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ORIGINAL ARTICLE

Differential expression of Isoesterases in leaves and roots of Vigna unguiculata L. in response to saline stress

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The present study was intended to reveal the differential isoesterase expression in leaves and roots of *Vigna unguiculata* L. in response to saline stress. Influence of NaCl (0, 3, 5, 7 and10 %) on seedlings of *Vigna unguiculata* L. was analyzed. The seedlings were grown in the centre for a period of 30 days. The randomly collected whole plants were used as a source for isoesterase isolation. The seedlings showed the maximum tolerance up to 10% of NaCl. The Poly acrylamide gel electrophoresis was performed by Anbalagan method. The staining and fixation of the enzyme was performed by the Sadasivam and Manickam method. Seedlings treated with various concentrations of NaCl showed different banding profile based on the concentration (0-10%) and duration (5-30 days) of salt treatment as follows: 32 bands with five active regions, 56 bands with eight active regions, 102 bands with eight active regions, 47 bands with seven active regions, 64 bands with nine active regions and 84 bands with nine active regions on 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} day respectively. The changing pattern of isozymes during development may be interpreted as evidence for differential timing of gene expression correlated with the physiological stress. The results of the present study concluded that the isoesterase patterns could serve as a useful biochemical marker of salinity.

Key words: Isoesterase; Salinity; biochemical marker; stress

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All organisms that continue to exist within natural environments are subject to stress at some points of their life (Bohnert *et al.,* 1995). At present, drought and salinity are the two important environmental factors that decrease plant productivity (Radic *et al.,* 2010). Soil salinity is a major limitation to control food production because it confines crop yield and restricts use of land previously uncultivated (Yokoi *et al.,* 2002). The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo, 1995). The problem is becoming more common as the intensity of agriculture increases (Bohnert *et al.,* 1995). Therefore, it is important to understand how plants respond and adapt to such types of stress. Change (usually increase) in the level of detoxification enzyme activity, such as peroxidase and esterase, have been used as a potential biomarker of many toxic

compounds like heavy metals, pesticides, but also of stresses caused by salinity, extreme temperatures, drought, ozone, etc. (Madhava Rao and Sresty, 2000; De Azevedo Neto *et al.,* 2006; Noriegax *et al.,* 2007; Dooslin mary *et al.,* 2009; Dooslin mary *et al*., 2010; Johnson *et al.,* 2011). In the present study esterase activity has been investigated as a potential biomarker of salt stress using *Vigna unguiculata* L. as a plant model. Esterases, a group of hydrolases, catalyze the formation or cleavage of ester bonds of water soluble substrates. Generally, these enzymes have a broad spectrum of substrates and act on a variety of natural and xenobiotic compounds (Cummins *et al.,* 2001). Esterases have been extensively studied in insects and vertebrates but much less in plants. Since esterases exist in different isoenzymes in plant and animal tissues, their electrophoretic pattern was also analyzed. The relationship between esterase activity and salinity has been investigated in several plant species, irrespective of their tolerance to salt stress (Thiyagarajah *et al.,* 1996; Hassanein, 1999; Dasgupta *et al.,* 2010). The molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (Sanders, 2000; Zhu, 2000; Hasegawa *et al.,* 2000). With this background the present study was intended to reveal the differential isoesterase expression in leaves and roots of *Vigna unguiculata* L. in response to saline stress.

MATERIALS AND METHODS

Seeds of *Vigna unguiculata* L. were obtained from the TNAU, Coimbatore. Liquid nutrient media of two different formulations Knudson (KC) and Murashige and Skoog's medium were used for the experiments. The pH of the media were adjusted to the suitable pH (5.8) using pH meter with 1 N NaOH / 1 N HCl before adding 0.5% (w/v) agar and dissolving it by heating at 80° C in a microwave over for solid media. The media were sterilized by autoclaving at 121° C under 1.1 kg cm-1 pressure for 15 min in an autoclave. The seeds were surface sterilized with 0.1% $HgCl_2$ & 0.1% sodium lauryl sulfate solution for 3-5 min and rinsed thrice with sterile distilled water. Influence of NaCl (0, 3, 5, 7 and10 %) on seedlings of *Vigna unguiculata* was analyzed. The seedlings were grown in the centre for a period of 30 days. The randomly collected whole plants were used as a source for isoesterase isolation. For Esterase (EC 1.11.1.7), 500 to 1000 mg of freshly harvested young leaves and root were taken and homogenized with 3.5 ml of ice-cold 0.1M phosphate buffer (pH 7.0) in a pre-chilled pestle and mortar and centrifuged at 12,000 rpm for 10 min and the supernatant was collected and used for isoesterase analysis. The Poly acrylamide gel electrophoresis was performed by Anbalagan (1999) method. The staining and fixation of the enzyme was performed by the Sadasivam and Manickam (1992) method. Each band of Esterase was named by capital letter E followed by a number for each isozyme. The MW-Rf value of each band was calculated by the following formula described by Sokal and Sneath.

RESULTS

The exomorphic character such as shoot length, fresh weight and dry weight were affected differently with reference to salt concentrations. The seedlings showed the maximum tolerance up to 10% of NaCl. The isoesterase banding pattern of the *V. unguiculata* seedlings under different salt conditions has been depicted in Fig 1A - G. Seedlings treated with various concentrations of NaCl showed different banding profile based on the concentration (0-10%) and duration (5-30 days) of salt treatment as follows: 32 bands with five active regions, 56 bands with eight active regions, 102 bands with eight active regions, 47 bands with seven active regions, 64 bands with nine active regions and 84 bands with nine active regions on

5th, 10th, 15th, 20th, 25th and 30th day respectively (Table 1-6).

Varietal Difference and Tissue Specificity of Esterase

Leaves

On 5th day, a total of 21 bands were observed in the leaf and their MW-Rf values ranged from 0.36 to 0.98 (Fig. 1; Table 1). Est 1 (0.36) and Est 4 (0.84) were showed their presence in all the four salt concentrations of treatments, whereas Est 2 (0.67), Est 3 (0.74), E6 (0.87) and Est 9 (0.98) were with specific response to salt stress at 3%. On 10th day, a total of 24 bands were observed in the leaf and their MW-Rf values ranged from 0.22 to 0.98 (Fig. 1; Table 2). Est 1 (0.36) and Est 4 (0.84) were with similar response to four different concentrations of salt treatments, whereas Est 2 (0.67), Est 3 (0.74), Est 6 (0.87) and Est 9 (0.98) were with unique response to salt stress at 3% concentration. On 15th day, a total of 42 bands were observed in the leaf of salt treated seedlings and their MW-Rf values ranged from 0.22 to 0.95 (Table – 3; Fig. 1). Bands of Est 2 (0.32), Est 10 (0.81) and Est 13 (0.96) were commonly present in leaves of seedlings treated with all concentrations of salt, while the band Est 18 (0.87) was present only in leaves of seedlings treated with 3% salt.

On 20th day, a sum of 27 bands were illustrated in the leaves and their MW-Rf values ranged from 0.05 to 0.99 (Table – 4; Fig. 1). Isoesterases with 0.07 (Est 2) and 0.09 (Est 3) Rf values were commonly present in leaves of all salt treated seedlings. While Est 4 (0.13), Est 10 (0.64), Est 11 (0.90) were present only in the seedlings treated with 7% of NaCl. The isoesterase with 0.41 (Est 6) and 0.46 (Est 7) showed their unique occurrence only in 5% NaCl treated seedlings leaves of *V. unguiculata*. The isoesterase Est 12 (0.94) was observed only in 10% NaCl treated seedlings of *V. unguiculata.* On 25th day, a total of 32 bands were observed in the leaves and their MW-Rf values ranged from 0.04 to 0.99 (Table – 5; Fig. 1). The isoesterase Est 2, Est 3, Est 12 and Est 13 showed their common presence in leaves of 3, 5, 7 and 10% NaCl treated seedlings. While Est 11 (0.92) was present only in 3% salt treated seedlings leaves.

On 30th d, a total of 38 bands were viewed in the salt treated seedlings leaf and their MW-Rf values ranged from 0.08 to 0.99 (Table – 5; Fig. 1). The isoesterase with 0.08 (Est 1), 0.13 (Est 2) and 0.99 (Est 13) Rf values were commonly present in all concentrations of salt treated seedlings leaves. Whereas Est 7 (0.56) and Est 8 (0.67) were restricted their expression only in 3% NaCl treated seedlings leaves of *V. unguiculata*. The isoesterase with 0.24 (Est 4) Rf values was present only in 7% NaCl treated seedlings leaves of *V. unguiculata.*

Root

On 5th day, a total of 11 bands were observed in the root of salt treated seedlings of *V. unguiculata* and their MW-Rf values ranged from 0.82 to 0.95 (Fig. 1; Table 2). The bands Est 5 (0.84) and E6 (0.87) showed their unique presence only in 5% and 7 % salt treated seedlings respectively. On 10th day, a total of 32 bands were observed in the root and their MW-Rf values ranged from 0.22 to 0.98 (Fig. 1; Table 2). Est 1 (0.22); bands Est 10 (0.81) and Est 13 (0.96) were common in seedlings treated with four different concentrations of salt treatments, whereas the bands Est 4 (0.40) and Est 5 (0.46) showed their unique presence only in 3% of salt treated seedlings. On 15th day, a total of 60 bands were demonstrated in the root of salt treated seedlings and their MW-Rf values ranged from 0.22 to 0.95(Table – 3; Fig. 1). Est 1 (0.22), Est 8 (0.53), Est 10 (0.65), Est 11 (0.66), Est 13 (0.74), Est 14 (0.76), Est 15 (0.79) and Est 16 (0.81) were jointly present in all concentrations of salt treated seedlings roots, while Est 2 (0.25), Est 12 (0.69)

and Est 18 (0.87) were present only in 3% salt treated seedlings root of *V. unguiculata*. The Est 5 (0.39) and Est 19 (0.90) were showed their unique expression only in 5% NaCl treated seedlings root. Whereas Est 3 (0.27) and Est 7 (0.49) were observed only in 7% NaCl treated seedlings. On 20th day, a sum of 24 bands were illustrated in the root of salt treated seedlings and their MW-Rf values ranged from 0.05 to 0.99 (Table – 4; Fig. 1). Isoesterase with 0.07 (Est 2), 0.09 (Est 3) and 0.25 (Est 5) showed their common presence in all concentrations of salt treated seedlings roots. While the bands Est 11 (0.90) and Est 12 (0.94) were present only in the seedlings treated with 7% and 5% of NaCl. On 25th d, a total of 32 bands were observed in the root and their MW- Rf values ranged from 0.04 to 0.99 (Table – 5; Fig. 1). The Est 1, Est 2 and Est 5 were showed their common presence in 3, 5, 7 and 10% NaCl treated seedlings roots with Rf values 0.04, 0.08 and 0.27. While Est 4 (0.22) and Est 6 (0.32) were present only in 5% salt treated seedlings roots of *V. unguiculata*. On 30th d, a total of 46 bands were observed in the salt treated seedlings root and their MW-Rf values ranged from 0.08 to 0.99 (Table – 6; Fig. 1). The isoesterase with 0.08 (Est 1), 0.13 (Est 2), 0.24 (Est 4), 0.85 (Est 11), 0.93 (Est 12) and 0.99 (Est 13) were jointly present in all concentrations of salt treated seedlings roots of *V. unguiculata*. Whereas Est 7 (0.56) showed its restricted presence only in 5% NaCl treated seedlings leaves of *V. unguiculata*.

Table 1. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 5th Day (32; 5)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.36	+	-	+	-	+	-	+	-	+	-
Est - 2	0.67	-	-	+	-	-	-	-	-	-	-
Est - 3	0.74	-	-	+	-	-	-	-	-	-	-
Est - 4	0.82	+	-	+	-	+	+	+	+	+	+
Est - 5	0.84	-	-	-	-	-	-	-	+	-	-
Est - 6	0.87	-	-	+	-	-	+	-	-	-	-
Est - 7	0.92	-	+	-	+	+	+	+	+	+	-
Est - 8	0.95	-	-	+	-	+	-	+	+	-	+
Est - 9	0.98	+	-	+	-	-	-	-	-	-	-

Table 2. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 10th Day (56; 8)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.22	-	+	-	+	-	+	-	+	-	+
Est - 2	0.25	-	+	-	-	-	+	-	+	-	-
Est - 3	0.32	+	-	+	-	+	-	+	-	+	-
Est - 4	0.40	-	+	-	+	-	-	-	-	-	-
Est - 5	0.46	-	-	-	+	-	-	-	-	-	-
Est - 6	0.57	+	-	+	-	-	+	+	+	-	-
Est - 7	0.66	-	-	-	-	+	+	-	+	-	-
Est - 8	0.75	-	-	+	-	-	-	-	-	+	-
Est - 9	0.78	-	-	+	-	-	-	-	-	-	-
Est - 10	0.81	+	+	+	+	+	+	+	+	+	+
Est - 11	0.86	-	-	-	-	-	+	-	+	-	+
Est - 12	0.92	-	-	-	-	-	+	-	+	-	-
Est - 13	0.96	+	+	+	+	+	+	+	+	+	+
Est - 14	0.98	-	-	-	-	+	+	-	+	+	-

Table 3. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 15th Day (102; 8)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.22	-	-	-	+	-	+	-	+	-	+
Est - 2	0.25	+	+	+	+	+	-	+	-	+	-
Est - 3	0.27	-	-	-	-	-	-	-	+	-	-
Est - 4	0.33	-	-	-	-	-	+	-	+	-	-
Est - 5	0.39	-	-	-	-	-	+	-	-	-	-
Est - 6	0.45	-	-	+	-	-	-	-	-	+	-
Est - 7	0.49	-	-	-	-	-	-	-	+	-	-
Est - 8	0.53	-	+	-	+	+	+	-	+	-	+
Est - 9	0.56	-	+	-	+	-	-	-	+	-	+
Est -10	0.65	+	+	+	+	+	+	-	+	+	+
Est -11	0.66	+	+	+	+	-	+	+	+	+	+
Est -12	0.69	-	-	-	+	-	-	-	-	-	-
Est -13	0.74	+	+	+	+	+	+	+	+	+	+
Est -14	0.76	+	+	+	+	+	+	+	+	+	+
Est -15	0.79	+	+	+	+	+	+	+	+	+	+
Est -16	0.81	+	+	+	+	-	+	+	+	+	+
Est -17	0.84	+	+	+	+	+	-	+	+	+	-
Est -18	0.87	+	+	+	+	-	-	-	-	-	-
Est -19	0.90	-	-	-	-	-	+	-	-	-	-
Est -20	0.95	-	-	-	-	-	+	-	+	-	+

Table 4. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 20th Day (51; 7)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.05	+	+	-	+	+	-	+	+	-	+
Est - 2	0.07	+	+	+	+	+	+	+	+	+	+
Est - 3	0.09	+	+	+	+	+	+	+	+	+	+
Est - 4	0.13	-	-	-	-	-	-	+	-	-	-
Est - 5	0.25	-	+	-	+	-	+	-	+	-	+
Est - 6	0.41	-	-	-	-	+	-	-	-	-	-
Est - 7	0.46	-	-	-	-	+	-	-	-	-	-
Est - 8	0.53	+	-	+	-	-	-	-	-	+	-
Est - 9	0.56	+	-	+	-	+	-	-	-	-	-
Est - 10	0.64	-	-	-	-	-	-	+	-	-	-
Est - 11	0.90	-	-	-	-	-	-	+	+	-	-
Est -12	0.94	-	-	-	-	-	+	-	-	+	-
Est - 13	0.99	+	+	+	+	-	+	-	-	-	-

Table 5. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 25th Day (64; 9)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.04	+	+	+	+	-	+	+	+	+	+
Est - 2	0.08	+	+	+	+	+	+	+	+	+	+
Est - 3	0.11	+	+	+	+	+	-	+	+	+	-
Est - 4	0.22	-	-	+	-	-	+	-	-	-	-
Est - 5	0.27	-	-	-	+	-	+	-	+	-	+
Est - 6	0.32	-	-	+	-	-	+	+	-	+	-
Est - 7	0.43	-	-	-	-	-	+	-	-	-	+
Est - 8	0.55	-	-	+	-	-	+	+	-	+	+
Est - 9	0.79	-	-	-	-	-	-	-	+	-	+
Est - 10	0.86	-	-	-	-	-	+	-	+	-	+
Est - 11	0.92	-	-	+	-	-	-	-	-	-	-
Est -12	0.95	+	-	+	-	+	+	+	-	+	+
Est - 13	0.99	+	-	+	-	+	+	+	+	+	-

Table 6. Isoesterase banding pattern for Different concentrations of NaCl treated *Vigna unguiculata* L. seedlings on 30th Day (84; 9)

ISO-FORM	MW-RF	Salt Concentrations in %									
		00		03		05		07		10	
		L	R	L	R	L	R	L	R	L	R
Est - 1	0.08	+	+	+	+	+	+	+	+	+	+
Est - 2	0.13	+	+	+	+	+	+	+	+	+	+
Est - 3	0.17			-	-	-	+	+	-	+	+
Est - 4	0.24		+	-	+	-	+	+	+	-	+
Est - 5	0.37	+	+	+	+	-	+	+	+	-	-
Est - 6	0.53	+	+	+	+	-	-	+	+	-	-
Est - 7	0.56			+	-	-	+	-	-	-	-
Est - 8	0.67			+	+	-	+	-	-	-	-
Est - 9	0.71	+		+	-	-	+	+	+	-	-
Est -10	0.77			+	-	-	+	+	+	-	+
Est -11	0.85	+	+	+	+	-	+	+	+	+	+
Est 12	0.93			+	+	-	+	+	+	+	+
Est -13	0.99	+	+	+	+	+	+	+	+	+	+

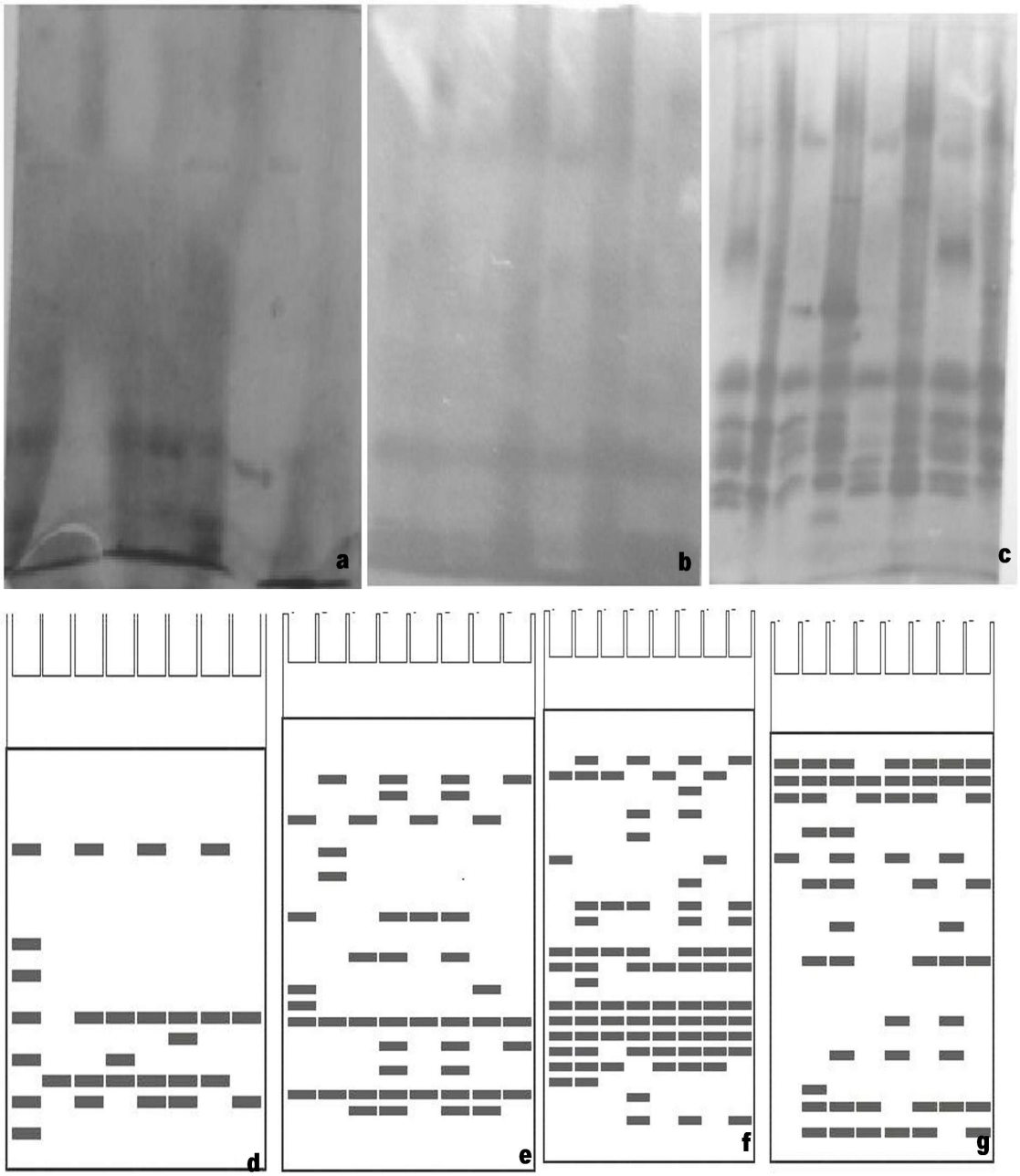

Figure 1. a - c: Differential expression of Isoesterases in leaves and roots of *Vigna unguiculata* L. in response to saline stress a. 10th d, b. 15th d and c. 25th d.

d -g. Zymogram of isoesterase banding patterns leaf and root of *Vigna unguiculata* L. in response to saline stress d. 5th d; e. 10th d; f. 15th d and g. 25th d.

DISCUSSION

The morphological variation, a result of genotype and the environment, is an important parameter, but much diversity, which remains unexpressed morphologically, can be revealed by biochemical methods. Study of isozymic variation is one such key and powerful procedure that has often been employed for this purpose. The esterases are a complex and heterogeneous group of enzymes, catalyzing the hydrolysis of the ester link. The isozyme forms of this enzyme, being the primary gene products, can be used to deduce gene

homology with precision by comparing variation in their expression patterns in different varieties. In the present study the isoesterase banding profiles are used to distinguish the salt tolerance of the *V. unguiculata* by comparing various concentrations of NaCl treated seedlings isoesterase profile. The response of plants to salt stress is based on the action of many defense proteins/enzymes (Converso and Fernandez, 1995). Plant isoesterases and esterase activity have been related to heavy metal and pesticide toxicity, pathogenesis, morphogenesis, embryogenic potential, NaCl and mannitol induced stress (Yang *et al.,* 1984; Yu, 1987; Suh *et al.,* 1997; Srivastava *et al.,* 2002; Johnson *et al.,* 2011). In the present study, esterase activity and isoesterase pattern were studied in leaves and roots of *V. unguiculata* subjected to NaCl induced stress.

Majority of new isoesterases appeared as late as at the end of second week (only few appeared after 10 day), suggesting de novo synthesis of new esterase isoenzymes. In confirmation with the postulate, induction of esterase activity caused by mannitol and salt was noticed after 10 and especially after 15 days. However, some saline treatments also induced new isoenzymes. However, the general pattern of appearance and disappearance of bands can be explained on the basis of gradual shifts of isozyme patterns in the salt treated seedlings taken as the course of development due to differential activation of genes involved in synthesis of these enzymes at various concentration of NaCl and duration of the treatment. Scandalios (1975) has listed 46 isozyme systems, in which the pattern of gene expression varies with the developmental condition. In the present study also the isoesterase expression was varied with reference to the salt treatment. The changing pattern of isozymes during development may be interpreted as evidence for differential timing of gene expression correlated with the physiological stress. The results of the present study concluded that the isoesterase patterns could serve as a useful biochemical marker of salinity.

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REFERENCES

Anbalagan, K. (1999) An introduction to electrophoresis, Electrophoresis Institute.

Bohnert, H. J. Nelson, D. E. and Jensen, R. G. (1995) Adaptations to environmental stresses. *Plant Cell.,* **7**, 1099–1111.

Converso, D. A. and Fernandez, M. E, (1995) Peroxidase isozyme from wheat grain: Purification and properties, *Photochemistry*. **40**, 1341-1345.

Cummins, I. Burnet, M. Edwards, R. (20010 Biochemical characterisation of esterases active in hydrolysing xenobiotics in wheat and competing weeds. *Physiol Plant*, **113**, 477–485.

Dasgupta, N., Nandy, P., Tiwari, C., Das, S. (2010) Salinity-imposed changes of some isozymes and total leaf protein expression in five mangroves from two different habitats. *J Plant Interaction,* **5(3)** 211-221

De Azevedo Neto, A. D, Prisco, J. T. Enйas-Filho, J. De Abreuceb and Gomes-Filho, E. (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ *Exp Bot* 56, 87–94

Dooslin Mary, D. Johnson, M & Gradin Jeyam. (2009) Effect of UV-B radiation on *Vigna radiata* L. var. K -851. *Biotechnology An Indian Journal,* **3(4)**, 207-211.

Dooslin Mary, D. Johnson, M. and Geradin Jeyam. (2010) UV-B Response of *Orysa sativa* var.

ADT (R) -45l Seedlings. *International Journal of Biotechnology and Biochemistry*, **6(3)**, 411-418.

Flowers, T.J. and Yeo, A.R. (1995) Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol*. **22**, 875-884.

Hasegawa, P.M. Bressan, R.A. Zhu, J.-K. and Bohnert, H.J. (2000b) Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 463-499.

Hassanein, A.M. (1999) Alternations in proteins and esterase patterns of peanut in response to salinity stress. *Biol Plant* **42**, 241–248.

Johnson, M. Dooslin Mary D.and Babu, A. (2011) Salt Stress responses on Protein Profile in *Vigna unguiculata* L. *Journal of Stress Physiology & Biochemistry*, **7(4)**, 241-249.

Madhava Rao, K.V. Sresty, T.V.S. (2000) Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan (L.*)Millspaugh) in response to Zn and Ni stresses. *Plant Sci,* **157**, 113–128.

Noriegax, Burgos, B. Purezf, J. (2007) Short day-photoperiod triggers and low temperatures increase expression of peroxidase RNA transcripts and basic peroxidase isoenzyme activity in grape vine buds. *Photochemistry*, **68**, 1376–1383.

Radic S. and Pevalek-Kozlina B. (2010) Differential esterase activity in leaves and roots of *Centaurea ragusina* L. as a consequence of salinity. *Periodicum Biologorum*. **112(3)**, 253–258.

Sadasivam, S. and Manickam, A. Biochemical methods for Agricultural Science, Chap 4.2. (Wiley Eastern limited & Tamilnadu Agricultural University, Coimbatore, India) 1992, pp 139.

Sanders, D. (2000) Plant biology: The salty tale of Arabidopsis. *Curr. Biol*. **10**, 486-488.

Scandalios, J.G. (1975) Genes, Isozymes and evolution, in Isozymes IV. Genetics and evolution, edited by L M Clement (Academic Press, New York), 1-7.

Srivastava, K.N. Rai, M. Tyagi, R. S. and Kaur, G. (2002) Enzyme analysis and isozyme pattern of basmati and non-basmati rice varieties, *Indian J Plant Physiol*. **7** , 227-233.

Suh, H.S. Sato, Y. I. and Morishma, H. (1997) Genetic characterization of weedy rice based on morpho-physiology, Isozymes and RAPD markers *Theor Appl Genet*. **94**, 316-321.

Thiyagarajah, M. Fry, S.C. Yeo, A.R. (1996) *In vitro* salt tolerance of cell wall enzymes from halophytes and glycophytes. *J Exp Bot*, **47**, 1717–1724.

Yang Hong, H. Xinli, S. and Xiang kun, W.(1984) Study on the centre of genetic diversity of Chinese cultivated rice *Agric Arch*. **4**, 2-8.

Yokoi Shuji, Ra, A. Bressan and Paul Mike Hasegawa. (2002) Salt Stress Tolerance of Plants. *JIRCAS Working Report*, 25-33.

Yu, W. J. (1987) Use of esterase isozyme to identify Chinese varieties, Zuower Pinzhong Ziyuan. **1**, 35-36.

Zhu, J.K. (2000) Genetic analysis of plant salt tolerance using Arabidopsis. *Plant Physiol*. **124**, 941-948.