

**NAME: Rita Bagheri**

**SUPERVISOR: Dr. Mohammad Irfan Qureshi**

**DEPARTMENT: Department of Biotechnology, Jamia Millia Islamia**

**TITLE: Investigating Proteomic Response of Spinach to Some Abiotic Stresses Using 2D Electrophoresis and Mass Spectrometry**

**ABSTRACT**

**BROAD AREA OF RESEARCH: Plant Biotechnology**

**SPECEIFIC AREA OF RESEARCH: Plant Proteomics, Metallomics, Mass Spectrometry, Plant Molecular Biology.**

**KEYWORDS: Proteomics, mass spectrometry, *Spinacia oleraea*, Cadmium, Salt, S-deficiency**

Plants are continually challenged to recognizing and responding to the environmental cues including signals generated by adverse changes in order to protect and avoid detrimental effects on growth and development. It is essential for an organism to alter the pattern of protein expression in order to get adapt and resist the changes induced by environmental fluctuations and stresses. Plants which are being used for the purpose of phytoremediation should be efficient enough to modulate at molecular and biochemical levels. Under such stresses, in general, plants modulate its defence mainly through overexpression of special proteins. The over-expressed or newly induced proteins might play a crucial role to cope up with stress or to shift plant metabolism onto an 'emergency mode'.

We used proteomics, coupled with biochemical measurements including levels of cellular antioxidants (enzymatic and non-enzymatic) to study the response of an important vegetable plant *Spinacia oleracea* (spinach) exposed to a numerous abiotic stresses in two set of experiments:

In first set of experiment, thirty-days-old *Spinacia oleracea* plants were exposed to NaCl stress (10 mg/g soil), cadmium stress 50 µg/g CdCl<sub>2</sub> and combined stress of both NaCl and Cd for 2 and 7 days. In the second set of experiment twenty-days-old *Spinacia oleracea* plants were divided into two groups for a period of 10 days on S-fed and S-deficient nutrient

media. After 10 days, half pots of every group subjected to CdCl<sub>2</sub> (50 µg/g soil) for a period of 2 and 4 days.

Two-dimensional gel electrophoresis was used for resolving leaf proteome and proteins of interest were identified using PDQuest software. Important proteins were tryptic-digested. Protein digest was analyzed peptide mass fingerprinting (PMF) and their match with protein databases using MASCOT.

More than 100 proteins were identified using peptide mass fingerprinting (PMF). Functional categorization of identified proteins shows 20% of proteins are associated with plant stress response and defence. Others were associated with photosynthesis (20%), protein synthesis (17%), signal transduction (13%), growth and cell division (9%), DNA/RNA metabolism (14%), energy (7%), transport (3%), secondary metabolism (3%) and cell death (4%). Several identified proteins were found associated with defence against cadmium and/or S-deficiency. A number of proteins showed requirement of appropriate amount of sulfur for better defence against Cd. Similarly, a coordination of protein network is essential for plant protection exposed to combinatorial stress of cadmium and salinity.

It can be concluded that *Spinacia oleracea* modulated its proteome profile down-regulating and up-regulating the expression of several proteins and activity of cellular antioxidants very efficiently under cadmium, salt and their combined stress. However, plant suffered a most significant loss in chlorophylls, protein content and biomass under dual stress of cadmium and salinity. Similarly, S-deficiency was proved to be very deleterious for plants; presence of Cd during S-deficiency further affected these parameters in plant. Proteomic data reveals that *Spinacia oleracea* upregulated or induced new and novel proteins specific to cadmium, salinity and S-deficiency or even to the multiple stresses occurring at a single time.