J. Phytol. Res. 11(2) : 179-181, 1998

ISOZYMIC VARIATIONS IN *VIGNA ACONITIFOLIA* UNDER SALINITY STRESS

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Under the salinity stress of NaCl salt and mixed salts ($MgSO_4$ + $CaCl_2$ + NaCl) the cultures exhibited enhanced tolerance to mixed salts stress as in these cultures shoot and root formation was as healthy as in control cultures and only the callus formation was delayed by one weeks period. On the other hand under NaCl salinity the regeneration of shoot bud was delayed to 5th week in comparison to 4th week in control and mixed salts stressed cultures. The regenerants of NaCl treated cultures were not as vigorous as in latter two cultures. The regeneration of shoot and root formation in both salinity stressed cultures was accompanied by the formation of isozymes XXXVII, XXXXVIII, LVI and LVII while in the cultures under control conditions the similar organogenesis was marked by the presence of isozymes XXXX-XXXXIII, LI and LII.

Keywords :Isozymes; Salinity stress; *Vigna aconitifolia*.

Introduction

The initiation of organised development in tissues cultured *in vitro* is associated with the variations in the activities of various enzymes and the level of cellular metabolites. Arnison and Boll[1] used isozymes patterns as tools to investigate specific traits in cell cultures of bush bean (*Phaseolus vulgaris* cv. Contender) and have shown that the study of isozymes was potentially a highly useful biochemical index by which to judge cell and tissue individuality. These isozymes provide a simple and easy approach to investigate into enzyme ontogeny and the mechanism regulating their temporal and spatial expression within the same organism. By studying the isoperoxidases one can elucidate various processes at genetic level and thereby desirable traits can be introduced by regulating the control mechanism. Isoperoxidases have been used as biochemical markers in leaf and root morphogenesis in Barley[2]. In the present investigation specific isoperoxidases related to particular morphogenetic events were detected.

Material and Methods

The Seeds of *V. aconitifolia* (Jacq.) Marechal *Var.* Jwala were surface sterlized using 0.1% mercuric chloride and transferrred aseptically on to the paper bridges in the test tubes containing distilled water. Leaf explants of 8 day old seedling were transferred to incubation medium i.e. Murashige and Skoog's (MS) medium[3] supplemented with 3 mg/l of kinetin (KIN) and 1 mg/l of indole-3-acetic acid (IAA) for regeneration of callus, shoot buds and roots.

For salinity treatment 0.1% NaCl salt for NaCl salinity and 0.1% of $MgSO_4$+ $CaCl_2$ + NaCl (ratio 1:2:7) for mixed salts salinity were added to the incubation medium.

The sample for biochemical analysis was prepared by homogenising 1.0g plant material (fresh) in 5ml of phosphate buffer (pH 7.0; 0.1 M) and centrifuging at 10,000 Xg for 20 minutes. The supernatant thus obtained was mixed with sucrose grains, a few drops of 0.01% bromophenol bule (BPB) and used as enzyme extract for electrophoresis.

Isozymes of peroxidase were separated by slab gel on polyacrylamide gel electrophoresis[4]. Peroxidase bands were visualised by o-dianisidine -H_2O_2[5].

Results and Discussion

It was observed that tissuses under the mixed salts salinity stress displayed enhanced tolerance to the salinity as compared to the tissues under NaCl salinity. In the cultures under mixed salts treatment though the callus formation was delayed to the 3rd week of

incubation in comparison to callus formation in 2nd week in cultures under controlled conditions, the shoots regenerated were as healthy as the shoots regenerated under controlled conditons. The NaCl salinity reduced shoot development in the cultures and the callus formation and shoot regeneration was delayed to 4th and 5th week respectively. This morphogenetic variation is marked by change in isozymic pattern in these cultures. In the cultures under salinity stresses (NaCl & mixed salts) shoot and root formation was accompanied by the appearance of isozymes XXXVII, XXXXVII, XXXXVIII, LVI and LVII (Fig. 1). However for similar organogenetic response in controlled cultures these isozymes were replaced by XXXX-XXXXIII, LI & LII (Fig. 1). This observation indicates the involvement of the isozymes in tolerance responses of the plants to the adverse conditons.

In present study the isozymes XXXVII and XXXXV were apparent only in mixed salt stressed cultures in addition to isozymes XXXVIII, XXXXVII, XXXXVIII, LVI and LVII which were of common occurrence for both NaCl and mixed salt stressed cultures, during delayed root and shoot formation (Fig. 1). All these isozymes were characteristically present in root and hypocotyl samples of the parent plant. These isozymes were present as intense bands in cultures under NaCl salt

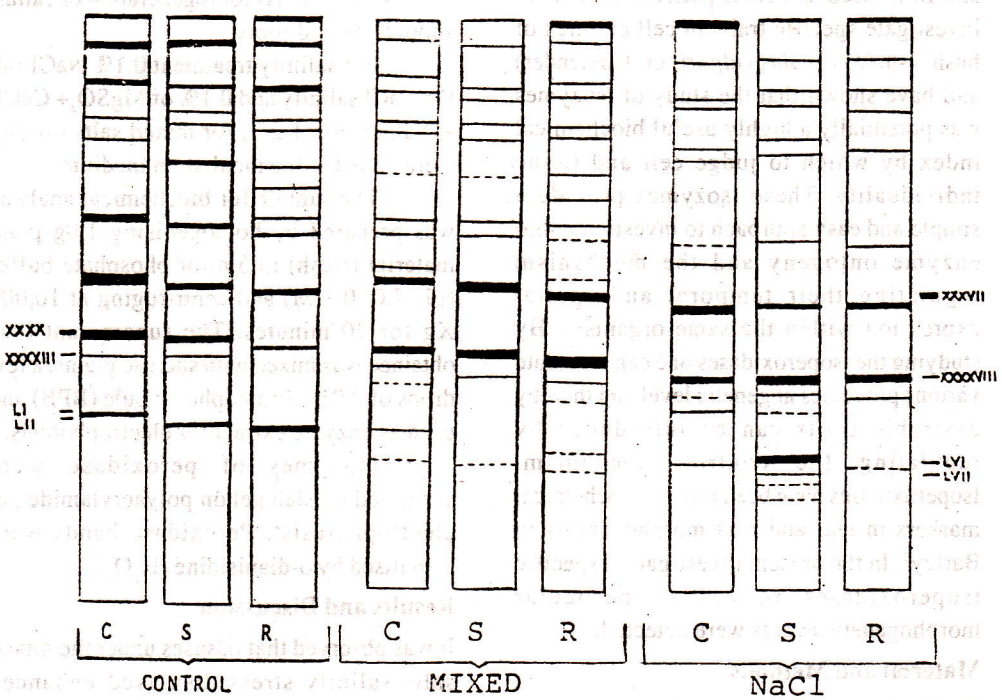

Fig.1. Zymogram representing isozymic variations in cultures of *Vigna aconitifolia* under mixed salts, NaCl salinity and control condition. C, S and R represens callus, shoot initiation and rooting stages.

treatment where stress conditions were more severe in comparison to mixed salts. It is well established that presence of extra Ca^{2+} lowers response to osmotic stress probably this effect has lead to enhanced tolerance under mixed salts salinity. Cramer *et al*[6] showed that addition of Ca^{2+} reduce the adverse effects of salinity on plants, as Ca^{2+} displaces Na^{2+} from the plasmalemma of salt stressed root cells. The alterations in isozyme pattern of cultures under NaCl salt stress as compared to the cultures under mixed salts stress was also due to the occurrence of delayed and reduced root, shoot formation. It has been shown by Brugnoli and Lauteri[7] that plant growth is strongly reduced by salinity. Total chlorophyll content, sugar content and relative turgidity decreased with increase in salinity[8].

Isozyme analysis has come up as genetic and biochemical tool in the study of plant physiology. By studying the isoperoxidases on can elucidate various process at genetic level and thereby desirable traits can be introduced by regulating the control mechanisms.

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