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Comparative Evaluation of Changes in Protein Profile of Sugarcane Varieties under Different Soil Moisture Regimes

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A B S T R A C T

Four commercial sugarcane varieties differing in their maturity were studied for the protein profiles under control (50% ASM), moisture stress of 40% ASM (mild stress) and 30 % ASM (severe stress). Both qualitative and quantitative differences were observed. Total soluble proteins content in leaves significantly increased at both 40% ASM levels (21.05 and 27.19%) and 30% (27.15 and 43.44%) as compared to 50% ASM level at 60 and 90 DAP, respectively. Severe stress of 30% ASM levels caused formation of new protein band of MW 18.56, 13.46, 31.6 and 36.6 kDa in leaves of variety Co 0238. In CoJ 64, one new polypeptide band of MW 38.5 kDa appeared and two polypeptide bands of MW 13.6 and 18.56 kDa disappeared. Variety CoS 767 showed the presence of four new band of 13.46, 15.6, 18.56 and 38.2 kDa. Likewise in variety CoH 128 two new polypeptide bands of MW 25.2 and 26.8 kDa appeared and 4 band of MW 13.46, 15.6, 18.17 and 54.6 kDa disappeared at 30% and 40% ASM levels. Appearance of new polypeptide(s) or their disappearance might be related to the genotypic stress tolerance or sensitivity.

Introduction

Sugarcane, a crop of great worldwide economic importance, accounts for approximately 75% of the global sugar production (Commodity Research Bureau, 2015). Being a high water demanding crop, sugarcane has necessitated the need to evolve drought tolerant varieties to sustain sugarcane production. Water is the major constituent of cane and approximately 2.97 lakh ha of cane area is prone to drought affecting the crop at one or other stage of growth in every state of India (Vision SBI, 2016). Water stress elicits a complex of responses beginning with stress perception, which initiates a signal transduction pathway(s) and causes multifaceted changes at the cellular, physiological and developmental levels (Bray, 2001). During drought, plant water status, water conductance and conservation are controlled by different mechanisms and protein synthesis plays major role as a biochemical signal in the adaptive response of plants (Singh *et al.*, 2013). SDS-PAGE is most economical simple and extensively used

biochemical technique for analysis of genetic structure of germplasm. The importance of protein profiling has long been acknowledged in plant abiotic stress studies and previous studies (Kiegel *et al.,* 2000) have provided useful information on stress-dependent changes in quantity, activity, as well as modifications of structural protein, protein-protein interactions, stress dependent protein movements, de novo synthesis and controlled degradation which in turn suggested that many proteins are specifically altered *de novo* under water stress as a part of the adaptive mechanism. There is net synthesis of some proteins and decrease in the others, with or without induction of unique stress proteins. These stress-induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with stress (Chandra and Tyagi, 2004).

Proteins which are synthesized in response to drought stress are called dehydrin (dehydration-induced). Most investigation on plants grown under drought stress showed the evidence of dehydrin proteins with molecular weight ranging from 22 - 60 kDa. These stress-induced proteins are involved in the biosynthesis of osmolytes, uptake and compartmentation of ions, hydroxyl-radical scavenging and protection of cellular structure (Joshi *et al.,* 2013). Thus, understanding the biochemical and molecular basis of drought tolerance will be helpful in developing strategies for improving drought tolerance in sugarcane. The present study was undertaken to see the effect of water stress on protein profiles of four sugarcane varieties differing in their maturity.

Materials and Methods

To study the effect of irrigations at different available soil moisture (ASM) levels on four sugarcane varieties, two under mid late group viz., CoH 128, CoS 767 and two under early group viz., Co 0238 and CoJ 64. After complete germination (40 days after planting), three levels of ASM were created i.e. irrigation at 50% ASM level (control), irrigation at 40% ASM level (mild stress) and irrigation at 30% ASM level (severe stress). These ASM levels were created only during pre-monsoon (in the month of April, May and June) period by withholding irrigation and later on i.e. post monsoon period (in the month of July), the crop was irrigated for stress revival as per requirement. Sampling was done at 60, 90 and 120 days after planting (DAP).

Extraction and determination of soluble proteins

One gram fresh samples from first TVD leaf was homogenized with the help of pre-chilled pestle and mortar in 2.5 ml of chilled tris buffer (0.1 M, pH 8.0) containing 0.1% polyvinyl pyrrolidone (PVP). The homogenate were then centrifuged at 10,000 g at 4°C for 15 min. The supernatant containing the proteins was taken in a chilled test tube. The amount of protein in the extract was determined following Bradford (Bradford, 1976).

Protein profile resolution (SDS-PAGE)

A 25 µl of crude protein extract, containing 50 µg of protein extract was transferred to an equal volume of Laemmli's 2X sample buffer (0.5 M Tris-HCl, pH 6.8) containing 20% glycerol, 4% SDS, 0.5% bromophenol blue (w/v) and 10% β-mercaptoethanol and heated at 100°C for 3 min and cooled. Electrophoresis was carried out by the method of Laemmli (Laemmli *et al.,* 1970). The cooled samples were then loaded on to a SDS-discontinuous gel system with a 0.1 mm thick stacking gel of 4% polyacrylamide in Tris-HCl buffer (pH 6.8) and a resolving gel of 10% polyacrylamide in Tris-HCl buffer

(pH 8.8). The gels were run at 15mA in the stacking gel and 25 mA in the resolving gel. After electrophoresis, gels were fixed and stained with 0.25% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol with 7% glacial acetic acid (v/v) and then destained in 10% methanol (v/v) with 7.5% glacial acetic acid (v/v). After destaining, the gels were stored at 7% glacial acetic acid (v/v).

Statistical analysis

All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Least significant difference test was applied at 5 per cent probability level to compare the mean differences.

Results and Discussion

Total soluble proteins content in leaves significantly increased at both 30% (27.15 and 43.44%) and 40% ASM levels (21.05 and 27.19%) as compared to 50% ASM level at 60 and 90 DAP, respectively. At 30% ASM level, significantly higher proteins content was recorded in variety Co 0238 (13.7 mg g^{-1} DW) followed by CoH 128 (13.36 mg g^{-1} DW) and CoS 767 (11.71 mg g^{-1} DW) whereas the lowest value was recorded in CoJ 64 (8.67 mg g^{-1} DW). After stress revival (at 120 DAP), non-significant difference was recorded at 30% and 40% ASM levels as compared to 50% ASM level (Table 1).

The alteration of protein synthesis or degradation is one of the fundamental metabolic processes that affect drought tolerance. Comparative evaluation of changes in protein profile was performed in sugarcane varieties (Co 0238, CoJ 64, CoH 128 and CoS 767) using SDS-PAGE analysis. The effect of different ASM levels were studied at different stages i.e. 60 DAP, 90 DAP and 120 DAP (stress revival) and clear differences was seen in protein profile pattern by the presence or absence bands to varied intensity of expression. At 60 DAP, in variety Co 0238, two new bands of MW 36.6 and 31.6 kDa appeared and in case of CoJ 64, one new polypeptide band of MW 38.5 kDa appeared at 30% ASM level. Whereas in CoH 128, two new polypeptide bands of 26.8 and 25.2 kDa appeared at 30% ASM level and CoS 767 showed the presence of one new band of 38.2 kDa at 30% ASM level and 40% ASM level in comparison to 50% ASM level (Figure 1).

These proteins might be synthesized either *de novo* in response to drought stress or may be present constitutively at low concentration and increase when these varieties were exposed to higher stresses. At 90 DAP, one new polypeptide band of MW 18.56 kDa appeared at 40% ASM level and one new band of MW 13.46 kDa appeared at 30% ASM level in Co 0238 whereas in CoJ 64, two polypeptide bands of MW 18.56 and 13.6 kDa disappeared at 30% ASM level. On the other hand in variety CoH 128, one band of MW 13.46 kDa disappeared at 40% ASM level and disappearance of 3 polypeptide bands of MW 54.6, 18.17 and 15.6 kDa was observed at 30% ASM level and in variety CoS 767, *denovo* synthesis of two new bands of MW 15.6 and 13.46 kDa were observed at 40% ASM level (Figure 2).

Such modifications might led to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear or disappear after stress treatment (Kumar *et al.*, 2015). Upon stress revival (120 DAP), no significant effect of different ASM levels were observed on protein profiling of different sugarcane varieties (Fig. 3). These results showed that varietal behaviour to moisture stress/drought was dependent on nature and concentration of stresses involved.

Table.1 Effect of different soil moisture regimes on total soluble protein content (mg g^{-1} DW) in sugarcane varieties differing in their maturity group

Varieties/Treatments	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean	CoH 128	CoS 767	Co 0238	CoJ 64	Mean
	LAI after 30 – 60 DAP					LAI after 30 – 60 DAP					LAI after 90 – 120 DAP (stress revival)				
Irrigation at 50 % ASM (Control)	7.64	8.85	8.83	8.13	**8.36^{B}**	11.65	8.26	10.24	8.23	**9.6^{C}**	9.50	8.90	10.61	9.43	**9.61**
Irrigation at 40 % ASM (Mild stress)	8.52	10.48	13.18	8.32	**10.12^{A}**	13.18	13.16	13.95	8.57	**12.21^{B}**	8.26	9.09	9.48	9.30	**9.03**
Irrigation at 30 % ASM (Severe stress)	9.07	10.80	13.97	8.70	**10.63^{A}**	15.25	13.73	16.89	9.21	**13.77^{A}**	8.37	8.98	9.93	9.75	**9.26**
Mean	**8.41^{C}**	**10.04^{B}**	**11.99^{A}**	**8.38^{C}**		**13.36^{A}**	**11.71^{B}**	**13.7^{A}**	**8.67^{C}**		**8.71^{C}**	**8.99^{BC}**	**10^{A}**	**9.49^{AB}**	
CV	**Varieties – 7.92; Treatments – 6.06**					**Varieties – 3.857; Treatments – 2.352**					**Varieties – 5.67; Treatments – 5.572**				
LSD	**V – 0.76**	**T – 0.67**	**T×V – 1.32**	**V×T – 1.31**		**V – 0.45**	**T – 0.32**	**T×V – 0.75**	**V×T – 0.78**		**V – 0.52**	**T – NS**	**T×V – NS**	**V×T – NS**	

Least significant difference test was applied at 5 per cent probability level to compare the mean differences.
(ASM – Available Soil Moisture; V – Varieties; T – Treatments; T × V – Treatments at the same level of varieties; V × T – Varieties at the same level of treatments)

Fig.1 Effect of different soil moisture regimes on polypeptide resolution (A) early sugarcane varieties (Co 0238; CoJ64) and (B) mid-late sugarcane varieties (CoH 128; CoS 767) at 60 DAP

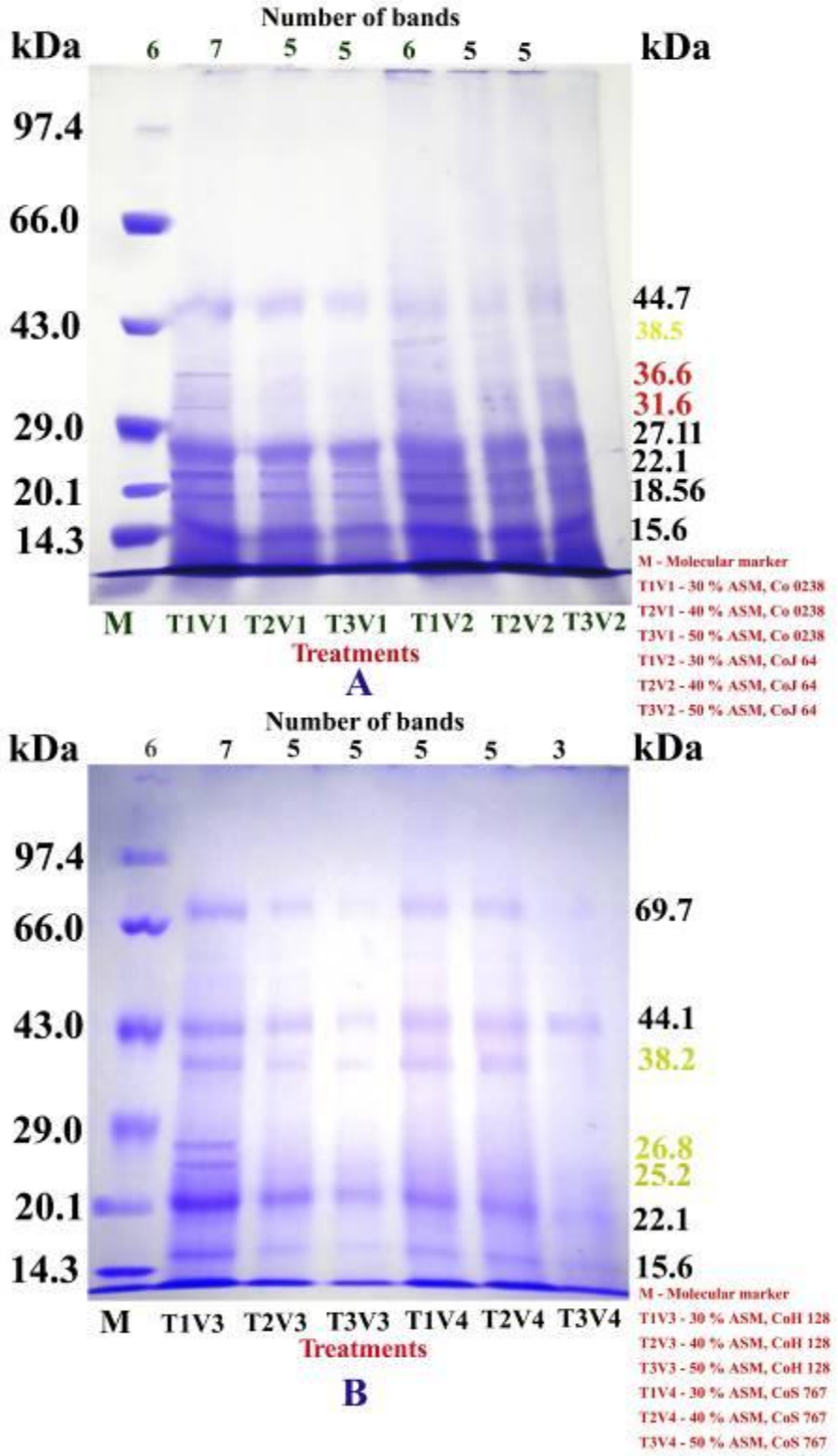


Fig.2 Effect of different soil moisture regimes on polypeptide resolution (A) early sugarcane varieties (Co 0238; CoJ64) and (B) mid-late sugarcane varieties (CoH 128; CoS 767) at 90 DAP

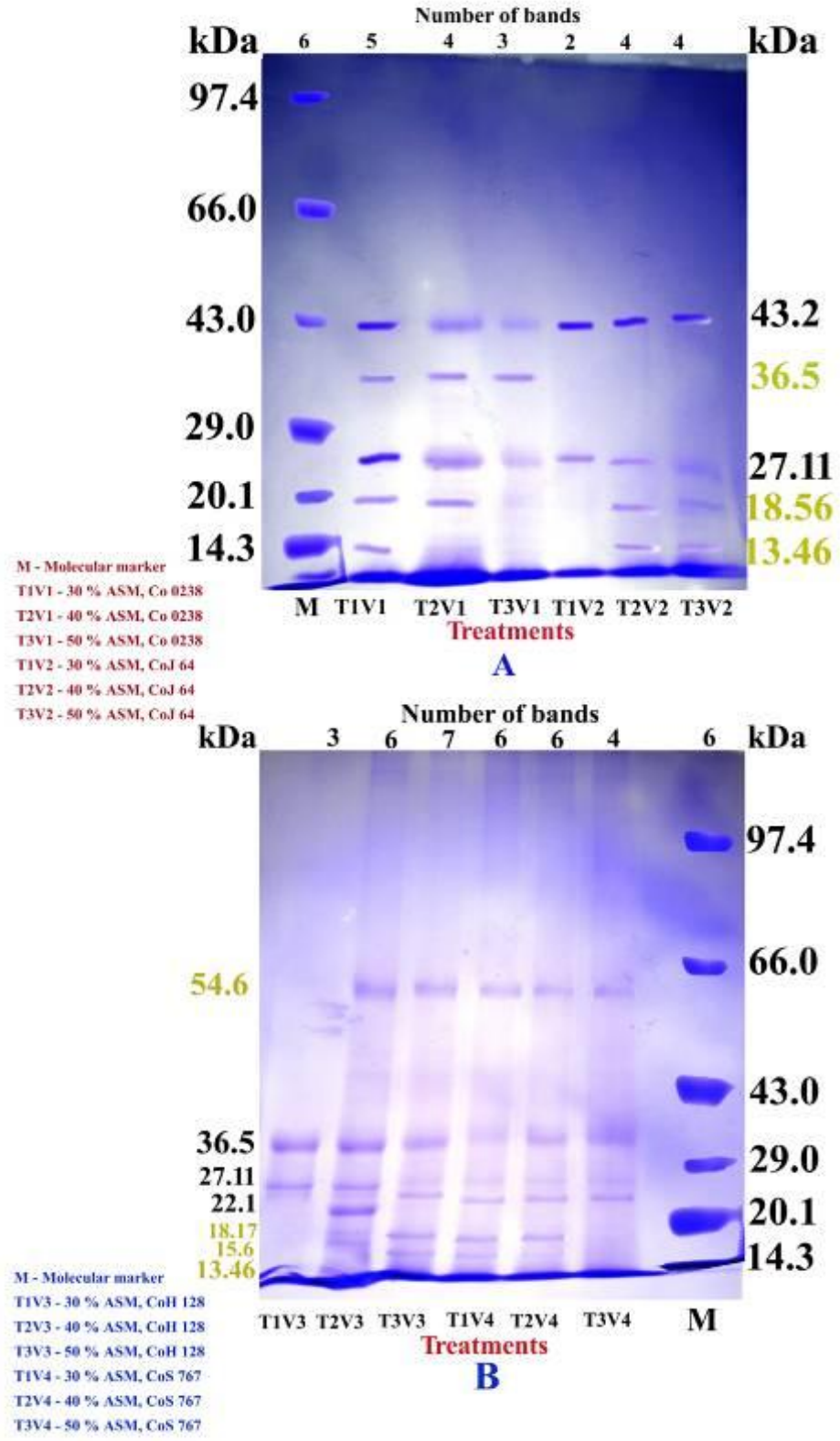
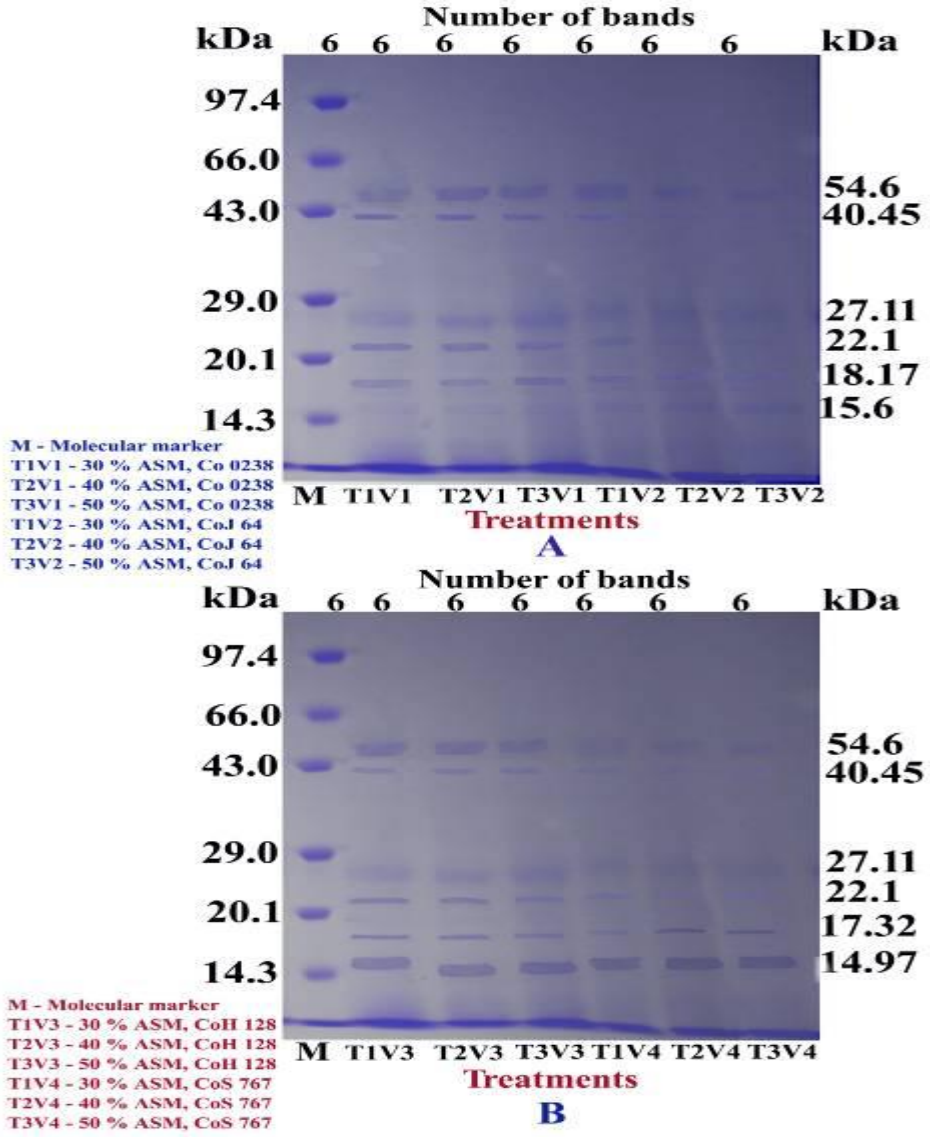


Fig.3 Effect of different soil moisture regimes on polypeptide resolution (A) early sugarcane varieties (Co 0238; CoJ64) and (B) mid-late sugarcane varieties (CoH 128; CoS 767) at 120 DAP (stress revival)



The bands which were present in the treated plants may be inherently associated with germination and growth processes. Their disappearance may affect the functional capabilities of plants to perform in the stress environment or it may suggest the negative effect of stress on protein/gene synthetic machinery.

The comparison of polypeptide profile of early and mid-late sugarcane varieties showed that four polypeptide bands (MW 54.6, 40.45, 27.11 and 22.1 kDa) appeared at 120 DAP (rewatering). This suggested that polypeptide expression varied depending upon the different developmental stages and the differential gene expression of concerned structural or regulatory gene (s). Our results were also in accordance with Jangpromma *et al.*, (2010) who reported accumulation of an 18 kDa protein in K86-161 sugarcane line which was subjected to progressive water

stress for 20 days.

The synthesis and accumulation of most of the polypeptides under moisture stress, in the present study suggests major mechanisms that underlie adaptation or tolerance to osmotic stress.

It is generally assumed that stress induced proteins may play a role in tolerance, but direct evidence is still lacking and the function of many stress responsive genes are unknown. Stress associated proteins are either synthesized *de novo* in response to stress or present constitutively at low level and their expression increases in response to stress.

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