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ORIGINAL ARTICLE

Drought Stress and Its Impact on Protein in Three Species of *Vitex*

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Drought is one of the most important natural phenomenon which affects on plant growth. When drought stress is imposed different molecular and biochemical responses took place in the plants. The protein profile of three species of *Vitex* (*Vitex trifolia* L., *Vitex altissima* L. and *Vitex negundo* L.) under normally irrigated condition and severe drought plants was analyzed through SDS-PAGE. Drought stress significantly affects proteins in plants when compared the normal conditioned plants. Several new protein bands were identified in the stressed plants. It seems that *Vitex* species can be adapted to drought stress conditions. Hence it was concluded that number of new proteins were synthesized in stressed plants for their adaptation in the stressed conditions. These proteins could be used as markers in identifying the stressed plants.

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Drought is one of the most significant factors among abiotic stresses that limit plant performance, growth and productivity (Chaves and Oliveira, 2004). Drought can be defined as the absence of rainfall or irrigation for a period of time sufficient to deplete soil moisture and injure plants. Drought stress results when water loss from the plant exceeds the ability of the plant's roots to absorb water and when the plant's water content is reduced enough to interfere with normal plant processes (Pierce, 2005).Nowadays many physiological, biochemical and molecular biology studies on the mechanisms of drought tolerance of agriculturally important crops have been performed (Yamaguchi-Shinozaki *et al.,* 2002). Plants being sessile have evolved specific acclimation and adaptation mechanisms to respond and survive short- and long- term drought stresses. Analysis of these protective mechanisms will contribute to our knowledge of tolerance and resistance to stress. The complex responses to

environmental stress, from perception, to transcriptional and physiological changes need to be considered at a global systems biology level to study the multiple interactive components in this biological process (Krishnan and Pereira, 2008). Hence the aim of this study was to analyze changes in protein pattern under normal and drought plants of three species of *Vitex* using SDS-PAGE.

MATERIALS AND METHODS

Materials:

Normal and drought *Vitex* species (*Vitex trifolia* L., *Vitex altissima* L., *Vitex negundo* L. and) were selected for the present study. The plants were identified and collected from in and around Tirunelveli regions. The collected materials were stored in deep freezer (-70^{0}C) for the SDS-PAGE analysis.

Methods:

Isolation of protein

250mg of stored plant materials were ground well in a mortar and pestle with extraction buffer (0.1M Tris-HCl, pH 8.0, 0.01 M $MgCl_2$, 18% (w/v) sucrose, and 40mM β-mercaptoethanol). The homogenate was centrifuged at 10000 rpm for 10 minutes. The supernatant was taken and the volume was noted. Equal amount of 10% TCA was added and kept in ice for 30 minutes. The mixture was centrifuged at 10000 rpm for 10 minutes. The pellet was collected and dissolved in 50µl of 0.2N NaOH. It was stored at -20^{0}C for further analysis.

SDS - PAGE analysis

SDS –PAGE of leaf protein was carried out in vertical slab gel discontinuous buffer system following the method of Laemmli (1970) using 10% acrylamide gel concentration. A total volume of 12µl protein extract solution was loaded into each well and electrophoresis was carried out at 100V until the bromophenol blue dye reaches the bottom of the gel.

Molecular weight Determination

The molecular weights of the dissociated polypeptides were determined by using the standard curve. The standard curve was plotted by calculating standard protein against the $\log_{10}$ of its molecular weight. Distance from the wells of the protein bands were found out. The molecular weight of the unknown protein bands and their Rf values were calculated using Total Lab 100 software.

RESULTS AND DISCUSSION

Drought is a meteorological term and is commonly defined as period without significant rainfall. In order to find out the effect of drought stress on proteins, this investigation was carried out.

The protein profile of normal *V. trifolia* has seven protein bands and stressed one has eight protein bands but the molecular weight and Rf value totally differ from normal plants. In *V. trifolia* six new proteins were synthesized in stressed plants (144.2558kD, 120.283kD, 101.963kD, 63.145kD, 41.777kD, 21.932kD) (Table 1). In *V.altissima* two new proteins which were absent in the normal plants, were found in the stressed ones (178.825kD, 149.105kD) (Table 2). With regard to *Vitex negundo* three new proteins were synthesized in stressed plants (172.122 kD, 90.443kD, 13.122kD) (Table 3). Pixel positions of both condition plants of all species of *vitex* are calculated (Fig 1-3).

Similar studies have been undertaken by earlier workers. Under stress condition the plant synthesize the heat shock proteins. The essential function of heat shock proteins is preventing aggregation and assisting refolding of non-active proteins under both normal and stress conditions (Hartl, 1996; Frydman, 2001).The synthesis of stress proteins in stress-tolerant plants occurs more intensive. Under temperature stresses the amount of HSPs in

mitochondria and chloroplast increased. Fast adaptive reactions in stress-tolerant plants promote their survival in unfavorable conditions. The observed distinctions in protein synthesis patterns suggest that stress response proteins could be useful as biomarkers of different ecological strategies.

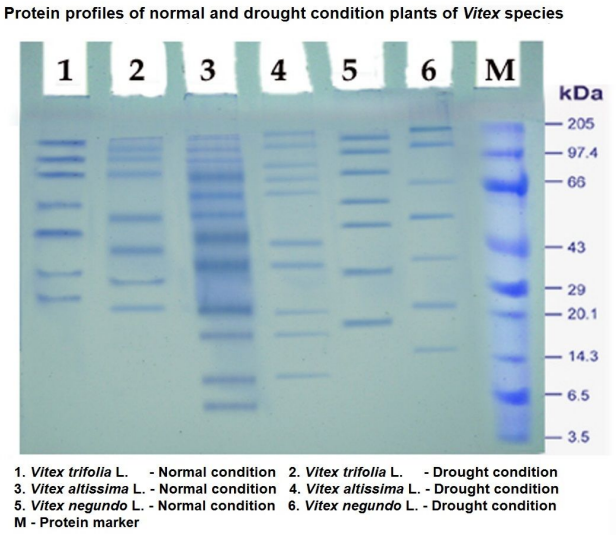

Figure 1. Pixel position of the protein bands present in *Vitex trifolia*

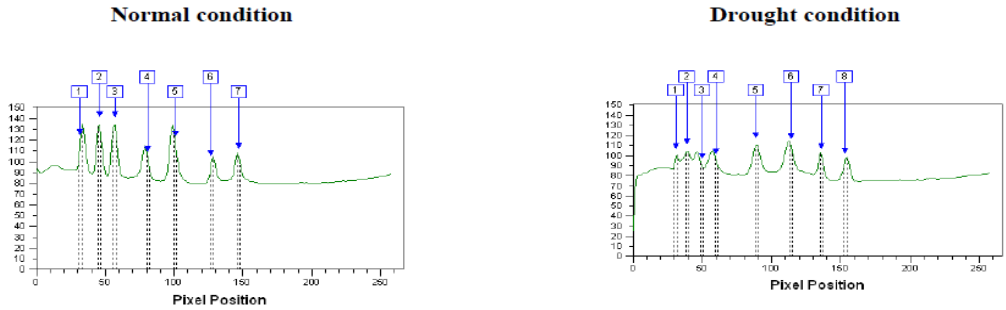

Figure 2 Pixel position of the protein bands present in *Vitex altissima*

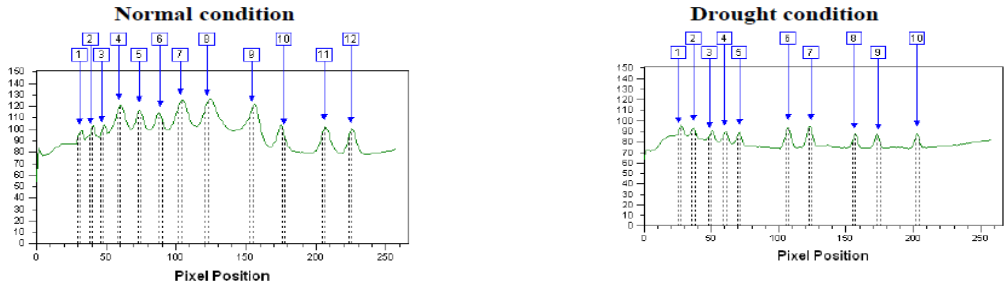

Figure 3 Pixel position of the protein bands present in *Vitex negundo*

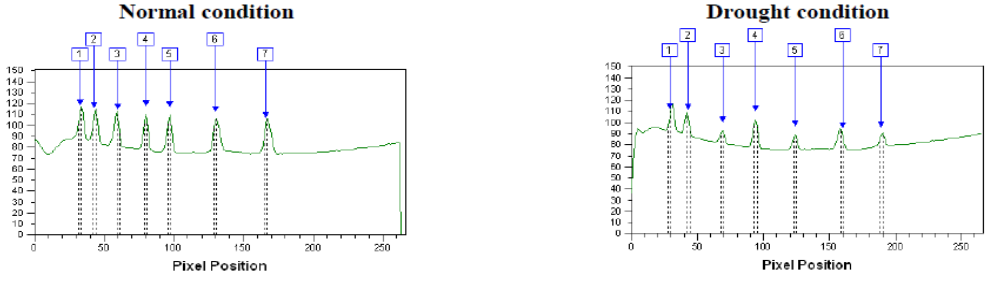

Table 1. Molecular weight & Rf values of proteins in *Vitex trifolia*

Ref Band No.	*Vitex trifolia*					
	Normal condition			Drought condition		
	Band No.	MW (kD)	Rf	Band No.	MW (kD)	Rf
1	1	161.947	0.124	1	164.645	0.12
2	-	-	-	2	144.258	0.151
3	2	128.501	0.178	-	-	-
4	-	-	-	3	120.283	0.194
5	3	107.145	0.221	-	-	-
6	-	-	-	4	101.963	0.233
7	4	72.069	0.314	-	-	-
8	-	-	-	5	63.145	0.345
9	5	51.788	0.391	-	-	-
10	-	-	-	6	41.777	0.442
11	6	33.701	0.492	-	-	-
12	7	24.217	0.57	7	29.045	0.527
13	-	-	-	8	21.932	0.593

Table 2. Molecular weight & Rf values of proteins in *Vitex altissima*

Ref Band No.	*Vitex altissima*					
	Normal condition			Drought condition		
	Band No	MW (kD)	Rf	Band No.	MW (kD)	Rf
1	-	-	-	1	178.825	0.101
2	1	163.793	0.121	-	-	-
3		-	-	-	149.105	0.143
4	2	143.448	0.152	-	-	-
5	3	125.631	0.183	3	122.287	0.19
6	4	102.967	0.23	4	101.963	0.233
7	5	80.298	0.288	5	85.017	0.275
8	6	62.619	0.346	-	-	-
9	7	49.649	0.401	6	46.9	0.415
10	8	36.234	0.475	7	35.414	0.481
11	9	21.317	0.599	8	20.871	0.605
12	10	14.559	0.689	9	15.502	0.674
13	11	9.002	0.802	10	9.6	0.787
14	12	6.57	0.875	-	-	-

Table 3. Molecular weight & Rf values of proteins in *Vitex negundo*

Ref Band No.	*Vitex negundo*					
	Normal condition			Drought condition		
	Band No	MW (kD)	Rf	Band No	MW (kD)	Rf
1	-	-	-	1	172.122	0.109
2	1	161.078	0.125	-	-	-
3	2	136.94	0.163	2	137.412	0.162
4	3	103.913	0.228	-	-	-
5	-	-	-	3	90.443	0.26
6	4	75.103	0.304	-	-	-
7	5	56.989	0.369	4	60.494	0.355
8	6	33.352	0.494	5	37.335	0.468
9	-	-	-	-	-	-
10	7	18.591	0.631	6	20.922	0.604
11	-	-	-	7	13.122	0.713

Abdollah *et al*., (2010) reported that water deficit stress is one of the important factors limiting chickpea production in arid and semiarid regions of West Asia and North Africa. When water deficit stress is imposed different molecular and biochemical responses take place. Probable stress responsive proteins in relation to imposed water deficit stress was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS - PAGE) method.

Abiotic stress usually causes protein dysfunction. Maintaining proteins in their functional conformation and the aggregation of non - native proteins are particularly important for cell survival under stress. Heat shock proteins (HSPS)/chaperones are responsible for protein folding, assembly, translocation and degradation in many normal cellular processes, stabilize protein and membranes and can assist in protein refolding under stress conditions. They can play a crucial role in protecting plants against stress by reestablishing normal protein conformation and thus cellular homeostasis (Wangxia**,** 2004).

Ali and Basha (1998) showed that the total protein content of the leaves significantly increased when peanut plants were subjected to water stress for 5 to 20 as compared to irrigated controls. Analysis of the leaf protein by SDS polyacrylamide gel electrophoresis showed higher levels polypeptides in stressed leaves.

From the results obtained in our study and based on the earlier reports it is understood that additional proteins are synthesized during stress conditions. This may be due to the stress response of plants. Additional proteins may be considered as marker proteins or stress resistant proteins.

CONCLUSION

This investigation helps to understand the response of plants to stress at the molecular level. The morphological responses are the result of the molecular changes. During stress, the plants not only show morphological changes, but also molecular changes. This investigation is useful to identify the stressed plants accurately. Further studies using molecular markers will help to understand the genetic variations due to stress.

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