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PROTEOMIC PROFILING OF *CARALLUMA FIMBRIATA* CELL WALL DURING DROUGHT CONDITION

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ABSTRACT

Water is one of the vital components of all living beings that triggered several biochemical changes by adding or withdrawal. Inside plants several oxidative injuries at the cellular level occurs as a result of water stress or other stresses. At cellular level, cell wall is one of the important components of plant cell that protect, support and involved in maintaining the permeability, integrity and physiological conditions inside the cell. In the present study we have applied expressional proteomic approach to find out up or down regulated cell wall proteins during drought condition. The immature plants of *Caralluma fimbriata* were used as a model organism and grown with water and without water up to their mature stage. Shoot parts of both plants were collected, homogenized, fractionated and cell wall fraction and their proteins were isolated with the help of standard biochemical techniques. The result obtained from these techniques clearly indicates that the basic proteins of water stress condition were induced than control. All these up-regulated basic proteins involved in transport and maintaining integrity during drought. So this type of approach can be extremely resourceful to interpret various growth conditions and it can be used as a prefractionation approach in proteome analyses.

KEYWORDS: ***Caralluma fimbriata,*** **cell wall proteins, drought condition, expression proteomics, prefractionation.**

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1. INTRODUCTION

Today, world's attention is shifted towards the elucidation of biological functional molecules of life i.e. proteins. For the reason that world scientist were run behind the genome projects, but after the completion of many genome projects they come to know that this genomic information is not sufficient to understand cellular mechanisms. It has a defined end point. It has unable to tell about the biological workhorse of cells. Actual the dynamics of all living beings lies in the biochemical structure and function of proteins. Therefore proteomics come forefront in the scientific disciplines to study all proteins and their diverse properties such as structure, function, expression, interaction, modification, localization, regulation, folding, etc.[1] This term was first coined by Mark Wilkins as like genomics and defined proteomics as is the study of entire protein complements expressed by a genome at given time and at given condition [2,3]. In addition to that, in eukaryotic, every cell of an organism contains a similar copy of genome i.e. genome is a constant feature of an organism whereas proteome is dynamic; it changes according to growth of development and environmental factors [4]. This is the reason that the old hypothesis 'one gene, one protein' of George Beadle and Edward Tatum is now changed and a new 'one gene, many protein' hypothesis is accepted [1,5]. That means the number of proteins present inside a cell are more in numbers than genes. Accordingly, the analysis of proteome is more complex than genome. It requires more advanced technologies, efforts and expertise than genomics [6]. The study of all these proteins at a time of an organism is a new challenge ahead to researches [7]. So there is a need to divide complex proteomics (organism proteome) into simple proteomics (subcellular proteome). Again proteomics can be restricted on type of study such as structural, functional, expression and interaction proteomics [1]. Therefore, we have selected plant as a model organism and tried to analyze which type of proteins are up or down regulated according to their surroundings.

In the present study we have taken *Caralluma fimbriata* as a model plant organism for the proteome analysis. It is a succulent plant in the cactus family *Asclepiadaceae*. It grows in wild, urban center in India and is often planted as roadside a shrubbery or boundary marker that has been used by hunting expedition. It is also found in Africa, Southern Africa, Arabla, Afghanistan, Spain and etc. It is well popular for their appetite suppressant and weight loss properties as well as their ability to lower blood sugar[8-10]. It also popular for their phytochemical constituents such as glycosides, flaonoides, megastigmane, saponins etc. and active components such as caratuberoide, bouceroside, tomentogenin, sitosterol, lecteolin-4-neohen, speridoside and kaempterol-7-0 neophesperidoside [11]. One of the advantages of this plant with the above importance is that it survives on minimum water and balance the metabolism against such conditions.

Initially we have collected immature plants of *Caralluma flmbriata* from hilly region of Ahmednagar district, Maharashtra, India. One plant was grown with water called as a control and another plant grown in water stressed condition as an altered or experimental up to their mature stage in college botanical garden. Equal amount of shoots of both conditions were cut, washed and homogenized in chilled condition. These homogenates were used for the isolation of cell wall and their proteins. Isolated cell wall proteins (CWPs) were then fractionated by chromatography and electrophoresis. The results obtained from these techniques clearly showed that the water stress condition synthesized some additional proteins that fight against the adverse situation. That means protein profile of altered is induced due to water stress. All these over expressed proteins are responsible for the sustaining plant in adverse condition. If we identify and make a complete database of each plant in different conditions than we can know which type of

protein and its respective gene have to express in particular condition. Therefore, this type of approach can be extremely resourceful to interpret various growth conditions.

2. MATERIALS AND METHODS

2.1: MATERIALS

2.1.1 Chemicals

All standard laboratory chemicals used in this work were obtained from Sigma (St. Louis, MD), SRL (Mumbai, India) and Qualigens (Mumbai, India) companies. All those were of Analytical grade.

2.2. METHODS

2.2.1: Collection and cultivation of plants

The immature plants of *Caralluma fimbriata* were collected from hilly region of Goregaon come under Parner taluka in Ahmednagar district, Maharashtra state, India. One plant was grown with water and another plant grown in water stressed condition in our botanical college garden. All the plants were grown during summer season. The average temperature of Ahmednagar district was around 41°C.

2.2.2: Homogenization-

After the maturation, the grown shoots of both plants were cut and washed 2-3 times with distilled water to remove foreign matter. 32 gm of each was taken and cut it into small pieces and then ground in mortal and pestle on ice bath. 50 ml of chilled phosphate buffer (0.01M, pH 6.8) containing 0.4 M sucrose, 5mM EDTA, 10mM DTT, 0.5% BSA, 1% PVP-40 was used as a homogenization buffer [12, 13]. Same procedure was used for altered growth condition.

2.2.3: Isolation of cell wall and cell wall proteins

The above homogenates were centrifuged at 4000 rpm for 10 min at 4°C (Remi cold centrifuge, Cat. No. C-24 BL). The supernatant was discarded and resultant residue was used for isolation of cell wall [14, 15]. This residue was mixed with extraction buffer 1 (0.01M, pH-5.5) containing 200mM $CaCl_2$ and 50mM sodium acetate. This mixture was kept in a shaker incubator for 30-45 minutes. After the incubation it was filtered through Whatmann filter paper 41. This filtrate was saved for further process as filtrate 1. The resultant residue was collected and repeats the same procedure as above. Again the filtrate was saved for further process as filtrate 2. Above filtrate 1 and 2 were mixed together. 1 ml extraction buffer 2 (0.01M, pH-5.5) containing 3M Lithium chloride and 50mM sodium acetate was added in it. This mixture was kept for overnight in refrigerator. Next day it was centrifuged at 1000 rpm for 10 min. at 4°C. The resultant supernatant was used as cell wall fraction for further analysis [16, 17]. Same procedure was used for altered sample.

2.2.4: Gel chromatography-

One gram of sephadex-100 was mixed with near about 50ml of distilled water and kept it for the purpose of swelling for one hour. The column (1.5 X 30 cm) was taken, cleaned with distilled water and glass wool was fixed at bottom end of column with the help of long glass rod. Then the swollen slurry of saphadex-100 was lodged into column. The distilled water was used to settle the matrix into the column. This packed column was then equilibrated with 25 mM sodium phosphate buffer (pH 6.8) in which sample was prepared. Before sample loading, the bed volume was determined and it was 21 ml. 0.5 ml sample was loaded on the column and connected with reservoir containing same equilibrium buffer. The flow rate was adjusted of 20ml/hour and 1.5 ml fractions were collected manually. Total proteins were eluted with same equilibration buffer on the basis of their molecular weight. The entire procedure was conducted at room temperature [18]. The column effluent was monitored and total protein content of pooled fractions was estimated by Lowry's method [19]. The same procedure and column was used for altered sample.

2.2.5: Cation-exchange chromatography-

The above chromatographically separated fractions were again fractionated on the basis of

its charges using ion exchange chromatography. Standard Amberlite (IR-120) cation exchange resin with medium mess was used for purification [20, 21]. This material was packed in 1.5 X 30 cm columns and equilibrated with 0.01mM phosphate buffer (pH 6.8) in which sample was prepared. The capacity and bed volume were determined before sample loading and it was 0.70 m. eq. /gm capacity and 18 ml respectively. 1.0 ml sample was loaded on the column and connected with reservoir containing same equilibrium buffer. Total non-adsorbed proteins were eluted first with same equilibration buffer. The remaining adsorbed proteins were eluted by equilibrium buffer containing increasing ionic strength of potassium chloride (0-0.5 M). The entire procedure was conducted at room temperature. The flow rate of the column was maintained at 6 ml/h and 1.5 ml fractions were collected. The column effluent was monitored and total protein content of pooled fractions was estimated by Lowry's method. The same procedure, column and fractions were used for altered sample.

2.2.6: Acetone Precipitation-

For further analysis by gel electrophoresis, only those fractions were selected from control and altered samples showing significant change in protein content. One volume of selected fraction and three volumes of ice cold acetone were mixed together and kept for two hours on ice bath. The precipitated proteins were collected by centrifugation at 2000 rpm for 10 min. at 4°C [22]. These proteins were then dissolved in distilled water and used for further analysis. Same procedure was used for remaining fractions.

2.2.7: Gel Electrophoresis-

The above precipitated or concentrated proteins were further fractionated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The equal volume of above concentrated proteins and sample buffer containing DTT, bromophenol blue and glycerol were mixed and heated in boiling water-bath for 5 minutes. It was cooled at room temperature and then 100µl samples were applied onto 12% SDS-PAGE gels[23]. Electrophoresis was performed on slabs (140cm x 160cm x 1mm) for approximately 8 hours at 20 mA. Gels were stained overnight with 0.1% coommassie Brilliant Blue R-250 in methanol, acetic acid and water (4:1:5) and destained with the same solution except Coommassie Brilliant Blue dye[1, 24, 25].

2.2.8: Estimation of cell wall proteins-

The proteins content of cell wall fraction and every step of fractionation were measured by well popular Lowry biochemical method [19]. Bovine serum albumin (BSA) was used as a standard.

3. RESULTS

3.1: Effect of water on Caralluma fimbriata

As described above, the collected immature plants of *Caralluma fimbriata* were grown up to their mature stage with water and without water. After the maturation, the grown shoots of both plants were cut, homogenized and cell wall fractions were isolated for proteome analysis. The protein contents of both homogenates and cell wall fractions were measured by Lowry method [19]. These obtained results clearly showed that the protein content of altered condition is induced as compare to control (Fig.1). Hence, primarily we can conclude that there is an induction in protein metabolism of altered than control.

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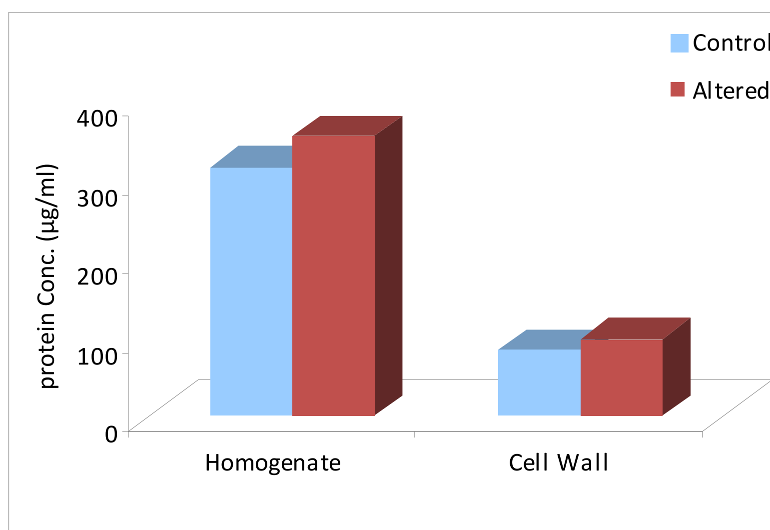

Figure 1
Effect of water on protein content of Caralluma fimbriata.

3.2: Expressional proteomic Profile of C. fimbriata Cell Wall during Drought Condition

The resultant cell wall fractions were used for the isolation and purification of CWPs using chromatography. Initially, all CWPs were fractionated on the basis of their molecular mass using gel chromatography. Typical protein profiles of both the conditions were obtained after evaluation of protein content from each pooled fractions (Fig. 2).

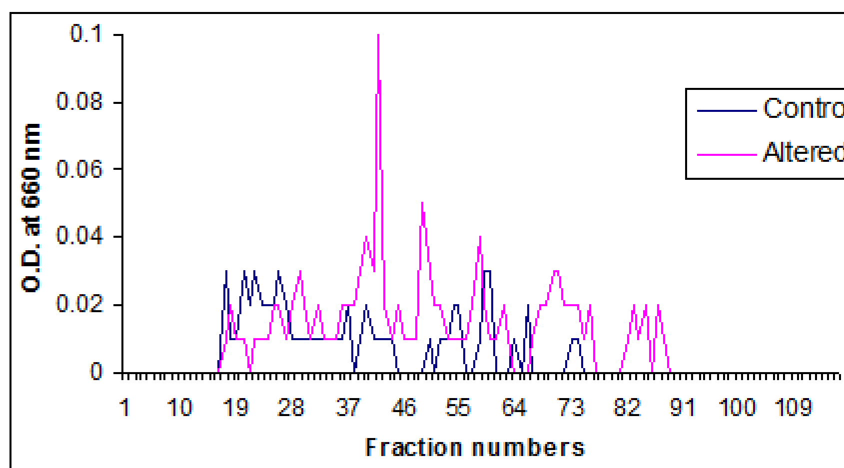

Figure 2
Gel chromatographic protein profiles of Caralluma fimbriata cell wall.

The obtained results showed that, the protein profile of water stressed condition are induced than control. This change in quantity of particular protein families is due to lack of water for maintaining the related internal environment for survive in hot condition or water stress condition. Secondly, we have proceeded to find out which protein or enzymes are over expressed due to the shortage of water. Therefore, we have used above chromatographically separated CWP fractions and again fractionated on the basis of their

charges using cation exchange chromatography (CEC). Those fractions of control and altered condition, which have given significant change in their protein contents they were selected for further separation. 1 ml fraction was loaded on a previously equilibrated Amberlite (IR-120) cation exchange column with 0.01M phosphate buffer (pH 6.8). A typical protein profile of both the conditions was obtained after evaluation of protein content from each pooled fractions. The non-adsorbed proteins that mean all those having negative charge were eluted first with same equilibration buffer (Fig. 3). All adsorbed proteins were eluted with increasing ionic strength of potassium chloride (0.1-0.5 M) of the same equilibrating buffer (Fig. 4-6). The same column and conditions were maintained for altered growth condition. Complete protein profiles of both the conditions were obtained at different washings after evaluation of protein content from each pooled fractions Fig. 7. The obtained results again showed that, the numbers of proteins in altered condition are up regulated due to lack of water. All these proteins are basic proteins of cell wall and participated in maintaining the conditions required for survival with shortage of water [26].

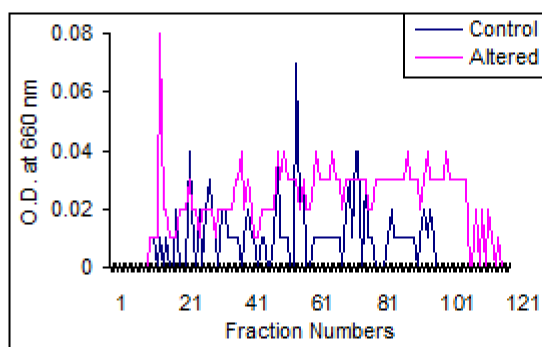

Fig. 3: Elution profile of non-adsorbed proteins of *Caralluma fimbriata*_cell wall using Amberlite (IR-120) cation exchange column.

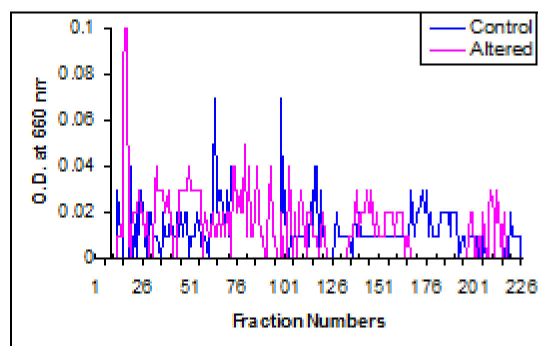

Fig. 4: Cation exchange chromatographic protein profile of adsorbed cell wall proteins at 0.1M KCl containing phosphate buffer (0.01M, pH 6.8).

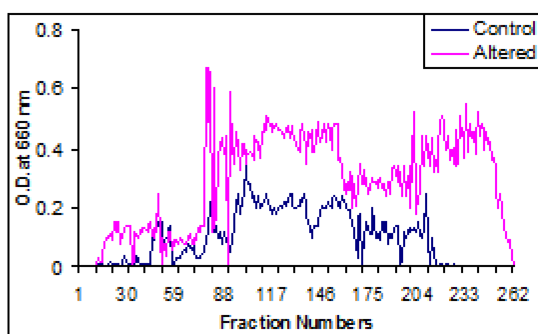

Fig. 5: Cation exchange chromatographic protein profile of adsorbed cell wall proteins at 0.3M KCl containing phosphate buffer (0.01M, pH 6.8).

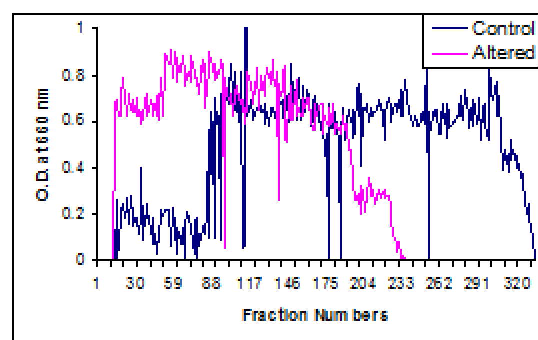

Fig. 6: Cation exchange chromatographic protein profile of adsorbed cell wall proteins at 0.5M KCl containing phosphate buffer (0.01M, pH 6.8).

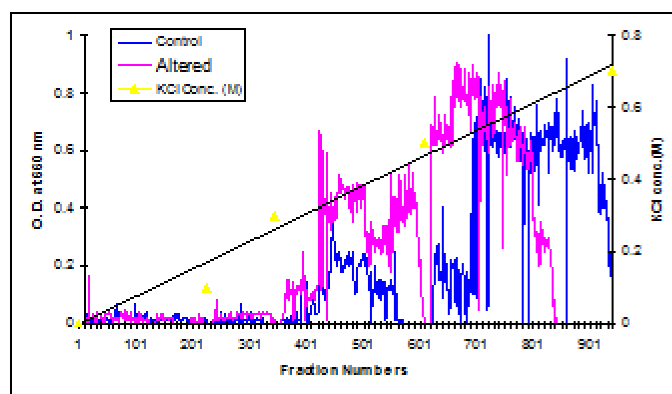

Figure 7
Expressional proteomic profile of Caralluma fimbriata cell wall proteins using Amberlite (IR-120) cation exchange column and eluted by increasing ionic strength of KCl (0.1M-0.5M) of the same equilibrating buffer (0.01M, pH 6.8).

3.3: Further purification of cell wall basic proteins using SDS-PAGE:

Above chromatographically fractionated proteins were further purified by 1-DE. Those fractions of control and altered condition showing significant changes in their protein contents were selected, concentrated and resolved on 12% SDS-PAGE. A typical protein banding pattern of each pooled fraction of both conditions was obtained after staining and destaining (Fig.8). Similar results were obtained after repeated experiments. The obtained results again showed that the protein profile of altered conditions contains more basic proteins than control. These altered basic proteins are extremely resourceful to interpret various growth conditions and overall metabolic patterns of proteins as well as genetic alteration such as gene deletion or over expression in cell wall proteome.

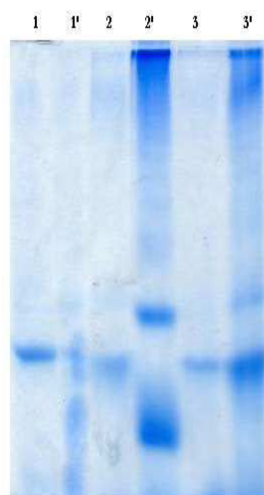

Figure 8
1-DE of chromatographically purified CWPs of Control (C) and altered (T) condition using 12% SDS-PAGE. (Lane No. 1 and 1': fraction No. 197 of C and T, Lane No. 2 and 2' fraction No. of 373 of C and T, Lane No. 3 and 3' fraction No. of 550 of C and T and Lane No. 4 and 4' fraction No. of 731 of C and T.)

The futuristic study will includes identification and characterization of drought resistant proteins of cell wall. For this the above electrophoretically separated proteins can be fragmented in gel with proteolytic enzymes and further analyze with matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) followed by bioinformatics tools such as various software, database and worldwide webs (www) [27-30].

4. DISCUSSION

Every plant needs water for the entire periods of growth but some stages of life are weaker to water shortage that directly affects the metabolism of plants [31]. In addition to that, any oxidative injury at the cellular level as a result of water or other stresses is a major cause of plant or crop damage [32- 34]. Under such conditions the whole metabolism of plant comes under stress. Some of the components of nucleic acid and protein metabolism are up or down regulated to sustain in such condition [35, 36]. Therefore water is one of the vital component of plants that triggered several biochemical changes by adding or withdrawal of water [37]. Due to lack of water the composition of cell compartments changes. In case of CWPs, it changes according to the surrounding. According to interactions of proteins with cell wall components there are three types of CWPs in every plant. First category of CWPs is loosely bound proteins with little or no interaction with cell wall components. These are mostly found in the intercellular space. Second category of CWPs is weakly bound to the cell wall components by Van der Waals interactions, hydrogen bonds, hydrophobic or ionic interactions. While the third category is strongly bound to cell wall components and become resistant to salt extraction [37, 38]. In the shortage of water these proteins are subjected to changes associated with the increase in permeability, loss of integrity and other subcellular components as well as change their functionality [39]. In the present study, we fractionated such loosely bounded basic CWPs from the rest of the proteins with the help of CEC and tried to purify with 1-DE. All the obtained results are clearly showed that the protein content of altered growth condition is induced than control. That means the water resistant proteins (WRPs) are up regulated and ultimately the genes of respective proteins may be expressed due to lack of water in altered condition. Early studies described that these types of proteins have interactions with negatively charged polygalacturonic acid residues of pectins in cell wall. When these proteins come under water stress causes a reduction in hydrostatic pressure and can cause an increase in abscisic acid in plant tissues [40]. This increase in negatively charged environment in cell wall can lead interactions with basic proteins and that can be help in maintaining the cell wall in altered growth condition. Some other study reported that the production of high levels of stress proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exposure of the cell to toxins (ethanol, arsenic, trace metals and ultraviolet light, among many others), nitrogen deficiency, or water deprivation [41, 42]. Our study clearly showed that the basic proteins and its related gene are over expressed in shortage of water. To assess the possible role of water stress, we have analyzed effects of water stress on the rate and pattern of protein synthesis in *Caralluma fimbriata*. It reduces the rate and changes the pattern of protein synthesis as evaluated by chromatographic and electrophoresis techniques. The results obtained from these techniques are clearly showed the induction in protein number of cell wall in altered condition. It includes synthesis of new enzymes, proteins as well genes are expressed to sustain these types of plants in water stress conditions [26].

One another advantage of this approach is that it helps to resolve low and high abundant proteins form the population of proteins in complex samples [43, 44]. Some analytical techniques are unable to separates very low and very high abundance proteins, extremely

basic or acidic proteins from complex mixtures. This approach can separate each and every protein on the basis of their mass, size and charges and help to reduce the complexity of proteomics [30].

5. CONCLUSION

Cell wall is the one of the important component of plants that play crucial roles during development and constitute the first barrier of defense against invading pathogens. Actually, the components of cell wall such as polysaccharides, lignins and proteins form a network and carry out all functions. But there is still no complete information about protein turnover and interaction between protein-carbohydrate or protein-protein interactions of cell wall to understand regulation of enzymatic activities and various transports during growth and environmental conditions. It is clear from the results presented in this sudy that the protein profiling of the cell wall of *Caralluma fimbriata* are responds to the changes in the availability of water. These changes in the CWPs during water stress reflects continuous metabolism and maintained all conditions in cell wall like permeability, rigidity, integrity of cell etc. This increase in proteins, presumably, helps to preserve the cell wall structure during the process of wilting. This may represent a survival mechanism for plants which grown under rain-fed conditions and have evolved to withstand extreme conditions of water stress. This type of expression proteomic approach helps in identifies and characterize up or down regulated proteins, which are responsible for generated problem or involved in overcome the generated problem. If we identify and characterize these types of proteins, which are involved to fight against such stress conditions then we can identify its respective gene to make sustainable plants for affected region and prevents loss of productivity as well as suicides of former. Therefore we have come to conclusion that this type of work will definitely helpful for the improvement of life of plants, quality, yield and survival in low rainfall and it may directly applicable to agriculture and biotechnology. In addition to that, this type of approach can be used as a prefractionation approach in proteome analyses for reduction of protein complexity before 2-DE or MS.

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