression of D.F. group was up to 5 (1701, 1601, 1903, 1503, 2501), down to12 (1501, 4801, 1901, 1902, 2903, 2901, 6401, 1401, 2902, 0301, 1801, 1502), and no expression was 13 (4903, 5501 3601, 4901, 3701, 4501, 4902, 5601, 5801, 6501, 3901, 0501, 5401).Compared with the Y group. The protein expression of C group was up to 15 (4801, 1901, 1902, 2903, 6401, 1401, 8902, 0301, 1801, 1701, 1601, 1502, 1903, 1503), down to 0, and no expression was 4 (3601, 4901, 3701, 0501).

3.3 Mass Spectrometry Identification of Differentially Expressed Protein Spots in Wheat Roots

The differential protein spots were identified by MALDI-TOF-TOF/MS mass spectrometry, and the peptide fingerprints (PMF) were obtained. Selection of partial differential protein spots (MALDI-TOF-MS) was identified by mass spectrometry, and the expression was obtained in Table 1.

Table 1. Identification of differential roots proteins in Triticum aestivum by MALDI-TOF-TOF/MS under salt stress and charcoal treatment

Spot	Theoretical Mr	Experimental Mr	Score	Protein name
4903	763.4195	763.4228	2142	ATP synthase subunit beta OS=Triticum aestivum GN=atp2 PE=2 SV=1
0501	728.4497	728.4545	2102	Chromosome 3B, genomic scaffold, cultivar Chinese Spring OS=Triticum aestivum GN=TRAES_3BF078000040CFD_c1 PE=3 SV=1
5401	859.4926	859.4949	1220	ATP synthase subunit alpha OS=Triticum aestivum GN=atp1 PE=3 SV=1
0301	893.4814	893.4858	812	Pathogenesis-related protein OS=Triticum aestivum GN=PRP PE=2 SV=1
2501	915.4141	915.4161	1421	Triosephosphate isomerase OS=Triticum aestivum PE=3 SV=1
1901	744.3916	744.3918	3782	Vacuolar proton-ATPase subunit A OS=Triticum aestivum PE=2 SV=1
1903	707.3697	707.371	3483	Chromosome 3B, genomic scaffold, cultivar Chinese Spring OS=Triticum aestivum GN=TRAES_3BF013300030CFD_c1 PE=4 SV=1
1902	728.442	728.4432	1798	Phosphoglycerate kinase OS=Triticum aestivum PE=3 SV=1
1502	707.3588	707.3602	2577	6-phosphogluconate dehydrogenase, decarboxylating OS=Triticum aestivum PE=3 SV=1
1503	1068.5658	1068.5676	1352	Cysteine synthase OS=Triticum aestivum GN=CYS1 PE=2 SV=1
4801	699.4466	699.4531	2959	Malic enzyme OS=Triticum aestivum PE=2 SV=1
4901	721.4182	721.4235	3262	Aconitate hydratase OS=Triticum aestivum PE=3 SV=1
5801	728.4375	728.4432	2083	Phosphoglycerate kinase, cytosolic OS=Triticum aestivum PE=2 SV=1
3901	754.4337	754.4335	2266	ATP synthase subunit alpha OS=Triticum aestivum GN=atp1 PE=3 SV=1
1401	699.4643	699.4243	1430	Triosephosphate isomerase OS=Triticum aestivum PE=3 SV=1
1701	1589.8148	1589.811	719	Vacuolar proton-ATPase subunit A OS=Triticum aestivum PE=2 SV=1
2903	1387.7347	1387.7286	704	Phosphoglycerate kinase OS=Triticum aestivum PE=3 SV=1
3601	728.4545	728.4518	2211	Chromosome 3B, genomic scaffold, cultivar Chinese Spring OS=Triticum aestivum GN=TRAES_3BF078000040CFD_c1 PE=3 SV=1

According to the information of the corresponding protein spots in the UniProtKB database, the 24 differentially expressed wheat seedling root proteins were classified into six types according to their functions. Class I: proteins related to energy metabolism, including ATP synthase (as ATP synthase subunit beta, SSP4903, ATP synthase subunit alpha, SSP5401 and SSP3901) and ATP (Vacuolar proton-ATPase subunit A, SSP1901 and SSP1701). Class II: proteins associated with glucose metabolism, inclouding Phosphoglycerate kinase, SSP1902 and SSP2903, Phosphoglycerate kinase, cytosolic, SSP580, Triosephosphate isomerase, SSP2501 and SSP1401, 6-phosphogluconate, dehydrogenase, decarboxylating, SSP1502, Malic enzyme, SSP4801 and Aconitate hydratase, SSP4901, Class III: Proteins related to amino acid metabolism.(Cysteine synthase, SSP1503). Class IV; proteins associated with genetic material (Chromosome 3B, genomic scaffold, cultivar Chinese Spring, SSP0501, SSP1903 and SSP3601). Class V; proteins related pathogenesis.

Class VI: proteins of unknown function.

IV. Discussion

4.1 Optimization of two Dimensional Electrophoresis Technique

In this study, we selected two methods to extract the total protein of the plant, which were TCA/ acetone precipitation method and urea / thiourea method. In the pre experiment, the content of the protein extracted by urea thiourea method was much higher than that extracted by TCA/ acetone precipitation method, But TCA/ acetone precipitation method ran out of the SDS-PAGE b and clear, is conducive to the subsequent biological analysis of protein spots. The experiments on the plants were treated with NaCl, experimental materials of salt ion concentration is higher, so need to take measures to improve the results of two-dimensional electrophoresis. In the experiment the plants were treated with NaCl, resulting in plant salt ion concentration is higher, so it is necessary to take measures to improve the results of two-dimensional electrophoresis. As in the setting of focus program, prolong the time of desalting; choose slow step-up in the boost; in addition to a focus in the bridge plate at both ends at the electrode, and closely contact electrode for removing salt.

4.2 Effects of Salt Stress and Charcoal on the Morphological Structure and Physiological and Biochemical of Wheat Seedling Roots

Salt stress can cause dwarfism, dry weight and fresh weight decreased, affecting plant photosynthesis, plant growth and early premature aging, [7], reduce the yield of crops [8] and affect ion transport in plants. With the increase of NaCl concentration, these symptoms become more severe [9] Because of the high concentration of NaCl in the response to salt stress, changes in carbohydrate metabolism and energy metabolism, membrane transport and balance of ions, the cytoskeleton reorganization and cell wall remodeling [10]. However, the study also found that a small amount of salt stress can enhance the antioxidant enzyme system, SOD, POD and CAT and other enzymes activity increased, and high content of salt can cause the plant antioxidant enzyme activity was inhibited. Many scholars have confirmed this phenomenon: 5% salt stress, with the time of salt stress, osmotic adjustment substances (soluble sugar, proline and soluble protein) content decreased, with the extension of stress time, POD, CAT, SOD activity increased at first and then decreased [11]. Using different concentrations of NaCl in the experiment, it was found that there was a significant inhibitory effect on Winter Wheat. The addition of NaCl makes the content of ROS in plant increased sharply, and the MDA content increased, which led to the destruction of membrane system and the increase of membrane lipid peroxidation, which caused the body to be damaged [12, 13]. And the higher the NaCl content, the more severe the situation, which can cause the death of the body, which further indicates that winter wheat 6339 is a salt sensitive strain [14]. Charcoal, as a new type of fertilizer which has no two pollution to the environment, has developed rapidly in recent years [15]. The addition of charcoal can increase the number of new roots of plants, increase plant fresh weight, plant height, leaf area, promote plant differentiation, improve crop yield [16, 12], At the same time, the content of MDA in plants decreased, and the activity of SOD, POD and CAT were increased significantly. At the same time, the accumulation of soluble sugar in roots of Winter Wheat under salt stress is the adaptation to salt tolerance, or the role of signal substances, it is still necessary to further study [17].

V. Conclusion

In this study, we successfully identified 24 protein spots, according to their functions can be classified as six types of protein.

Class I: energy metabolism related proteins, including ATP synthase (such as ATP synthase subunit alpha, SSP4903; SSP5401 and SSP3901) and ATP (Vacuolar proton-ATPase subunit an enzyme, SSP1901 and SSP1701). These proteins were down regulated or not expressed under salt stress, and some of them were up-regulated in charcoal treatment, which indicated that salt stress could inhibit the synthesis of ATP and weaken the life activities of plants.

Class II: sugar metabolism related proteins, including triosephosphate isomerase (Phosphoglycerate, kinase, SSP1902, SSP2903 and Phosphoglycerate kinase, cytosolic, SSP5801), Triosephosphate isomerase, SSP2501 and SSP1401 6-phosphogluconate dehydrogenase, decarboxylating, SSP1502、Malic enzyme, SSP4801、Aconitate hydratase, SSP4901. These proteins will lead to reduced expression or no expression of protein under salt stress, while charcoal can alleviate this situation, so that some protein spots to be raised.

Class III: proteins related to the metabolism of amino acids, such as Cysteine synthase, SSP1503.Protein expression was inhibited by salt stress, while D.F. expression was up-regulated, which indicated that salt stress inhibited the synthesis of protein related to amino acids.

Class IV: proteins related to genetic material, such as Chromosome 3B, genomic scaffold, cultivar Chinese Spring, SSP0501, SSP1903 with SSP3601. These protein spots can control the genetic performance, so that the chromatin to maintain a certain structure.

Class V: Pathogenesis-related protein, SSP0301, a typical stress protein, can enhance the resistance of the plant body to a typical stress protein. The protein expression was down regulated, plant susceptible disease, decreased immunity with the condition of salt stress.

Class VI: unknown function protein will be further discussed in the following study.

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