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Evaluation of leaf proteome in wheat genotypes under drought stress

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Abstract
Drought stress in plants, the change (increase or decrease) in the production of plant proteins. Proteomics in recent years one of the most powerful tools that help us to study the changes in protein In order to investigate the proteome of wheat leaves in response to terminal drought, two genotypes susceptible and resistant wheat genotypes were evaluated under irrigated (non-stress) and rain-fed (stress) conditions. After applying stress and extraction of leaf proteins, two-dimensional gels were prepared and scanned. Analysis of gel images was performed using Same Spot Progenesis. About 657 protein spots were identified by the software. After alignment of the spots and their correspondence, 148 spot were identified visually and by using the software and statistical analysis was carried out. Five spots with Fold $\geq$ 1/5 at P $\leq$ 0/05 were identified, of which 4 spots were significant at P $\leq$ 0/05 and 1 spot was significant at P $\leq$ 0/01.

Keywords: drought stress, proteome, wheat

1- Introduction

Drought is an important environmental factor that limit plant performance, growth and productivity (Chaves and Oliveira, 2004). With dwindling water resources, the Breeders breeding programs for varieties adapted to drought stress increased (Li *et al*., 2011). Among the changes that occur due to drought stress in plants, the change (increase or decrease) in the production of plant proteins (Donnelly *et al*., 2005 Jangpromma *et al*. 2007). Drought stress decreased soluble proteins whit high molecular weight, while soluble proteins whit low molecular weight increase (Farshadfar *et al* 2008, Zimmerman 1998, Sujin and Ray wu. 2004). Rubisco accounts for about 30-60 % of the total soluble protein in plants that can be quickly remobilized under stress and senescence. (Demirevska *et al*. 2008; Feller *et al*., 2008). Proteomics in recent years one of the most powerful tools that help us to study the changes in protein (Suoyi *et al*.2009). Two dimensional electrophoresis (2-DE) is the most commonly used technology to separate hundreds of proteins from plant tissues (Gorg *et al*., 2004). In studies on rice plant, more than 700 protein spots on the gel were partitioned using the software Image Master-2D, 57 proteins were identified. Then the proteins were analyzed by MALDI-TOF MS, which lead to the identification of 52 proteins (Li *et al*., 2011). Donnelly *et al* (2005) in wheat leaf total of 277 proteins were examined on two-dimensional gels. This research aimed to evaluate changes in proteins in wheat leaves under drought stress were measured.

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2- Materials and methods

Experimental location and Plant material

This research was carried out using bread wheat genotypes during 2010 at research farm and laboratories of Razi university and medical biology research center Kermanshah university of medical sciences. At grain filling stage, plants were selected and flag leaf samples were harvested. The experiments on both susceptible (Hamam-4) and resistant (Pishgam (Bkt/Zhong)) genotypes of wheat in irrigated (non-stress) and rain-fed (stress) conditions was performed.

Protein Extraction and Two-dimensional Electrophoresis (2-DE)

Protein extraction and preparation are basic steps for the success of the 2-DE (Isaacson *et al*., 2006). The modified method Damrval *et al* (1986) was used to extract proteins. After stress, 1g of leaves were powder in liquid nitrogen, then transferred into microtubes and extraction buffer were added. The mixture was placed at -20C ° for 1 h. Then at 12000 rpm for 20 min at 4 ° C. Then at 12,000 rpm for 20 min at 4 ° C was centrifuged. the supernatant discarded, the precipitate was washed. Then for 15 min at -20C ° was mixed. Then at 12,000 rpm for 20 min at 4 ° C. The mixture was centrifuged and the supernatant was discarded. the precipitate was repeated three times. The samples were incubated in acetone to evaporate it. The microtubes of 400 µl Lysis buffer containing urea, thiourea, Tris, ampholine pH 3-10, CHAPS and DTT was added. The protein concentration by the Bradford (1976) method was determined. Rehydration for every 360 ml of samples, DTT 0/077 g and IPG Buffer 5 ml was added. Rehydration was performed for 12- 24 h. After Rehydration the strips were washed and for Iso Electic Focusing was placed on of multiphor II. two-dimensional electrophoresis was on gels 12% with dimensions 18 × 18 cm. After placing the strips on the gel, melted agarose gels to hang on the strips and the second dimension was added to the flow rate was set. Before staining solution, the gel was placed overnight in a solution stability. coomassie brilliant blue used for staining. The scanned images (Figure 1) analysis were performed using the software Same spot progenesis.

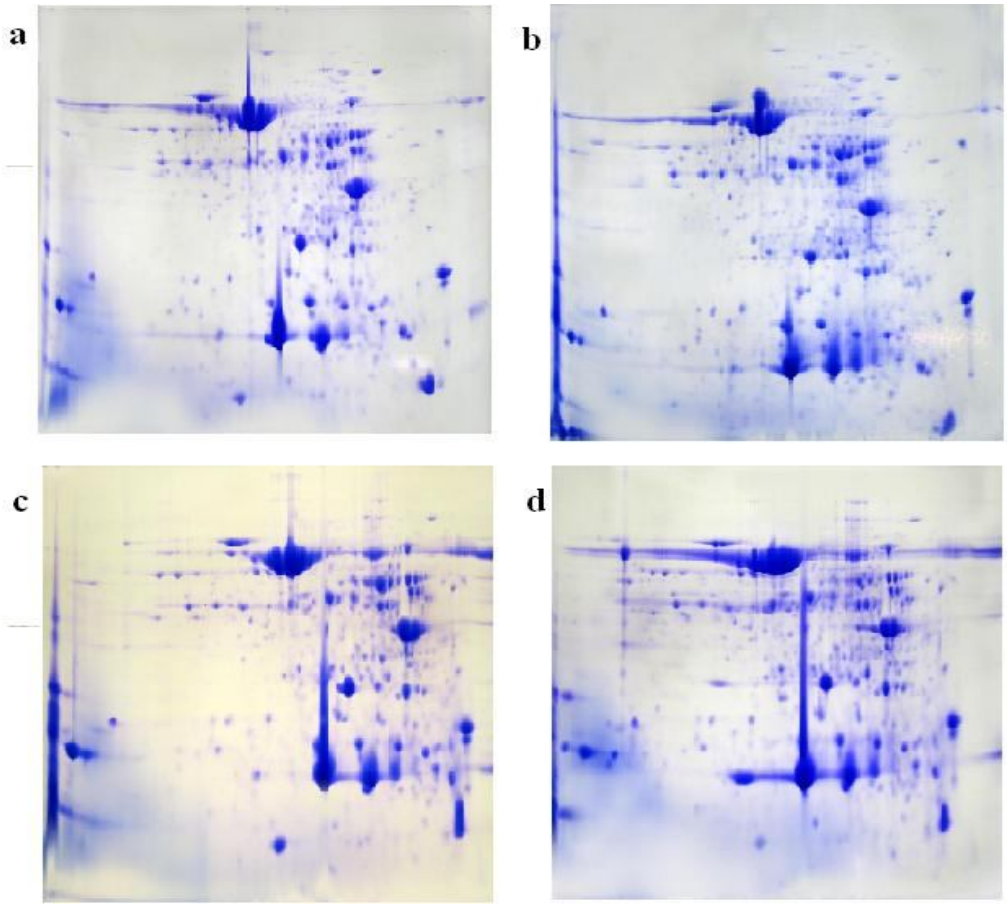

Figure 1 - Two-dimensional electrophoresis pattern in the range of PH 3-10 a- susceptible genotype in non-stress conditions b- susceptible genotype in stress conditions c- resistant genotype in non-stress conditions d- resistant genotype in stress conditions

3- Results and discussion

In order to investigate the wheat leaf proteome, both susceptible and resistant genotypes of wheat in irrigated (non-stress) and rain-fed (stress) conditions were evaluated. Two-dimensional gels were prepared and scanned the stress, Tiff format images to analysis using Same spot progenesis software company became Nonlinear England. The statistical design was composed as follows:

1. Comparison of protein spots in susceptible genotype in stress and non-stress conditions
2. Comparison of protein spots in resistant genotype in stress and non-stress conditions

Gel image of resistant genotype under irrigated conditions analysis was selected as the reference image. Approximately 657 spots were identified by the software. Alignment of the 148 spots software and match them with software to help visually confirmed to be compliant were detected (Figure 2), and statistical analysis were performed on them. The analysis 5 spots $P \leq 0/05$ and Fold $\geq 1/5$ were identified.

Changes in protein expression were analysis by the software to the spot anywhere in the gel image of the studied groups were compared. Information about changes in protein expression levels for each spot on the graph as " Log normalised volume" was drawn up. Statistical analysis showed that the level of 5% ($P \leq 0.05$) show a significant difference in the number 5 spots. a spot at the 1% statistical level showed significant differences (Table 1). Two-dimensional electrophoresis of proteins on tea leaves 750 spots using PDQuest 8.0.1 software was detected on gel, 61 protein spots showed significant differences. The analysis of the protein spots were identified by mass spectrometers 30 protein spots, of which 26 protein spots in the metabolism of carbon, nitrogen and sulphur are important photosynthesis defense proteins were identified (Li *et al*, 2011). In a study induced changes on the proteome of wheat seeds by two dimensional electrophoresis and quantitative analysis using software Melanie-3 600 protein spots on the reproducibility was assessed for 153 of those parts of the response was significantly stress showed. Among these proteins, 78 protein spots including storage proteins, heat shock proteins, proteinase inhibitors, detoxification enzymes, aldehyde dehydrogenases and LEA proteins by MALDI TOF / TOF were identified (Haj Heydari *et al*, 2005).

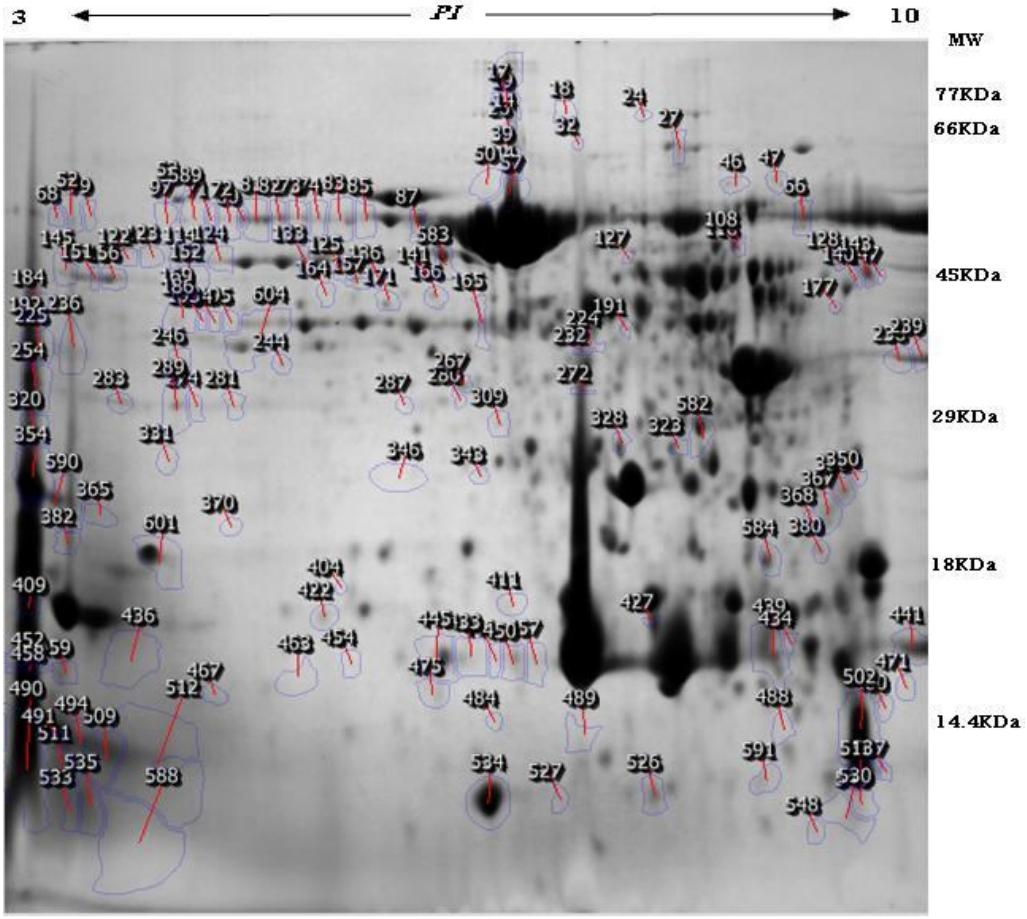

Figure 2 - 148 spots detected by the software

Table 1 - Characteristics of the P-value, Fold and protein expression levels of significance in level 0.05 (**) and 0.01 (*)

Spots	Anova (p)	Fold	Tags	pI	MW	Average Normalised Volumes	
						Condition 1	Condition 2
80	0.007**	2.6		4.88	53	804.829	2059.331
164	0.023*	4.9		5.5	42	524.002	2582.680
26	0.043*	14.1		6.88	66	2107.188	149.322
287	0.043*	2.3		6.1	31	522.801	1220.651
509	0.048*	3.7		3.75	13	2.268e+004	6088.264

4- Conclusion

The 164, 26, 287 and 509 spots at the level of 5% are significant. The 164 (Figure 3) and 287 (Figure 4) spots were increased due to drought. while 26 (Figure 5) and 509 (Figure 6) spots showed decreased protein expression. 80 (Figure 7) spot showed a significant change at the level 1% That Protein expression was higher in drought stress. In this research can be obtain more information whit isolation, sequencing and identification of the expression proteins in drought conditions.

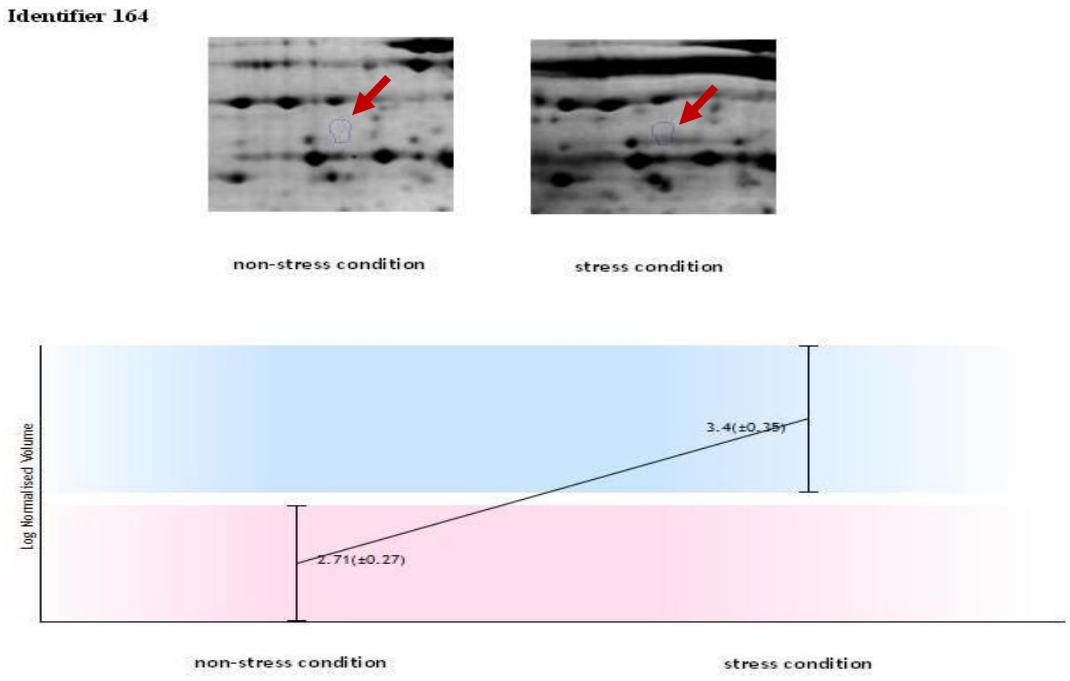

Figure 3 - 164 spot and chart " Log normalised volume" in non-stress and stress conditions

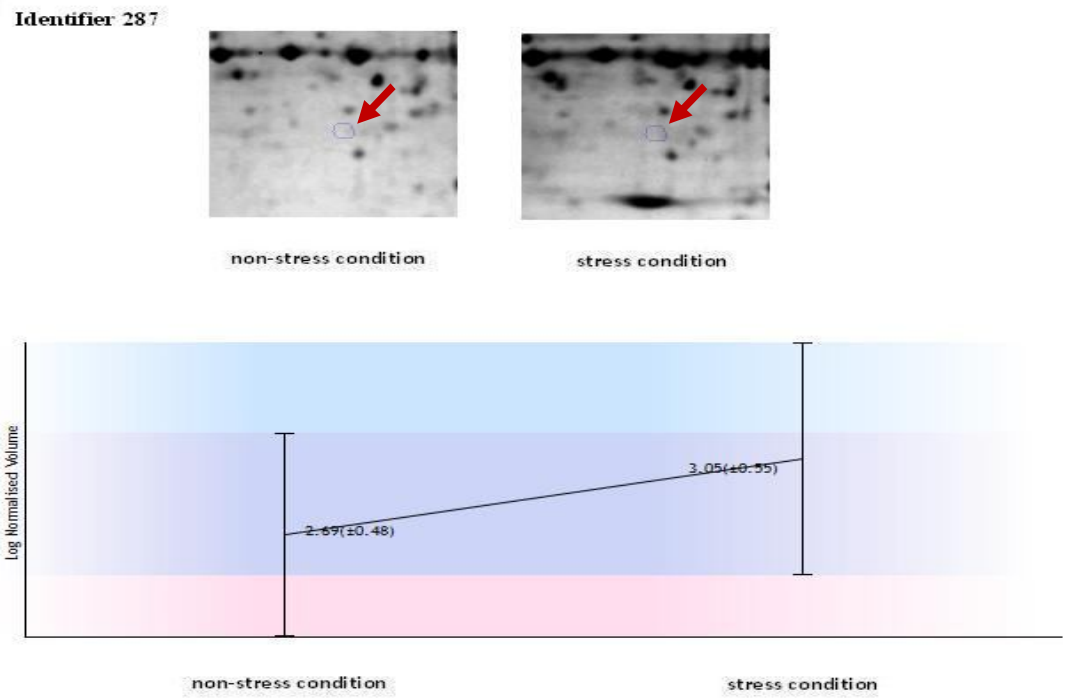

Figure 4 - 287 spot and chart " Log normalised volume" in non-stress and stress conditions

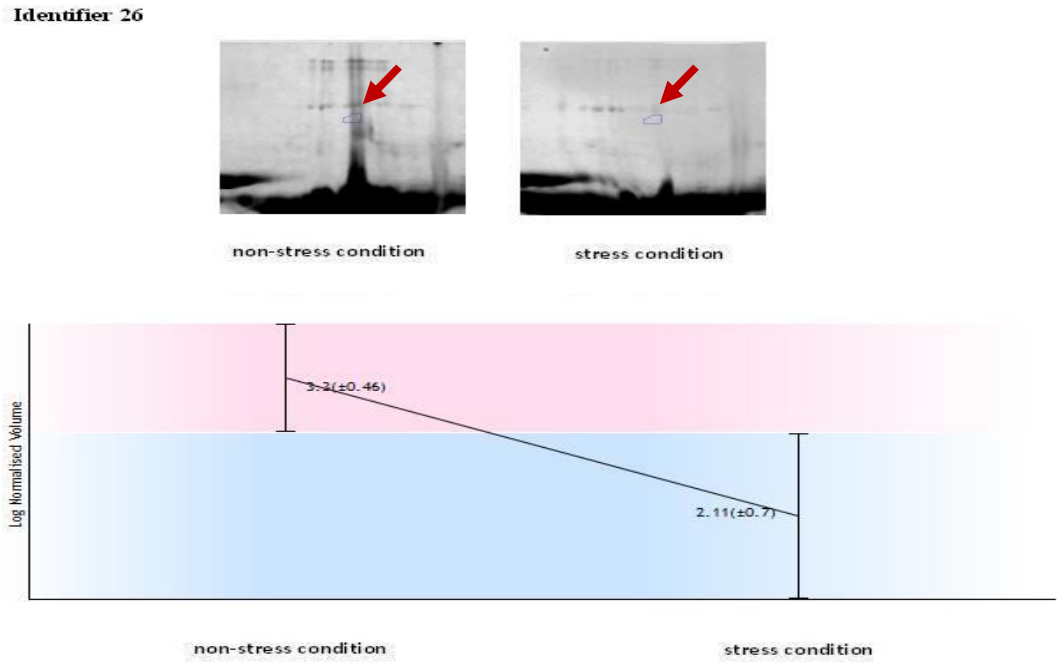

Figure 5 - 26 spot and chart " Log normalised volume" in non-stress and stress conditions

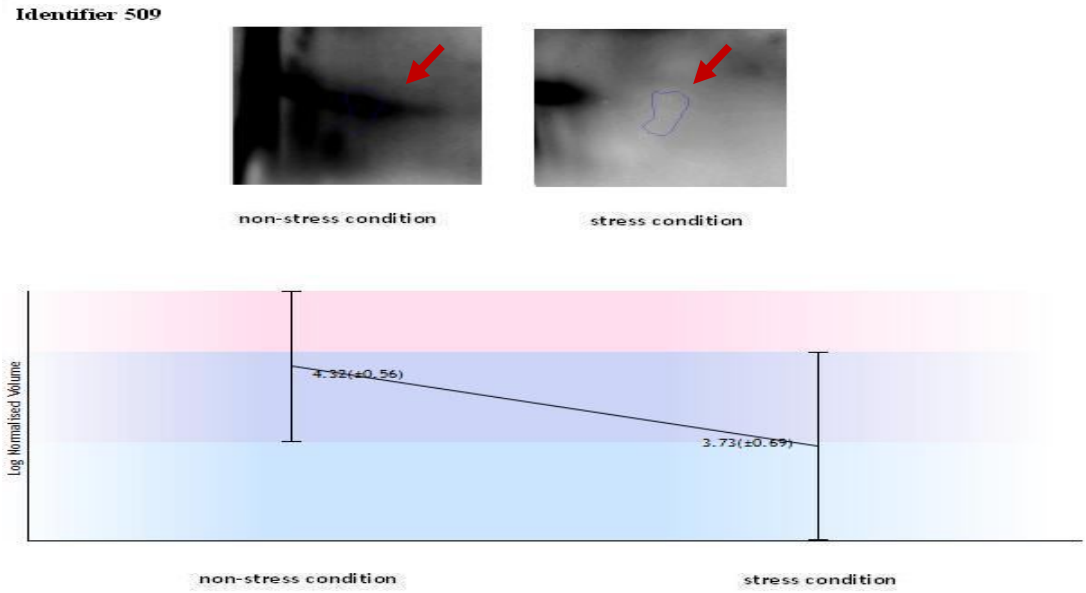

Figure 6 - 509 spot and chart " Log normalised volume" in non-stress and stress conditions

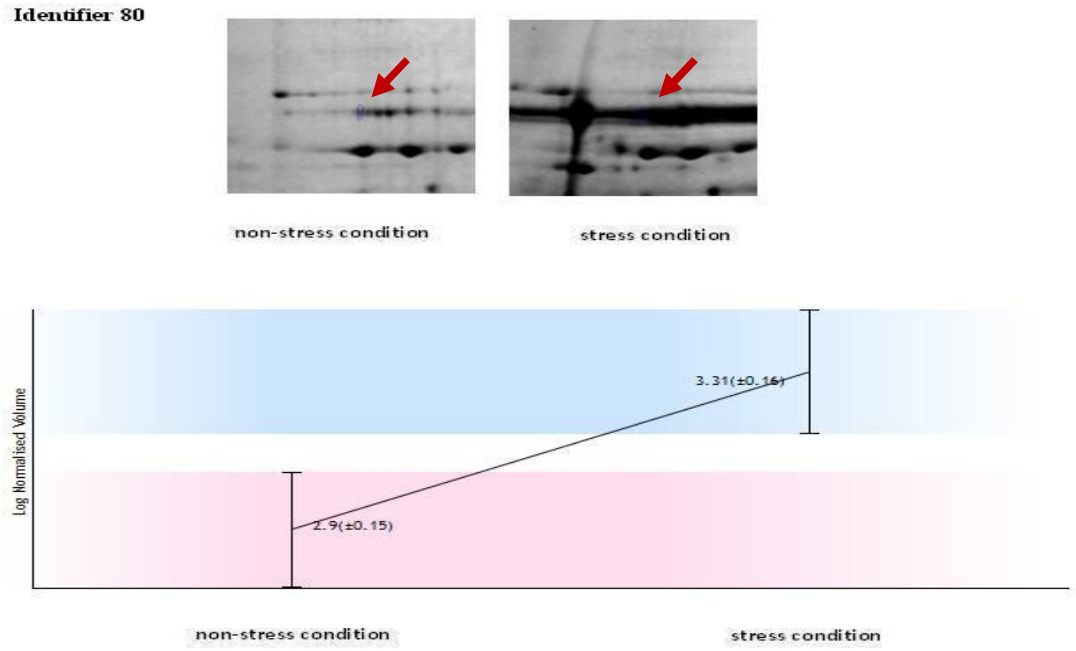

Figure 7 - 80 spot and chart " Log normalised volume" in non-stress and stress conditions

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