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The proteome response of barley root (*Hordeum vulgare L.)* to cold stress

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Abstract

Low temperature limits distribution and productivity of plants, and causes genetic, morphologic and physiologic changes. The aim of this research is to investigate the cold stress effect of 4°C on morphological traits and proteome profile of root in a cold tolerant barley cultivar (EC83-1215). For this purpose, the seeds were grown in greenhouse, and the cold stress was imposed at seedling stage. The root morphological characteristics and proteome profile were examined, after 48 hours of imposing cold stress and compared with plants grown under 25°C as control. Two-dimensional electrophoresis (2-DE) was employed for proteomic analysis and total protein was extracted using TCA-acetone method. The first dimension of the electrophoresis performed as iso-electric focusing in tube gels and the second dimension carried out using slab sodium dodecyl sulfate polyacrylamide gels. Examination of the 2-DE gels showed that among 72 repeatable protein spots, 15 proteins indicated significant changes in which, 10 and 5 protein spot were up-and down-regulated, respectively. Statistical analysis revealed that applying 4°C cold stress on plants did not change the root related traits including number of roots, root fresh weight, root volume, root surface and root diameter, statistically. Therefore, lower temperatures may be needed if we wish to see any changes in these traits.

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Introduction

Abiotic stresses such as drought, salinity and cold as well as minerals impose devastating effects on growth and metabolism of plants, which eventually interfere with plants life. Under such conditions, plants make change in their metabolic processes, and engage defensive mechanisms in order to face the stresses. Responses to abiotic stress occur across the plant (Ghosh and Xu, 2014). Under normal condition, roots perform the lifetime task of assimilation and allocation of water and minerals from soil, important for maintenance of cellular homeostasis (Ghosh and Xu, 2014). Upon stress imposition, roots constitute the first and the most important organelles in plants confronting abiotic and biotic stresses by creating cellular defense. Besides, root proteins initiate immediate responses comparable to proteins existing in leaves and stems (Lee *et al*., 2009).

Proteomic approach provides powerful tool for separation and identifying proteins responding to stress (Hashimoto *et al*., 2009), yields a better understanding of plant's response to stress condition at molecular level, helps take a closer look at inside the plant resistance to stress, by identifying proteins involved in cold tolerance (Xing *et al*., 2002; Wang *et al*., 2011). Hashimoto and Komatsu (2007) investigated proteome profile of rice seedlings under cold stress condition. From 1000 spots detected on slab 2DE gels, only 400 were associated with roots. Up-regulated proteins in root were UDP-pyrophosphorylase, Adenylate kinase, Cysteine protease and Peptidyl prolyl isomerase cpy250. Most cold responding proteins in roots were different from other tissues', implying root specific mechanisms underline reaction to cold stress. Lee *et al*. (2009) studied cold influence on the roots of rice seedlings. In that study, 37 protein spots exhibited differential accumulation, and 27 proteins were identified in mass spectrometry. Majority of identified proteins were involved in energy production and metabolism. Despite using polyethylene glycol in degradation and disaggregation during samples preparation, only one protein regulated by cold was detected at a low frequency. Bae *et al*. (2003), analyzing nuclear proteome response in Arabidopsis to cold stress, grew plant materials in 22 ℃ for three weeks, and then transferred them into a dim environment at 4 ℃ for 6 hours. The extract - of nuclear proteins- was analyzed using 2DE and *Peptide mass fingerprinting* (PMF). From total of 184 proteins extracted, 54 proteins exhibited at least a twice as much change in expression, with 40 proteins were up-regulated and 14 down-regulated. These proteins were involved in protein synthesis, RNA metabolism, protein shaping and forming, and transcription regulation.

Barley is the fourth in the rank of global production, only after wheat, rice and corn. It is considered a very important crop plant in the world, which displays tolerance to heat, alkaline and salt, compared to its peers. Its cultivation is also important in marginal, infertile lands stricken by drought, low temperatures and salinity (Baum *et al*., 2004).

Cold stress is very important in the sense that it influences plant's development (Hashimoto *et al*., 2009), and the damages incurred are considered a global problem which calls for outcome-oriented methods towards mitigating the problem through increase in the number of crop plants tolerant to cold. In the meantime, identification of proteins involved in the process seems to be the first step towards reaching the goal. Therefore, the goals of this study have been to determine 2-DE protein profile of barley root and investigate quantitative changes of proteins under cold stress. The paper would explain the role of differentially changed proteins involved in mitigation of cold effects.

Material and methods

Plant material preparation

Seeds of barley (cold resistant cultivar EC83-1215) were used as the plant material. After the initial germination on filter paper, seeds were planted in pots with 15 cm diameter and 50 cm of length containing fine sand and perlite (ratio 10:1) in an experimental greenhouse located at the department of

Plant Breeding and Biotechnology, University of Tabriz under 70% humidity, 16 hours daylight and 25°C and irrigated regularly up to three-leaf stage. Afterwards, cold stress of 4°C was applied to a number of randomly chosen pots in a freezing test machine for 48 hours. Plants Root were harvested and compared with that of grown in greenhouse conditions at normal temperature to measure the morphological traits, proline content as well as proteome analysis.

Measurement of morphological traits

The maximum length of the main root (Taproot length) was measured using millimeter ruler. Root volume was recorded by putting the roots in a graduated cylinder with a specific volume, and subtracting the volume of water before from after submerging the roots. Fresh and dry weights were measured after drying in 72°C for 48 hours. Root area was calculated using the following formula (Alizadeh, 2004):

$$\text{Root area} = 2(\text{root length} \times \pi \times \text{root volume})^{0.5}$$

Root diameter and root area density were calculated, respectively as (Hajabbasi, 2010):

$$\text{Root diameter} = (\frac{4 \times \text{fresh weight}}{\pi \times \text{root volume}})^{0.5}$$

$$\text{Root area density} = \pi \times \text{root diameter} \times \text{root length}$$

Proline content was measured according to Bates *et al.* (1973).

Extraction of proteins

Total protein extracts were isolated from approximately 0.5g of frozen root per biological replicate to obtain a fine powder, which was, then, suspended in cold aceton containing 10% TCA and 0.07% 2-Mercapthoetanol. The resultant powder dissolved in lysis buffer containing 7M Urea, 2 M thiourea, 2% CHAPS, 60 mM DDT and 1% ampholyte (pH: 3-10). Protein concentration was determined by Bradford assay (Bradford, 1976).

Two-dimensional electrophoresis

Iso-electric focusing (IEF) of proteins performed with 400µg of protein extract using tube gels 11cm in length and 3 mm in diameter (O'Farrell, 1975). IEF gel solution consisted of 8 M urea, 3.5% polyacrylamide, and 2% NP-40, 2% ampholines (pH: 3.5-10 and pH: 5-8), ammonium persulfate and TEMED. The voltage setting of the IEF was a 200 V gradient for 30 min, a 400 V gradient for 16 h, and a 600 V for 1h, consecutively. Proteins in the tube gels separated on the basis of their pI, isoelectric point. After this, tube gels were subjected to the second dimension of electrophoresis after being transferred onto a 15% acrylamide separating gel and 5% acrylamide stacking gel. After electrophoresis, proteins were stained with silver nitrate. The resulting gel images were performed with PDQuest software (BioRad). The pI/Mw (pI, isoelectric point and Mw, molecular weight) of each protein were determined using 2D-PAGE markers (BioRad, Hercules, CA, USA). After normalization, t-test were used to identify the statistically changed proteins between control and stressed plants ($p<0.05$). Identifications of proteins were obtained from NCBI, Expasy and Swiss- 2DPAGE databases.

To evaluate root morphological characteristics, a trial with seven replications was conducted. A t-test was used to test the statistical difference between cold stress and control pots, after clearing homogeneity of within-group variances. Data analysis was performed using SPSS software.

Results and discussion

The effect of cold on root characteristics

After being sure of normality of the data, t-test was performed and the results showed that the difference between the control group (25°C) and cold stress (cold 4°C) for five morphological characteristics associated with root, including the number of roots, root fresh weight, root volume, as well as root area and diameter, was not statistically significant, meaning 4°C cold stress did not impact these traits significantly within 48 hours of exposure to stress.

This is probably due to the shortness of time plants have been impacted by cold 4°C and that more time is needed in order for stress to bring about changes to morphological traits. The difference between the control and cold-stressed plants for 4 morphological traits (root dry weight, root maximum length, average root length and root surface density) was significant at 5% level of significance (Table 1).

Tab. 1. Summary of information pertaining to morphological traits of barley root studied under normal (25 °C) and stress (4°C) conditions.

	Root number	Root Dry weight (gr)	Root Fresh weight (gr)	Root volume (cm^3)	Maximum root length (cm)	Average root length (cm)	Root surface (cm^2)	Root diameter (cm)	Root surface density (gr/cm^2)	Proline (gr/fresh weight)
Control (25°C)	6/13[a]	0/0037[a]	0/0244[a]	0/034[a]	19[a]	8/60[a]	2/146[a]	0/512[a]	13/76[a]	0/982[b]
Stress (4°C)	5/30[a]	0.0026[b]	0/0163[a]	0/026[a]	10/93[b]	4/63[b]	1/213[a]	0/030[a]	4/43[b]	3/043[a]

Individual letters represents significance level at 5%, and for proline the significance level is set to 1%.

Root dry weight plummeted from 3.7mg of control to 2.6mg under cold stress. Experiment conducted by Ganjeali *et al.* (2008) on root traits in two genotypes of pea plants under waterlogging stress showed that the dry weight of roots in both genotypes had dropped, in comparison to control conditions. The reduction in root storages under stress condition, yellowing and loss of leaves under such circumstances, results from deactivation of the roots so they cannot intake water and nutrients from the soil and transfer them up to the shoots, where they will be used in photosynthesis. Obviously, again, under such conditions, plant undergoes general weakness and root and shoot biomass may shrink away subsequently. Maximum and average length of the root dropped, respectively, from 19 and 8.6 cm under normal condition to 10.93 and 4.63 cm under stress conditions. It has been reported that flooding stress has left a significant adverse effect on aggregate length of the roots and length of the main root in Pea (Ganjeali *et al.*, 2008).

Generally, abiotic stresses such as drought, salinity and cold have a significant impact on the growth and metabolism of plants, and can, eventually, interfere in plant's life. For plant to survive these stress conditions, modifications in metabolic pathways seems necessary (Ghosh and Xu, 2014).

Cold effect and proline quantity

The results of the current research showed that for the content of proline in root, there was a significant difference between the control (25°C) and cold stress (cold 4°C) conditions, at 1% (Table 1), as stress increased the amount of proline.

Poustini and the colleagues (2007) reported an increase of proline content in wheat under salinity and stated that this increase has been far more noticeable in sensitive cultivars than resistant ones, concluding proline cannot hold a protective role against salt stress. Silveira *et al.* (2003) reported that increase in proline concentration in wheat is bigger in varieties sensitive to salinity, and such changes occur differently in roots and shoots and in different growth stages.

Proteome analysis of root

Changes in the expression of proteins in barley roots (cold resistant cultivar EC83-1215), between control (25°C) and cold stress (4°C), were studied using a proteomic approach and two-dimensional electroph-

oresis techniques. Comparison between two-dimensional electrophoresis gels from control and cold stress conditions in the aforesaid cultivar led to emergence of 72 repeatable protein spots (Fig. 1). T-test was used to identify significant protein spots with significant expression.

Besides, IF indicators were used in order to identify spots with significant decrease or increase in expression. of the 72 appeared repeatable protein spots, 15 were significant both statistically and in case of inducing factor (Table 2).

Tab. 2. Description of proteins with modification in expression in barley cultivar EC83-1215.

hange in expression	Spot number	homologues	pI/MW(kDa) practical	pI/MW(kDa) theoretical	Access number
increase	2	Glysine-rich RNA-binding protein 7	5.4 /14	6/16	Q03250
decrease	1102	Germin-like protein	6.05 / 28	6.8/22	gil1755184
decrease	2503	ATPase beta subunit	6.2 / 54.5	5.5/54	gil13938810
increase	3301	NADP-specific isocitrate dehidrogenase	6.35 / 43	6.3/46	NP-917313
increase	3302	Froctose-bisphosphate aldolase, cytoplasmic isozyme	6.4 / 40	6.6/39	O65735
increase	4604	aldehyde dehydrogenase ALDH2b	6.65 /60	6.33/59.27	BAB 19052
decrease	5101	Glyceraldehyde-3-phosphate dehydrogenase C subunite(GAPC)	6.7 / 23	6.62/37	At3g04120
decrease	5302	Putative fructose-bisphosphate aldolase	6.67 / 37.5	7.57/37.69	AP004279
increase	5401	Actine isoform B	6.6 / 47	5.3/42	gil6683504
increase	5605	Catalase	6.75 / 56	6.93/57	AY339372
increase	6101	Adenylate kinase a	6.9 / 25	8.49/26.67	ABA96905
increase	6401	UGP (UDP-glucose pyrophosphorylase) UTP: glucose-1-phosphate uridylyltransferase	6.8 / 48	5.8/52	gil5228498
increase	6402	Putative cysteine proteinase	6.8 / 46.2	6.89/45.88	AP006170
increase	6503	Phosphoglycerate kinase	6.97 / 49	6.58/49.98	X73528
decrease	7101	Putative 6-phosphogluconolactonase	7.1 / 26	5.44/28.02	SGN-U313292

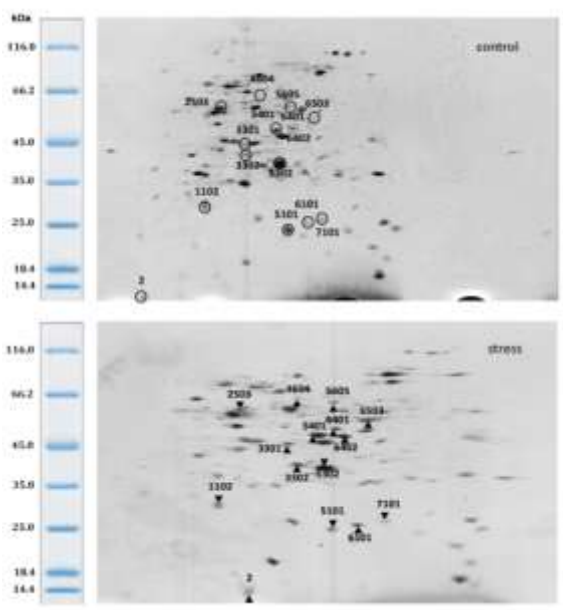

Fig. 1. Pictorial proteome profile of barley root cultivar EC83-1215 under normal and cold stress.*Functional grouping of identified proteins.*

Proteins identified here were assigned to three groups of proteins functioning in metabolic pathways and energy, proteins involved in the metabolism of RNA, cell wall and skeleton and those related to the energy paths, which respectively accounted for 60%, 26.6 % and 13.4% of the proteins identified (Fig. 2). Spot number 3301, in comparison between control and stress conditions, showed a statistically significant raise in expression under stress condition. This protein, NADP-specific isocitrate dehydrogenase, is involved in metabolism. Lee *et al.* (2009) reported that in rice roots, during 24- 48 hours of cold stress, proteins associated with metabolism including NADP-specific isocitrate dehydrogenase increased in expression. Isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to alpha-ketoglutarate which requires NAD^+ or $NADP^+$ and produces NADH or NADPH. These two plays an important role in conservation of the cells from

oxidative damage. It has been reported that NADPH-ICDH activities increased in response to temperature fall in roots of rice under cold stress (Lu *et al.*, 2005). According to the results obtained, it is clear that, under cold stress, plants need to produce more energy and regulate proteins involving in more energy production engaged in defense system. Spot Number 4604, in comparison between control and cold stress conditions, showed statistically significant changes in expression; increased in expression was observed in the state of stress. This spot identifies aldehyde dehydrogenase ALDH2b. Agzalyl-CoA decarboxylase and aldehyde dehydrogenase are enzymes responsible for aldehyde injuries caused by the attack of reactive oxygen species (ROS), a cumulative product in environmental stresses, to lipids, proteins and oxalic acid (Sunkar and Kirch, 2003).

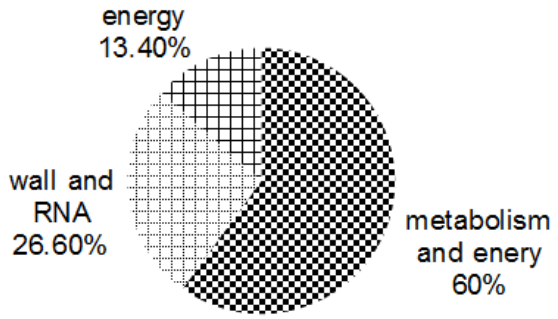

Fig. 2. Classification of proteins based on their known biological function.

Spot number 5302 showed a statistically significant change in expression, down-regulating under cold stress compared to control. This spot represents Putative fructose-bisphosphate aldolase. Abbasi and Komatsu (2004) reported that fructose-bisphosphate aldolase is not only down-regulated in response to salt stress, abscisic acid and cold stress can reduce its expression in rice leaf sheath. According to the researchers, a similar regulatory system functions in leaf stipules and leaf sheath.

Spot number 5605, in the comparison between control and stress, showed statistically significant increase in expression when plants experienced cold stress. This protein spot represents Catalase. Under salt stress, catalase activity in leaves of cowpea exhibited a sharp decline, but there was no change in roots (Hashimoto and Komatsu, 2007). But the activity of this enzyme recovered subsequently in leaves (Abbasi and Komatsu, 2004). In a research by Hashimoto and Komatsu (2007), catalase expression in leaf stipules increased only under cold stress. It seems that the catalase activity in response to cold stress is initially inhibited, and then increases. Spots numbers 6101 and 6402, showed significant increase in expression under stress, compared to control. These represent Adenylate kinase and a Putative cysteine proteinase, respectively, whose functions remain unknown under cold stress. Adenylate kinase catalyzes a reversible phosphorylation reaction of turning ADP to ATP and AMP (Pradet and Raymond, 1983). The output of this activity is production of pure ATP and, thus, ADP (Roberts *et al.*, 1997). In another study (Hashimoto and Komatsu, 2007), the quantity of adenylate kinase increased during cold stress. These results provide ample evidence as to ATP synthesis and energy metabolism in plants are affected by environmental change.

Cysteine proteinase has been examined in terms of plant responses to environmental stresses such as drought and frost (Harrak *et al.*, 2001). It has been also suggested that cysteine proteinase may preferentially accumulate in the roots under cold stress (Hashimoto and Komatsu, 2007).

Spot number 6503, representing Phosphoglycerate kinase, increased significantly in expression under cold stress than the control. The quantity of Phosphoglycerate kinase have increased in rice leaves under salt stress, and it seems that this protein enzyme is responsive to both salinity and cold (Parker *et al.*, 2006). Spots numbers 5101 and 7101, are related to proteins Glyceraldehyde-3-phosphate dehydrogenase C subunite (GAPC) and Putative 6-phosphogluconolactonase that have been down-regulated during cold stress. Spots numbers 2, 1102, 5401 and 6401 showed significant changes in expression, with spots 2, 5401, 6401 increased in

expression, and spot number 1102 was down-regulated under cold stress. Spot number 2 is Glysine-rich RNA-binding protein 7, 1102 Germin-like protein, 5401 Actine isoform B, and spot number 6401 is UGP (UDP-glucose pyrophosphorylase) UTP: glucose-1-phosphate uridylyltransferase, all describe proteins involved in RNA metabolism and cell wall skeleton. RNA metabolism, including RNA processing, its transferring from the nucleus to the cytoplasm and stabilizing mRNA structure, may be impaired under cold stress (Zhu *et al.*, 2007).

Mousavi and Hotta (2005) reported that the GRPs play an important role in modifying gene expression post-transcriptionally and in defense mechanisms in different plants after undergoing stress conditions. GPR7 expression falls in sensitive cultivars and increases in resistant ones. GPR7 is involved in mRNA exit from the nucleus to the cytoplasm in Arabidopsis under cold stress (Kim *et al.*, 2008). According to Mauro *et al.* (1997), two GRPs, including GPR7 and GRP1A, in T. halophila increased in expression under cold stress conditions, hence cold acclimation process can be enhanced with the departure of mRNA from nucleus. Protein spots numbers 2503 and 3302 respectively represent ATPase beta subunit and cytoplasmic isozyme of Froctose-bisphosphate aldolase, which have been down-regulated under cold stress, and both are involved in energy path.

Conclusion

Generally, it can be concluded that the treatment with cold stress at 4°C for 48 hours at three-leaf stage did not impose statistically significant change in the characteristics related to root, root dry weight, maximum root length, root average length and root surface density at 5%. In this experiment, tolerant barley cultivars treated with 4°C cold stress displayed an increase in proline. Performing two-dimensional electrophoresis (2DE) in the study on the cultivar, based on isoelectric point led to the identification of 15 protein spots with significant changes in expression. The proteins involved in metabolic pathways and energy accounted for most of the proteins with significant changes in expression.

References

Abbasi FM, Komatsu S. 2004. A proteomic approach to analyze salt-responsive proteins in rice leaf sheath. Proteomics **7,** 2072-2081.

Alizadeh A. 2004. Soil, water, plant relation-ship. Emam Reza University Press.

Bae MS, Cho EJ, Choi EY, Park OK. 2003. Analysis of the Arabidopsis nuclear proteome and its response to cold stress. The Plant Journal **36,** 652-663.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and soil **39,** 205-207.

Baum M, Grando S, Ceccarelli S, Backes G, Jahoor A. 2004. Localization of quantitative trait loci for dryland characters in barley by linkage apping. Challenges and Strategies of Dryland Agriculture, CSSA Special Publication **32,** 191-202.

Beck EH, Heim R, Hansen J. 2004. Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. Journal of Biosciences **29,** 449-459.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry **72(1),** 248-254.

Ganjeali A, Palta J, Turner NC. 2008. Effects of water logging on root and shoot growth of chickpea genotypes *[Cicer drietinum L.].* Gorgan University of Agricaltural Sciences and Natural Resources **15,** 78-89.

Ghosh D, Xu J. 2014. Abiotic stress responses in plant roots: a proteomics perspective. Frontiers in plant science **5**:6. doi: 10.3389/fpls.2014.00006.

Hajabbasi MA. 2010. Tillage effects on soil compactness and wheat root morphology. Journal of Agricultural Science and Technology **3,** 67-77.

Harrak H, Azelmat S, Baker EN, Tabaeizadeh Z. 2001. Isolation and characterization of a gene encoding a drought-induced cysteine protease in tomato (*Lycopersicon esculentum*). Genome **44,** 368-374.

Hashimoto M, Komatsu S. 2007. Proteomic analysis of rice seedlings during cold stress. Proteomics **7,** 1293-1302.

Hashimoto M, Toorchi M, Matsushita K, Iwasaki Y, Komatsu S. 2009. Proteome analysis of rice root plasma membrane and detection of cold stress responsive proteins. Protein and peptide letters **16,** 685-697.

Kim JS, Jung HJ, Lee HJ, Kim K, Goh CH, Woo Y, Kang H. 2008. Glycine-rich RNA-binding protein7 affects abiotic stress responses by regulating stomata opening and closing in Arabidopsis thaliana. The Plant Journal **55,** 455-466.

Lee DG, Ahsan N, Lee SH, Lee JJ, Bahk JD, Kang KY, Lee BH. 2009. Chilling stress-induced proteomic changes in rice roots. Journal of Plant Physiology **166,** 1-11.

Lu B, Yuan Y, Zhang C, Ou J, Zhou W, Lin Q. 2005. Modulation of key enzymes involved in ammonium assimilation and carbon metabolism by low temperature in rice (*Oryza sativa* L.) roots. Plant science **169,** 295-302.

Mauro S, Dainese P, Lannoye R, Bassi R. 1997. Cold-resistant and cold-sensitive maize lines differ in the phosphorylation of the photosystem II subunit, CP29. Plant physiology **115,** 171-180.

Mousavi A, Hotta Y. 2005. Glycine-rich proteins. Applied biochemistry and biotechnology **120,** 169-174.
O'Farrell PH. 1975. High resolution two-dimensional electrophoresis of proteins. Journal of biological chemistry **250,** 4007-4021.

Parker R, Flowers TJ, Moore AL, Harpham NV. 2006. An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. Journal of Experimental Botany **57,** 1109-1118.

Poustini K, Siosemardeh A, Ranjbar M. 2007. Proline accumulation as a response to salt stress in 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Genetic Resources and Crop Evolution **54,** 925-934.

Pradet A, Raymond P. 1983. Adenine nucleotide ratios and adenylate energy charge in energy metabolism. Annual Review of Plant Physiology **34,** 199-224.

Roberts JK, Aubert S, Gout E, Bligny R, Douce R. 1997. Cooperation and competition between adenylate kinase, nucleoside diphosphokinase, electron transport, and ATP synthase in plant mitochondria studied by 31P-nuclear magnetic resonance. Plant physiology **113,** 191-199.

Sunkar R, Bartels D, Kirch HH. 2003. Overexpression of a stress-inducible aldehyde dehydrogenase gene from Arabidopsis thaliana in transgenic plants improves stress tolerance. The Plant Journal **35,** 452-464.

Wang Y, Kim SG, Kim ST, Agrawal GK, Rakwal R, Kang KY. 2011. Biotic stress-responsive rice proteome: An overview. Journal of plant biology **54,** 219-226.

Xing T, Ouellet T, Miki BL. 2002. Towards genomic and proteomic studies of protein phosphorylation in plantpathogen interactions. Trends in plant science **7,** 224-230.

Zhu J, Dong CH, Zhu JK. 2007. Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation. Current opinion in plant biology **10,** 290-295.