

**STUDIES ON OSMOREGULATION
IN CULTURED CELLS OF VIGNA RADIATA
(L.) WILCZEK VAR. RADIATA**



By

VINOD KUMAR

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in partial fulfilment of the requirements for the Degree of*

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IN
GENETICS**

**COLLEGE OF BASIC SCIENCES AND HUMANITIES
HARYANA AGRICULTURAL UNIVERSITY
HISAR
1987**

To
My
Parents

CERTIFICATE - I

This is to certify that this dissertation entitled, "Studies on osmoregulation in cultured cells of Vigna radiata (L.) wilczek var. radiata" submitted for the degree of Doctor of Philosophy, in the subject of Genetics of the Haryana Agricultural University, is a bonafide research work carried out by Shri Vinod Kumar under my supervision and that no part of this dissertation has been submitted for any other degree.

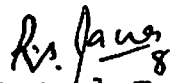
The assistance and help received during the course of investigation have been fully acknowledged.


(D.R. Sharma)
Major Advisor

CERTIFICATE - II

This is to certify that the dissertation entitled, "Studies on osmoregulation in cultured cells of Vigna radiata (L.)Wilczek var. radiata" submitted by Shri Vinod Kumar to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in the subject of Genetics has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.


Major Advisor 8.4.88


8.4.88
External Examiner


22/4/88
Head of the Department


27.4.88
Dean, Postgraduate Studies

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(Vinod Kumar)

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INTRODUCTION

Drought and salinity are age old stress conditions for plants. These are in fact, the major barriers to productivity in many environments. Therefore, attempts are being made world over to evolve plants which can tolerate elevated levels of salinity.

It is a common observation that there is genotypic variability within a species and some genotypes are fairly salinity tolerant. Biochemical investigations have revealed that usually these stress tolerant types show accumulation of some sugars, amino acids or some other osmoprotectants and exhibit osmoregulation. In bacteria, genetic basis of osmoregulation has to some extent been elucidated. However, the genetic/biochemical basis of uptake, synthesis, accumulation and degradation of these osmoprotectants is not properly understood in plant cells. Although considerable amount of information has been gathered regarding stress response in whole plants, organizational complexity of the plant has been a predicament to resolve the cell responses in isolation from neighbouring tissues. During the mid seventies, attempts were made to use isolated cell and callus cultures to find out whether stress tolerant cell lines could be developed under in vitro conditions and if so how did they differ from the normal cell lines (Dix and Street, 1975; Nabors et al., 1975; Croughan et al., 1978). It appears that comparison of

stress tolerant cells with nontolerant cultures isolated from the same initial explants would enable measurement of the capacity and range of cellular tolerance which may be applied for more specific in vitro selection (Chandler and Thorpe, 1987). So far most of detailed studies of tolerance to stress in vitro have been carried out with only a few model systems such as tobacco (Widholm, 1972a, 1976; Nabors et al., 1975, 1980; Dix et al., 1984), tomato (Tal et al., 1978; Tal and Katz, 1980; Tal, 1983) and citrus species (Kochba et al., 1980; Kochba et al., 1982).

In the mean time discovery of Osm (osmotic tolerance) genes in bacteria, their role on conferring resistance to salinity and transfer of such genes through plasmids have raised new hopes for the discovery of similar genes or set of genes in plants. In that case genetic manipulation of plants for stress tolerance would become straight forward. However, in lack of the discovery of stress tolerance genes in plants, it was intended to undertake detailed studies on cellular adaptation to osmotic stress caused by the increased levels of NaCl in the medium and to compare this response with cell adaptation to antimetabolite stress caused by thioproline, an analogue of proline. It has been demonstrated in several cases that amino acid analogue resistant cells usually are overproducers of the corresponding

amino acids (Widholm, 1972b; Widholm, 1976; Riccardi et al., 1981, 1983; Cella et al., 1982; Dix et al., 1984; van Swaaij, 1986). The cell lines resistant to thioproline are most likely to be over producers of proline which acts as an osmoprotectant. Similarly, NaCl resistant cell lines would also exhibit osmoregulation by synthesizing osmoprotecting molecules. It was, therefore, contemplated to screen NaCl and thioproline resistant cell lines in mungbean (Vigna radiata (L.) Wilczek var. radiata) which otherwise is a salinity sensitive glycophyte and to compare these lines between them as well as with the sensitive wild type cells. Attempts have been made to characterize these lines for their growth patterns under stress and non-stress conditions, ion contents, soluble carbohydrates, soluble proteins and a few enzymes and proline. Efforts have also been made to compare the osmotic potentials of these lines under stress and non-stress conditions.

The main objectives of the present investigation were:

- a) to determine whether or not genetic variability be created for stress tolerance,
- b) to compare and contrast thioproline resistant and sodium chloride resistant callus lines with normal sensitive ones, and to find out
- c) whether unified concept of osmoregulation/stress tolerance is upheld or not ?

REVIEW OF LITERATURE

One of the major barriers to productivity in many environments is the harmful effects of salt and water stress. This has been realized as severe national as well as world-wide problem. Therefore, a lot of attention is being devoted for understanding the physiological, biochemical and genetic basis for the stress tolerance of different types so that enhancement in the productivity of biological system could be accomplished.

Biological systems exposed to environments with reduced water availability (salinity, drought, extreme temperatures) have adaptive responses which involve organic and inorganic osmoregulation. Osmotic adjustment by organisms in response to stress is known to involve regulation of the intracellular levels of various carbon compounds (sugars, organic acids, Polyols), nitrogenous compounds (amino acids, tertiary nitrogenous substances) and inorganic ions.

Accumulation of organic osmotica

Several workers (Stewart and Lee, 1974; Chu et al., 1976; Hellebust, 1976; Bar-Nun and Poljakoff-Mayber, 1977; Flowers et al., 1977; Wyn Jones and Storey, 1978; Jefferies, 1980; Aspinall and Paleg, 1981; Wyn Jones and Storey, 1981; Goas et al., 1982; Yancey et al., 1982) have hypothesized that a class of small organic molecules (glycine

betaine, proline betaine, proline and others) found in scores of higher plants behave as osmoprotectants. These molecules accumulate during osmotic stress and prevent damage from cellular dehydration by balancing the osmotic strength of the cytoplasm with that of environment. Though tolerance is frequently associated with the accumulation of betaines and/or amino acids, it is not invariably so. The compounds like sorbitol accumulate in Plantago maritima (Ahmad et al., 1979), sucrose in sweet pea (Nightingale and Farnham, 1935-36), barley (Gauch and Eaton, 1942) and carrots (Bernstein and Ayers, 1953) upon subjecting them to salt or osmotic stress.

Proline accumulation in salt stressed cultured cells

Watad et al. (1983) and Lerner (1985) studied the intracellular proline content of the sodium chloride sensitive and resistant cell lines of Nicotiana tabacum (with cytoplasm from gossii). They observed that salt-selected cell lines accumulated more proline than the sensitive cell lines upon exposing them to increasing concentrations of sodium chloride. Moreover, proline accumulation was found to be reversible phenomenon because salt-selected cell lines when transferred sequentially to lower and lower NaCl concentrations, their proline content fell to the level corresponding to the new NaCl concentration. Further, the NaCl-selected cells responded to water stress

(i.e. added mannitol) by accumulating markedly more proline than did the wild type. From this study, they concluded that proline accumulation was not the visible result of damage, e.g. to mitochondrial membranes (Aspinall and Paleg, 1981) but accompanied survival and growth in a saline and drought environments. Handa et al. (1982a) too reported reversibility of proline accumulation in a tomato cell line upon subjecting the cells to water stress.

Pandey and Ganapathy (1985) observed that NaCl-tolerant callus line (R-100) of Cicer arietinum accumulated free proline in response to increasing sodium chloride concentrations, while the sensitive line C-0 did not. Further, the tolerant callus line grown on NaCl free medium accumulated proline when recultured on NaCl medium. Dix and Pearce (1981) followed the levels of proline and other amino acid accumulation in their NaCl-resistant and sensitive cell lines of Nicotiana sylvestris. Both the cell lines rapidly accumulated free proline when placed on medium containing 1.5 per cent NaCl; however, the amount was not sufficient to act as osmoticum even if localized in the cytoplasm. Thus they concluded that proline accumulation in resistant cell line could not be accounted for an adaptive response in stress resistance.

Recently, Duncun and Widholm (1987) reported that embryogenic callus of certain inbred lines of maize accumulated

proline to levels 2.1 to 2.5 times that of control callus when subjected to mannitol-induced water stress, cool temperature (19°C) and abscisic acid (ABA). Their studies indicated that proline and conditions which induced proline accumulation increased cold tolerance of regenerable maize callus.

But Chandler and Thorpe (1987) observed that proline accumulation was correlated with the inhibition of growth, and there was a negative correlation between proline concentration and culture age for tolerant Brassica napus callus.

Exogenous application of proline

The protective effects of exogenously applied proline seem to be dependent on tissue organization. Mathur et al. (1980) working on the isolation of NaCl-tolerant plantlets of Kickxia ramisissima under in vitro conditions and Bar-Nun and Poljakoff-Mayber (1977) investigating germination of root growth in Pisum sativum were able to counteract the effect of NaCl with proline. Similarly, van Swaaij et al. (1985) reported that exogenous application of proline to one of the genotypes of Solanum species resulted in the accumulation of the proline in the leaves of shoot cultures, resulting in an increase in frost tolerance. This has not been possible in several studies with callus cultures.

Tal and Katz (1980), Rosen and Tal (1981) in tomato and Dix et al. (1983) in tobacco actually found that in the absence of NaCl, proline was inhibitory to salt-resistant callus, relative to sensitive callus of Lycopersicon and Nicotiana sylvestris, respectively. However, in neither case it was possible to protect sensitive callus from salt stress, using proline. The difference between whole plant and callus responses is further substantiated by comparing Nicotiana sylvestris callus and seedlings (Dix et al., 1984). Handa et al. (1986) observed that addition of exogenous proline to the medium during water stress and during osmotic downshock alleviated the normally resulting inhibition of growth in cultured tomato cells. Their results suggested a positive role for proline accumulation in adaptation of cells to changing external water potentials. Pandey and Ganapathy (1985) reported that addition of 10 mM exogenous proline to the medium containing 100 mM NaCl increased fresh and dry weights, and free proline content of the sensitive C-0 as well as tolerant R-100 callus lines of Cicer arietinum. But at 200 mM NaCl, addition of exogenous proline to the medium had no significant effect on the growth of either callus line.

Thus one can conclude that proline is implicated in the salt resistance of nonhalophytes, but its role is unclear and

its effect may depend on the translocation and metabolic capacities of differentiated plant structures.

Ions and Osmoregulation

The involvement of ions in osmoregulation has been extensively reviewed from time to time (Hellebust, 1976; Flowers et al., 1977; Osmond, 1979; Jeschke, 1984; Pitman, 1984). According to these workers ions are equally important in cell turgor and cell volume regulations.

The ion uptake has been held responsible for playing a major role in the osmotic adjustment of the plants which tolerate and grow in saline environment (Greenway, 1973). Epstein et al. (1963) proposed system-1 and system-2 responsible for ion uptake. The system-1 has high affinity for K^+ ions and mediates its uptake from low concentrations, whereas, system-2 exhibits less pronounced selectivity for K^+ - Na^+ but both the ions inhibit their uptake mutually. A large number of workers have reviewed relationship between K^+/Na^+ selectivity and salinity (Rains, 1972; Greenway and Munns, 1980; Jeschke, 1979, 1984; Wyn Jones, 1980; Flowers and Yeo, 1986). Similarly different, possibly related mechanisms of K^+/Na^+ selectivity at the cellular level have also been proposed (Jeschke, 1984).

Potassium has been found to be nutritionally important especially in salt stressed glycophytes where high cytoplasmic

Na^+/K^+ ratio account for metabolic damage (Flowers et al., 1977; Winter and Preston, 1982) and high Na^+ concentrations may interfere with K^+ acquisition (Greenway, 1965; Rains and Epstein, 1967; Orton, 1980b). Importance of potassium is further strengthened from the fact that leaves and shoots of a number of different salt tolerant glycophytic varieties accumulate lower Na^+ and Cl^- and higher K^+ concentrations than the sensitive ones (Bernstein and Ayers, 1953) in carrots; Greenway (1965) in barley, Luchli and Wieneke (1979) in soybean; Sharma et al. (1984) and Joshi et al. (1985) in wheat; Sharma (1985, 1986) in rice. Similarly Rana et al. (1976) in wheat and barley, Hegde and Joshi (1974) in rice and Janardan et al. (1976) in cotton have reported higher K^+/Na^+ ratio in the tolerant varieties than the sensitive ones. This observation led them to conclude that higher K^+/Na^+ ratios can be taken as a suitable criterion for testing salt tolerance. But Greenway and Munns (1980) reported high concentration of Na^+ in one of the tolerant varieties of rice. Likewise Lessani and Marschner (1978) were unable to detect significant differences in the ion concentration of leaves of different varieties of maize which otherwise exhibited different degree of tolerance towards salinity. Greenway and Munns (1980), therefore, concluded that in such cases varietal differences in the salt tolerance could be due to either compartmentation of ions

in the leaves or concentration of ions in the roots or distribution of ions among the leaves of different ages.

Calcium is also essential for selective transport of alkali cations (Epstein, 1961; Läuchli and Epstein, 1970). LaHaye and Epstein (1969) reported that calcium cations play an important role during the selective transport of K^+ . They grew salt-sensitive bean, Phaseolus vulgaris, in solution cultures salinized with sodium chloride at 50 mM, at various concentrations of calcium. At the higher calcium concentration (3 and 10 mM) the plants grew as well as did those in the unsalinized control cultures. The presence of calcium kept the entry of sodium proportional to growth and at a level that the plant tolerated. At six weeks the plants looked healthy and produced beans in profusion (LaHaye and Epstein, 1971). The observations of Waisel (1972) further substantiate these results.

Another mechanism in salt tolerance is the exclusion of specific ions from the plant cells so that their sensitive cytoplasmic components are protected from the toxicity of such ions (Levitt, 1980). For example, sodium is excluded from the leaves of many plant species (Jacoby, 1964) including legumes (Brown and Hayward, 1956; Gates et al., 1970; Läuchli and Wieneke, 1979; Lauter et al., 1981; Winter and Läuchli, 1982). Jacoby (1964) demonstrated that bean plants exclude sodium in the basal parts of the plants

but readily translocate chloride to the tops. Retention of sodium in the stems became gradually saturated with increasing salt concentration in the medium. Similarly, chloride is also known to be excluded in some legumes (Abel and Mackenzie, 1964; Gates et al., 1970; Winter and Läubli, 1982). Abel and Mackenzie (1964) showed that varietal differences in salt resistance were correlated with exclusion of chloride, the resistant varieties controlling chloride contents in stem and leaves to a low level. Abel (1969) showed that chloride transport to the tops of soybean varieties is under genetic control. In particular, chloride exclusion in soybean cv. "Lee" was proposed to be controlled by a dominant, single gene pair. But Van Steveninck et al. (1982) reported that two lupin species exhibited opposite response. Lupinus luteus appeared more salt-resistant and less effective in excluding sodium chloride than Lupinus angustifolius. Thus some legumes can adapt to moderate salinity by exclusion of sodium and/or chloride from the leaves, whereas the salt-resistant Lupinus luteus may be termed a salt accumulator and thus features a halophytic response.

Subcellular ion localization

Many halophytes avoid specific ion toxicity resulting from excessive uptake by accumulating ions in the vacuoles. The osmotic requirement for a high concentration of

cytoplasmic solutes is then fitted by non-toxic organic molecules, such as glycine betaine (Storey and Wyn Jones, 1975; Hall et al., 1978), proline (Buhl and Stewart, 1983; Voetberg and Stewart, 1984), sugars like sucrose (Gauch and Eaton, 1942; Bernstein and Ayers, 1953). Likewise osmotic adjustment of non-halophytes to a saline environment could also be through localization of the ions in the vacuole (Yeo, 1983).

Ionic regulation of salt stressed cultured cells

The difference in the ionic content between NaCl-selected and non-selected cells has been monitored to explain mechanism used by the NaCl-selected cells for growing on normally lethal levels of NaCl (Croughan et al., 1978; Stavarek and Rains, 1982; Watad et al., 1983; Pandey and Ganapathy, 1984a, 1984b).

Croughan et al. (1978) reported that NaCl-selected cell line maintained a higher K^+ content at all levels of NaCl than the nonselected cell line of alfalfa. Further, they observed that at low NaCl concentration, the NaCl-selected cell line exhibited elevated levels of NO_3^- . Besides this, the salt-selected cells required 0.50 per cent NaCl for optimal growth and grew poorly in the absence of salt. These observations are known to be highly correlated with salt tolerance in both halophyllic bacteria and halophytic plants (Epstein, 1969; Rains, 1979; Greenway and Munns, 1980).

Thus they concluded that growth and ionic uptake patterns of the salt-selected cell line indicated a general shift towards a halophytic mode of salt tolerance. Stavarek and Rains (1982,1984a) observed that as osmotic stress increased in the salt-selected cell lines of alfalfa, the K^+ uptake also increased whereas the capacity of nonselected cell line to take up K^+ decreased under identical NaCl stress. Thus, they concluded that this mechanism of maintaining K^+ uptake by the salt-selected cell line was an important factor for their tolerance towards the increased salinity. Similarly Tal et al. (1978) observed that the callus derived from the salt-tolerant tomato plant accumulated low levels of Na^+ and Cl^- but high levels of K^+ at a given external NaCl concentration.

In Cicer arietinum, Pandey and Ganapathy (1984a,1984b) reported that salt-selected calli accumulated more Na^+ and Cl^- than salt-sensitive ones. But K^+ and PO_4^{3-} levels decreased in both the calli with increasing external NaCl. However, salt-selected calli maintained higher concentrations of K^+ and PO_4^{3-} ions than did the cells of salt-sensitive line despite high Na^+ in the system. These observations led them to conclude that more retention or maintenance of an appreciable levels of K^+ in the selected calli was responsible for their increased tolerance than the sensitive calli.

Watad et al. (1983) reported that both the salt-selected

as well as wild type cell lines of Nicotiana tabacum/gossii showed steady rise in internal Na^+ content as a function of external NaCl concentration. Also the wild type cells lost a considerable part of their K^+ content and the extent of loss increased with rise in external NaCl concentration. The selected cells, in contrast, lost no K^+ at external NaCl concentrations below 50 mM external NaCl, and at higher concentrations lost less than the wild type. Studies of Lerner (1985) based on solute compartmentation in Nicotiana tabacum/gossii also revealed similar type of results. These findings led them to suggest that selectivity for K^+ accumulation in the cytosol of salt-selected cell line was the possible mechanism of the increased tolerance towards NaCl stress.

Stavarek and Rains (1982) observed that Ca^{++} concentration of the NaCl-selected and nonselected alfalfa cells decreased as NaCl concentration was increased in the media. The salt-selected tissue, however, always maintained a higher Ca^{++} concentration than the nonselected tissue. Thus, they concluded that higher concentration of Ca^{++} was an important factor contributing to the tolerance of salt-selected cell lines.

Some species may tolerate the salt stress by partial avoidance of external NaCl. This was the conclusion drawn from the studies of Ben-Hayyim and Kochba (1983) on cultured

Citrus cells. They observed that R-10 (NaCl-resistant) cell line accumulated less Na^+ and Cl^- than did the L-5 (NaCl-sensitive) cell line. Further, there were no differences with respect to their internal K^+ concentration and Cl^- ion was found to be toxic element for cell growth in the presence of NaCl.

Ion exclusion mechanism for salt tolerance does not seem to operate at the cellular level. Dix et al. (1983) observed that the kinetics of Na^+ uptake was almost identical in the salt-tolerant (NCR 120) and salt-sensitive (NS) cell lines of Nicotiana sylvestris. Similar pattern of Na^+ uptake has been reported for callus of Nicotiana tabacum (Umiel et al., 1980). Similarly, Stavarek and Rains (1981) have observed that NaCl-selected alfalfa cells accumulated approximately the same amount of Na^+ and Cl^- as the nonselected tissue when grown on different levels of NaCl. It appeared to them that the salt-selected cells did not exclude salts as the mechanism of tolerance towards salinity but were more efficient at the compartmentation of toxic ions than the nonselected cells. The above findings suggest that salt tolerance is not associated with ion exclusion at the cellular level.

Heyser and Nabors (1981) reported that there were no obvious qualitative differences with regard to the osmotic adjustment of NaCl adapted and non-adapted cells of

Nicotiana tabacum var. Samsum. They concluded that probably a quantitative gene mutation might be held responsible for salt tolerance in such cases.

Effect of salt stress on metabolism/metabolic pathways

A lot of literature is available regarding the effects of salt stress on the enzyme activities and isoenzyme patterns of important metabolic pathways among different plant species. Sharma and Garg (1985) observed that wheat cv. WH-157 plants grown under saline conditions resulted in an increase in the activity of aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase enzymes in roots and leaves. Studies of El-Fouly and Jung (1972,1981) in wheat and Aggarwal (1986) in barley have also reported increase in the activities of certain key enzymes during salinity stress. Sheoran and Garg (1979) too reported that in mungbean activity of peroxidase increased in embryo axis and leaves but decreased in cotyledons and roots with different salt treatments to varying concentrations. Further, the salt treatment resulted in the appearance of new isoenzymes of peroxidase in all the plant organs except roots where it remained unchanged. Weimberg (1970) reported that isoenzyme pattern of peroxidase from roots of salt grown plants of pea altered in that two of the five detectable isoenzymes migrated more slowly than those in extracts from nonsaline plant tissues. Weimberg (1967) found that an

isoenzyme of malate dehydrogenase isolated from pea seeds, was stimulated by NaCl if the concentration of salt did not exceed 20 mM. Higher concentrations of salt inhibited the enzyme activity.

Hason-Porath and Poljakoff-Mayber (1969) observed that Na_2SO_4 and NaCl type salinities induced increase in NADP coupled activity of the mitochondrial malate dehydrogenase in pea roots. The NAD coupled activity, however, was depressed except in cytoplasmic fraction of plants. Further, Na_2SO_4 stress did not alter the isoenzyme pattern whereas NaCl stress resulted in the appearance of new isoenzymes. Plaut (1974) reported reduction in nitrate reductase activity in NaCl stressed wheat seedlings. But after removing stress, the enzyme activity was restored.

Weimberg (1970,1975) has studied the effect of NaCl and several other salts on the activities of certain key enzymes of respiratory metabolism and photosynthetic apparatus of pea seedlings. Their findings indicated that these enzymes did not show any alteration either in their activities or isoenzyme patterns. Likewise, Greenway and Osmond (1972) reported that malate dehydrogenase, aspartate transaminase, glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase extracted from Atriplex spongiosa, Salicornia australis, and the glycophyte Phaseolus vulgaris

did show similar in vitro NaCl sensitivity despite great differences in salt tolerance of the plants.

Flowers et al. (1977) have reviewed the loss of in vitro activity of malate dehydrogenase with increasing NaCl concentration from large number of different halophyllic species. Treichel et al. (1974) also reported that in vitro and in vivo responses of the PEP (phosphoenolpyruvate) carboxylase were not similar because enzyme activity was inhibited when NaCl was added to assay and magnitude of this inhibition was not found to be correlated with salt tolerance.

Poljakoff-Mayber et al. (1981) studied the ratio between glycolytic and pentose phosphate pathway (PPP) in the root tips of different cultivars of Pisum sativum as well as Pisum elatius and Pisum fulvum. The different cultivars and species differed in the ratio between these two pathways and their responses to exposure to salinity during growth. Laxton progress (most salt-sensitive cultivar) exhibited decrease in the absorption capacity for external glucose and increase in the activities of PPP compared with glycolytic pathway when subjected to salt stress. Pisum elatius (most tolerant) also showed similar response when grown under a salt stress that inhibited growth. They could not find out a clear cut correlation between salt sensitivity, or salt tolerance and the enzymes studied in response to salinity, however, the best correlation seemed to be between sensitivity

to salinity and increase in the activity of 6-phosphogluconic dehydrogenase. Porath and Poljakoff-Mayber (1964,1968) have also studied the effect of chloride and sulphate types of salinity on respiratory mechanisms of pea roots. The sodium chloride salinity resulted increase in the percentage of pentose phosphate pathway whereas sulphate type salinity did not. Decrease in the activities of enzymes like malate dehydrogenase coupled to NAD (Krebs cycle) and most of the enzymes of glycolytic pathway was also observed under the salt stress of both the types. Moreover, mitochondrial and supernatant glucose-6-phosphate dehydrogenase coupled to NADP, showed increase in the activity under both the types of salinity. This finding led them to conclude that increase in the activity of this enzyme may be due to increased participation of PPP during respiration under such types of salinities. Apart from interfering with the enzyme activity, salinity does affect the uptake and transport of different amino acids, ions and nutritionally important metabolites. Moreover, incorporation of amino acids in proteins and protein synthesis itself may be drastically reduced under such circumstances.

Reddy and Vora (1985) reported that protein and ribonucleic acid content decreased by salinity whereas

soluble proteins showed a little variation but amino acid content, protease and ribonuclease activities were found to be higher in the salinized bajra leaves. Kahane and Poljakoff-Mayber (1968) have observed that pea roots when grown at high salt concentrations, uptake of amino acids and their incorporation was affected even if they were carried out in the absence of salt. They found that NaCl and Na₂SO₄ affected uptake, incorporation and metabolism of ¹⁴C leucine in different ways and moreover preliminary indication revealed that different types of proteins may be synthesized under salt stress conditions. Benzioni et al. (1967) too reported that uptake of L-leucine and its incorporation into protein chains was considerably reduced in the salt stressed leaf discs of Nicotiana rustica. Lubin and Ennis (1964) observed that mutant E.coli B cells when depleted of K⁺, cell division and protein synthesis stopped but ribonucleic acid synthesis continued and Na⁺ was found to antagonise the action of K⁺. Similar type of observations were also made by Wyn Jones et al. (1979) in wheat. Aslam et al. (1984) studied the effect of NaCl and Na₂SO₄ salinity on NO₃⁻ assimilation in young barley seedlings. Both Cl⁻ and SO₄²⁻ salts reversibly inhibited uptake of NO₃⁻. Likewise, K⁺ and Na⁺ salts inhibited NO₃⁻ uptake, hence, the process seemed more sensitive to anionic salinity than to cationic salinity. Further, unlike NO₃⁻ uptake, NO₃⁻ reduction was not

affected by salinity and thus they concluded that susceptibility to injury of NO_3^- transporter rather than NO_3^- reduction system, may be a critical factor to plant survival during salt stress. Helal and Mengel (1979) reported that salinization in young barley plants, impaired growth and incorporation of labelled N into the protein fraction paralleled by accumulation of inorganic N. But addition of potassium enhanced N uptake and incorporation into protein and reduced the accumulation of inorganic N and improved the growth of salinized plants. These findings suggested that impairment of protein (enzyme) metabolism was an important aspect of salt stress which was probably induced by the disturbance of K/Na balance of the tissue under saline conditions.

Genetic regulation of salt tolerance

The response of plants towards the salt stress varies from species to species and variety to variety. Surprisingly, very little information has accumulated among plants so far, regarding the genetic control of salt resistance and one must not forget in order to formulate any breeding programme, it becomes equally essential to have a thorough understanding of the different genetic mechanisms of salt tolerance operating at cell level.

Only few single gene mutations that may be involved in the control of mechanisms related to salt resistance are known. Epstein (1972) and Scholz and Bohme (1980) have

advocated that differences due to single gene mutations could be employed for understanding regulatory mechanisms of salt tolerance. Mutations affecting aspects possibly related to osmoregulation include (i) a proline-deficiency mutation, in Zea mays (Gavazzi et al., 1975), (ii) a salt-resistant mutation in Salmonella typhimurium which accounts for the production of high amounts of proline (Csonka, 1980), and (iii) a low cellular osmotic pressure mutation in Arabidopsis thaliana (Langridge, 1958).

Genes governing the ion transport are well studied in microorganisms like bacteria (Slayman, 1973; Hall and Silhavy, 1981; Laimins et al., 1981; Kennedy, 1982; Le Rudulier et al., 1984). Similarly reports are available among plants regarding the genes responsible for transport and metabolism of minerals during salt stress (Kruckeberg, 1959; Myers, 1960; Gerloff, 1963; Epstein and Jefferies, 1964; Epstein, 1972; Läuchli, 1976). Single gene mutations involved in the regulation of either uptake, transport or utilization of the ions of the most prevailing salt (i.e. NaCl) in saline soils and water resources are very rare among plants. Abel (1969) has reported a recessive single gene mutation (ncl) in soybean. Plants homozygous recessive for ncl mutation did not prevent the transport of Cl^- ions from the root to shoot and thus were found to be salt-sensitive. Another recessive mutation related to ion

balance, Scabrous diminutive (sd) has been described in pepper (Tal et al., 1974). Tal et al. (1976) found that the difference in stomatal behaviour between the sd mutant and normal genotype was correlated with differences in K^+ content in their guard cells. Na^+ substituted for K^+ in its function in stomatal movement in mutant plants grown in the saline medium. Tal and Benzioni (1977) and Benzioni and Tal (1978) reported that Na^+ exclusion and K^+ uptake in the sd mutant roots of pepper was less efficient than in the roots of normal genotype. This observation led them to suggest that the defect in the mutant resulted from either an impaired Na^+/K^+ carrier system and/or an impaired membrane permeability. Plasma membrane ($Na^+ + K^+$) activated ATPase play a role in cellular tolerance by mediating the energy required for cation uptake across the membrane of plant root cells as suggested by Kylin and Quatrano (1975) and Hodges (1976). Similarly Morton (1979) gave indications of lower Ca-ATPase activity in the sd mutants of pepper. Apart from these studies, single gene was also held responsible for absorption and transport of Fe^{++} in a soybean variety (Weiss, 1943) and genetic control of Fe^{++} was found to reside in the roots of soybean (Brown, 1963). Though the absorption of Na^+ and Cl^- in crop plants appeared to be under the control of single genes but the different effects of osmosis as a result of NaCl stress appeared to be more complex and under the control

of large number of genes (Bernstein, 1975). Likewise, incomplete to complete dominance and nonallelic gene interactions in barley (Maddur, 1976), and complementary gene action, incomplete dominance to complete dominance and nonallelic gene interactions in sorghum (Ratanadilok et al., 1978) have been held responsible for salt tolerance. Transfer of NaCl-resistant genes from wild to the cultivated species in wheat (Dvorak et al., 1985) and in tomato (Rush and Epstein, 1981) have also appeared in literature.

Thus, it is reasonable to accept the suggestion of Ramage (1980) that salt tolerance is a complex character and its expression largely depends upon the genetic background of the species in question. He has further suggested that in order to breed for salt resistant genotypes, it is better to follow recurrent selection.

Selection for salt-resistant cell lines

Melchers (1972), for the first time, discussed the advantage of tissue culture technique for selecting salt-resistant mutants. Zenk (1974) employed this approach successfully by selecting a cell line from the haploid cultures of Nicotiana sylvestris that was able to grow on a medium containing 0.17M NaCl. on which control cells did not grow.

Nabors et al. (1975) selected NaCl-resistant cell lines from Nicotiana tabacum suspension cultures treated by Ethane methane sulphonate (EMS) and then grown in a medium containing 0.03M

NaCl. Cells derived from these lines even resisted concentrations as high as 0.09M NaCl in the medium. Most of the control cultures did not grow on the saline medium. Few of them, however, remained alive, and after several transfers started to grow fast. The appearance of spontaneous dominant mutations were held responsible for this growth. Later Nabors et al. (1980) reported the isolation of NaCl-resistant cell line from the suspension cultures of Nicotiana tabacum that could tolerate 0.88 per cent NaCl. Further, the regenerated plants obtained from the NaCl resistant culture also inherited this trait and the level of resistance in the progeny of the regenerated plants was higher than in the original cells in culture. Though resistance was maintained in the progeny of the regenerants for the two subsequent generations examined but it did not conform to simple genetic ratio. Another successful selection was reported by Dix and Street (1975), who selected a number of cell lines of Nicotiana glauca and Capsicum annuum capable of growing in liquid medium containing upto 0.34M NaCl. Some of these lines retained the resistance to salt after several subcultures in media lacking NaCl. Dix (1980) regenerated plants from these resistant cultures previously grown for 2 to 6 passages in media containing 0.34M NaCl. Further, calli initiated from the leaves of these two groups of regenerated plants were tested for their ability to grow in NaCl medium.

While callus of Nicotiana sylvestris showed only limited sectorial growth, if any, that of Capsicum annuum grew as the original resistant cell lines. In evaluating disappointing inheritance data for F_1 and F_2 generations from salt-tolerant regenerants of Nicotiana sylvestris, Dix et al. (1983) suggested that epigenetic variation frequently produced stable-resistant lines and that care should therefore be taken to identify true genetic resistance.

Similarly Oono and Sakaguchi (1978,1980) in Oryza sativa, reported the isolation of NaCl-resistant cell line on a medium containing 1.5 per cent NaCl. The plants regenerated from this resistant cell line were found to tolerate high levels of salinity. This observation led them to conclude that probably NaCl-resistant trait might be heritable in these regenerants. Plants have also been regenerated from NaCl-tolerant cells of Oats (Avena sativa), rice (Oryza sativa), wheat (Triticum aestivum) and sorghum (Nabors et al., 1982; Nabors, 1983; Bhaskaran et al., 1983). The salt tolerance was reported to be retained in some of these regenerants. Employing the method of Maliga (1978), for mutant cell selection, Yoshida et al. (1983) were able to select NaCl-resistant cell lines of many cultivars of Oryza sativa. The regenerated plants from the selected mutant cell lines and those derived from the seeds of these regenerants, were found to be more tolerant to salinity in comparison to the nontolerant plants of mother

variety. Regeneration of plantlets from the salt-selected callus cultures has also been reported in Basmati rice cv. 27814 (Reddy and Valdyanath, 1985) and Oryza sativa (Kucherenko, 1980).

It has also been reported in the literature that NaCl-resistant trait may be either lost when the selected cells are allowed to grow on NaCl free medium for some passages or it is unable to express among the regenerants or if at all expresses in the regenerants, there is no improvement in the degree of resistance. Hasegawa et al. (1980) have reported that Nicotiana tabacum cells resistant to 1 per cent NaCl, lost their tolerance once they had been grown away from salt for five cell mass doublings. Smith and McComb (1983) observed that the regenerated plants obtained upon differentiation of one of the NaCl-resistant culture of alfalfa did not show any improved resistance and same was true even for the cell cultures derived from these regenerated plants. The differential growth behaviour of NaCl-tolerant and nontolerant cells, normally expected to be present in variant cell line, under stress and nonstress conditions has been held responsible for the instability of NaCl-resistant trait among the variant cell lines (Simon et al., 1984). It is also quite possible that NaCl resistance in the selected cell lines may simply represent a physiological habituation rather than the genetic alteration(s) in their genome.

Croughan et al. (1978) isolated stable, NaCl-resistant cell lines, which could grow on a medium containing 0.17M NaCl, from a cell culture of alfalfa. Stavarek et al. (1980) were able to regenerate plants from this long time maintained cell culture of alfalfa, by sequential transfer on new media. The regenerated plants were accompanied with many lethal genetic changes. The plants were slow growing, stunted and susceptible to many diseases. Further, Croughan et al. (1981) were unable to determine fully the amount of NaCl resistance expressed in the regenerated plants.

Watad et al. (1983) developed a NaCl-resistant cell line from the suspension cultures of Nicotiana tabacum/gossii by stepwise increase in NaCl concentration in the medium. Resistance showed stability through at least 24 generations in the absence of added NaCl. Rains et al. (1980b) selected a NaCl-resistant cell line of Oryza sativa at 0.26M NaCl, a concentration lethal for unselected cells. The selected cell line tolerated high levels of other salts and was found to be fairly stable even after many generations of growth. Gosal and Bajaj (1984), Pandey and Ganapathy (1984b) in Cicer arietinum and Zhou Jia-Ping et al. (1981) in Glycine max have too reported the isolation of stable NaCl-resistant cell lines. But Gosal and Bajaj (1984) also reported the isolation of unstable NaCl-resistant cell lines of Vigna radiata and Pisum sativum. Tyagi et al. (1981) reported the isolation of

stable NaCl-tolerant cell line from the haploid callus cultures of Datura innoxia. The tolerance was expressed among the regenerants of selected cell line. Likewise Yamada et al. (1983) have selected stable resistant cell lines at 37.5 per cent sea water in certain cultivars of Oryza sativa. The resistance was also expressed among the regenerants.

Kochba et al. (1980,1982) reported the isolation of "Shamouti" orange (Citrus sinensis (L.) Osb.) callus lines with increased resistance to NaCl. The resistance was maintained in embryos obtained from these lines. These observations are further supported from the findings of Ben-Hayyim and Kochba(1982,1983) in Citrus . Spiegel-Roy and Ben-Hayyim (1985) observed that the regenerated plantlets obtained upon the differentiation of salt-resistant cell line of Citrus sinensis retained the tolerance character.

Handa et al. (1982a,1982b) reported the isolation of Lycopersicon esculentum cells that were able to grow in polyethylene glycol (PEG)-containing media of osmotic potential as low as - 39 bar or in 600 mM NaCl. Stable differences in PEG tolerance were detected in unselected cells, but when selection pressure with PEG was applied, there was also a rapid adaptation of the intolerant cells such that the stable tolerant cells did not have a selective advantage (Handa et al., 1983). Adaptation involved changes in the degree of cell expansion as well as in the accumulation of salts and

organic compounds (Bressan et al., 1982; Binzel et al., 1983).

Apart from these reports, Liu and Yeh (1982) in Saccharum officinarum, Rangan and Vasil (1983) in Pennisetum americanum and Nabors and Dykes (1985) in Avena sativa, Oryza sativa and Triticum aestivum also reported the isolation of NaCl-tolerant cell lines. Similarly, McHughan and Swartz (1984) in Linum usitatissimum callus cultures, Nyman et al. (1983) in Colocasia esculentia shoot tip cultures, Yamaguchi and Matsumoto (1984) in Pocitrus trifoliata hypocotyl explant cultures and Mathur et al. (1980) in Kickxia ramosissima internodal explant cultures are other such reports available where NaCl-resistant cells have also been selected.

Thus we find that direct selection for resistance to NaCl in cultured cells has been attempted in many species, but the results are encouraging in some whereas disappointing in other species. The main areas where problems have been encountered in producing stress tolerant genotypes are:

- (a) metabolic and structural adaptation of cultured cells,
- (b) non-regeneration of selected cell types or gross phenotypic changes in regenerated plants, (c) non-expression of tolerant phenotypes in the regenerated plants, and (d) obscure transmission of salt-tolerant phenotypes to progeny plants.

Growth characteristics of salt stressed cultured cells

The cultured cells either grow poorly or exhibit growth inhibition kinetics upon exposure to increasing levels of

salinity (Nabors et al., 1975; Croughan et al., 1978; Tal, 1983; Stavarek and Rains, 1984b). These observations are found to be fairly consistent with the reports available from whole plant studies (Bernstein, 1963, 1975; Greenway, 1965, 1973; Clemens et al., 1983).

Salt tolerance manifested in callus cultures of several species, such as, tomato, barley and sugar beet is reflected in the whole plant (Tal et al., 1978; Orton, 1980b; Smith and McComb, 1981a). Smith and McComb (1981a) have compared plants and their corresponding callus cultures of a salt-tolerant glycophyte (Beta vulgaris), a salt-sensitive glycophyte (Phaseolus vulgaris), and two halophytes (Atriplex undulata and Suaeda australis) to increasing levels of NaCl (0-250 mM). Beta vulgaris (both callus and plant) showed an increase in growth at intermediate salt levels and a decrease at higher levels, whereas Phaseolus vulgaris (both callus and plant) decreased in growth with increasing levels of salt. But for both the halophytes, the callus growth decreased with increasing levels of salt, while the plants showed increased growth under identical conditions. From this study, they concluded that Beta vulgaris seems to have a cellular mechanism of salt tolerance and the two halophytes have whole plant mechanisms (salt glands, succulence) for dealing with salt and hence tolerance is not expressed in their callus.

The degree of growth inhibition of salt stress. cultured cells varies with species to species, cultivar to cultivar as has also been observed at whole plant level (Tal, 1985). Orton (1980b) while comparing the salt tolerance of Hordeum vulgare and Hordeum jubatum in whole plants and callus cultures, observed that callus response paralleled the plant as NaCl was increased to 0.17M. Moreover, the callus cultures of the two species did not show growth inhibition at 0.17M NaCl but did exhibit differential response towards their recovery after 30 days on the medium supplemented with 0.17M NaCl i.e. Hordeum jubatum callus cultures did recover after 30 days, whereas, Hordeum vulgare failed to do so. Smith and McComb (1981b) have compared the effect of NaCl on the growth of the callus cultures and plants in nutrient solution cultures of several lines of Medicago sativa L. cvv. Hunter River, Cuf 101, Hasawi and W75RS, and Trifolium repens L. cv. Ladino, all of which are considered salt-sensitive species, and Trifolium repens L. cv. Palestine which is considered moderately salt-tolerant. They observed that the callus growth of all lines of Medicago sativa, except W75RS, was depressed by the addition of 62.5 mM NaCl to the media. Medicago sativa cv. W75RS showed some tolerance, as callus growth was comparable at the 0 NaCl control and at the 62.5mM NaCl level. Similarly, callus of Trifolium repens and Trifolium fragiferum were also reduced in growth by NaCl. Moreover, the whole plants exhibited similar trends in fresh

weight as the callus cultures. From this study, they concluded that there was differential line/variatal response of the cultured cells towards external NaCl concentration and tolerance, or sensitivity in culture appears to be reflected by the whole plant.

Pandey and Ganapathy (1984a) in Cicer arietinum callus cultures and Hasegawa et al. (1980) in Nicotiana tabacum cultured cells have shown slight stimulatory effects on the growth upto 50 mM and 0.17 mM NaCl concentrations, respectively. Moreover, both showed cell growth inhibition as a function of increasing NaCl concentration in the culture medium. Similar stimulations on relative growth at low NaCl concentrations have also been reported in bean suspension cultures (Gale and Boll, 1979) and in callus and suspension cultures of Cicer arietinum and Pisum sativum (Gosal and Bajaj, 1984). The stimulation of growth at lower NaCl concentrations may be due to the osmoregulatory role of Na^+ as suggested by Huq and Larher (1983) though they had worked with whole plants of Phaseolus. But von Hedenström and Breckle (1974) have also shown that the growth of Suaeda maritima and Salicornia europaea callus cultures improved with the addition of increasing NaCl concentration in the medium.

Though the salt used in the majority of salinity studies is NaCl but several workers have compared the response to callus to different types of salts. Goldner et al. (1977)

using Daucus carota callus cultures and Chen et al. (1980) using Nicotiana tabacum callus cultures, found different responses when they compared growth on synthetic sea water, sea water, mannitol, NaCl and other Cl^- and SO_4^{2-} salts. Their findings led them to suggest the use of multiple salts or synthetic sea water as a selection pressure to better parallel the salinity under field conditions.

Kochba et al. (1982) have reported that NaCl-tolerant citrus callus cultures when tested on other salts were found to be sensitive to KCl but grew well on Na_2SO_4 , MgSO_4 and K_2SO_4 . Stavarek and Rains (1982,1984a) in alfalfa and Stavarek and Rains (1984a) in rice have compared the growth of NaCl-selected cell lines on different salts (NaCl, KCl, Na_2SO_4 and K_2SO_4). The growth of the NaCl-selected alfalfa line was inhibited at high levels of all salts except NaCl whereas the NaCl-selected rice cells could tolerate the high levels of all the salts tested. These studies led them to conclude that NaCl-selected rice cells had adapted a different mechanism for dealing with growth in saline environment than the NaCl-selected alfalfa cells.

Pandey and Ganapathy (1984a) reported that the increase in Na^+ and Cl^- levels of salt stressed callus of Cicer arietinum was almost linear to increasing salinization of the culture medium and most of the accumulation of ions occurred by the first week whereas significant growth inhibition became

apparent only by the third and fourth weeks. These findings conformed to the suggestion of Greenway (1962) according to whom stress originated at the point of ion balance.

Apart from the growth inhibition of salt stressed cells, it has also been observed that growth inhibition concentrations of NaCl exerted cytogenetic abnormalities such as increased polyploidization in the cultured cells of Crepis capillaris (Kulieva et al., 1975). Moreover, these polyploid cells once selected upon subsequent growth on NaCl supplemented medium were found to be more salt-tolerant than the corresponding diploid cells.

Resistance to amino acids and amino acid analogues

Amino acid toxicity is caused by inhibition of the synthesis of related amino acids whereas resistance is obtained by the relaxed control of the amino acid biosynthetic pathway for which the cell is normally starved. Resistance to antimetabolites can arise through one or more of several mechanisms.

First, overproduction of the naturally occurring compound may dilute the effect of the antimetabolite. Overproduction of an amino acid, for example, is commonly a result of deregulation of enzymes normally subject to feedback inhibition. Carlson (1973) selected tobacco cells resistant to methionine sulfoximine by plating haploid cells or protoplasts in agar-solidified medium containing methionine sulfoximine at

inhibitory concentrations. Plants were regenerated from three stably resistant cell lines and leaves from two of these plants contained about six times the normal level of free methionine. Chaleff and Carlson (1975) also selected cultured rice callus which grew on normally inhibitory concentrations of aminoethyl-L-cysteine, a lysine analogue. The resistant cells accumulated about twice the normal level of free lysine and the protein lysine levels were elevated. Zenk (1974) reported the selection of Nicotiana sylvestris cultured cells resistant to 10-fold higher concentrations of DL-methionine, a methionine analogue. Preliminary results indicated that the free methionine levels were increased in these resistant cells. Palmer and Widholm (1975) reported a p-fluorophenyl-alanine (Phenylalanine analogue) resistant cell line which accumulated about six times the normal level of phenylalanine. Similarly, Widholm (1972a, 1972b) selected the suspension cultured carrot and tobacco cells that were resistant to 5-methyltryptophan (tryptophan analogue), as most of these oversynthesized tryptophan, as a result of an altered control enzyme, anthranilate synthetase. Chowdhury (1982) reported the selection of p-fluorophenylalanine (analogue of phenylalanine) and 5-methyltryptophan (analogue of tryptophan) resistant cells of Vigna radiata that were having higher amounts of free phenylalanine and tryptophan, respectively than the wild type cells. Reish et al. (1981) have isolated

ethionine (analogue of methionine) resistant cell lines from the suspension cultures of diploid Medicago sativa. Some of the lines showed resistance to growth inhibition by lysine plus threonine and some also contained increased levels of free methionine. Widholm (1976) selected tobacco cell lines resistant to the lysine analogues S-2-aminoethyl-L-cysteine and Δ -hydroxylysine and carrot lines resistant to a methionine analogue, ethionine. The lysine-analogue resistant tobacco lines and methionine-analogue resistant carrot lines had more than 10 times the normal levels of free lysine and methionine, respectively. The alteration in the feedback control was suggested as the possible mechanism of their resistance to the analogues.

Second, resistance may arise if the metabolite is effectively excluded from the cell, e.g., through a mutation affecting membrane permeability. Variant cell lines with altered transport have been isolated. Widholm (1974) reported the isolation of carrot cell line which did not have an altered enzyme activity but apparently discriminated against 5-methyl-tryptophan at the uptake level. Berlin and Widholm (1978a) have also observed that the transport variants in tobacco cell line showed resistance to more than one amino acid analogues.

Third, a compound may be detoxified through degradation

before it is incorporated into the protein chains. For example, Berlin (1980) reported a tobacco cell line resistant to p-fluorophenylalanine (PFP) which degraded PFP by phenylalanine ammonia lyase.

Fourth, increased synthesis of secondary metabolites can result in analogue resistant mutants. Berlin and Vollmer (1979) reported that the increased activity of p-fluorophenylalanine resistant cell line of tobacco could result in increased synthesis of phenolic compounds as in Nicotiana tabacum (Berlin and Widholm, 1977) and Acer pseudoplatanus (Gathercole and Street, 1976, 1978) or polyphenols as in Nicotiana tabacum (Berlin and Widholm, 1978b). Palmer and Widholm (1975) found that a tobacco cell line resistant to p-fluorophenylalanine, had an altered control enzyme, chlorismate mutase, which apparently made excess phenylalanine, but this was converted into phenolic compounds which did accumulate. These secondary metabolites when produced in excess can interfere with plant regeneration. For example, Sung (1979) observed that a thirty-fold increase in indoleacetic acid (IAA) synthesis in 5-methyltryptophan resistant cell lines of Daucus carota was responsible for inhibiting embryogenesis.

Reports are also available in which correlations between amino acid or amino acid analogue resistant with specific enzyme activities have been made. For instance,

Widholm (1972a) in Nicotiana tabacum and Widholm (1974) in Daucus carota has correlated decrease in feedback inhibition of anthranilate synthetase by tryptophan with the resistance to 5-methyltryptophan. Scott et al. (1979) detected a 1.5 fold increase in anthranilate synthetase activity and a two-fold increase in tryptophan synthetase activity in Catharanthus roseus cell lines with resistance to 5-methyltryptophan. In atleast one variant cell line anthranilate synthetase was less sensitive than the wild type enzyme to feedback inhibition by tryptophan in callus initiated from regenerated plants. But anthranilate synthetase isolated from the whole plant was found to exhibit similar degree of sensitivity to tryptophan as wild type enzyme (Widholm, 1974). Thus, it is found that the cell cultures and plants regenerated from cells may respond differentially to the same type of antimetabolite due to expression of different isoenzymes. In addition to this, Matthews et al. (1980) have reported alteration in the activities of aspartokinase and dihydropicolinic acid synthetase in S-aminoethylcysteine resistant cell lines of Daucus carota and Hibberd et al. (1980) observed that aspartokinase activity was resistant to feedback inhibition by lysine in lysine plus threonine resistant cell lines of Zea mays.

Widholm (1974) observed that the plants regenerated from a 5-methyltryptophan resistant cell line of carrot were

sensitive to the analogue. But Flick et al. (1981) in Nicotiana tabacum and Evans and Gamborg (1979) in Datura innoxia have regenerated p-fluorophenylalanine (PFP) resistant plants from PFP resistant cell lines. Likewise, Ranch and Widholm (1983) have reported that regenerants from 5-methyltryptophan resistant cell culture of Datura innoxia did express the resistance trait. Apart from this, Ahmad et al. (1986) have isolated L-ethionine (analogue of methionine) resistant cell lines from the chemically mutagenised cell suspensions of Vigna sinensis.

Some information regarding the genetic analysis of amino acid or analogue-resistant mutants has also accumulated in the literature. Of the methionine sulfoximine resistant cell lines isolated by Carlson (1973), two were found to be semidominant nuclear mutations and one was due to two successive nuclear alleles (Carlson et al., 1973). Lawyer et al. (1980) reported that glycine hydroxamate resistance behaved as dominant Mendelian trait in Nicotiana tabacum. Similarly, resistance to isonicotinic acid hydrazide (an inhibitor of the pathway in which glycine is converted to serine) in Nicotiana tabacum was also a dominant mutation (Berlyn, 1980). Hibberd and Green (1982) attributed Zea mays resistance to lysine plus threonine to a single dominant nuclear allele. Bourgin (1978) reported that valine resistance in Nicotiana tabacum was

inherited as either a single dominant or semidominant nuclear allele. Similarly, resistance to trans-4-hydroxyproline in R5201 mutant of barley was found to be due to a single partially dominant nuclear gene (Kueh and Bright, 1981).

Proline analogue resistance

Like any other amino acid analogue, proline analogues such as hydroxyproline, azetidine-2-carboxylic acid, 3-4-dehydroproline and many others do get incorporated into protein chains (Fowden, 1963; Fowden et al., 1963; Holleman, 1967; Cleland and Olson, 1968) and thereby can inhibit the cell growth. Even Hewitt and Notton (1967) have reported that azetidine-2-carboxylic acid prevents the formation of nitrate reductase activity under the reducing conditions. But elevated levels of endogenous proline apart from providing resistance to these analogues can also confer salt and frost resistance (van Swaaij et al., 1986). Similarly increased levels of proline in proline analogue resistant mutants (e.g., hydroxyproline resistant Hordeum vulgare plants isolated by Kueh and Bright, 1981) may lead to plants with increased resistance to water stress. In addition to this, resistance to proline analogue such as hydroxyproline can also be conferred by the enzymatic conversion of free hydroxyproline into the proline (Varner, 1980; van Swaaij et al., 1986).

Widholm (1976) isolated hydroxyproline resistant cell line from the mutagen (ethylmethane sulfonate) treated suspension cultures of Daucus carota. The resistant cell line contained 15 to 30 times the normal free proline levels and was cross resistant to other proline analogues, like L-azetidine-2-carboxylic acid, L-thiazolidine-4-carboxylic acid and 3,4-dehydroproline. Moreover, hydroxyproline resistant trait was found to be quite stable as the resistant cells when grown away from hydroxyproline for 50 generations did not alter the resistance of this cell line significantly.

Nielsen et al. (1980) selected L-azetidine-2-carboxylic acid cell line of carrot that had higher concentration of free proline but were unable to regenerate plants for biochemical and genetic analysis. Dix et al. (1984) reported the isolation of hydroxyproline resistant cell lines of Nicotiana sylvestris which overproduced proline and their regenerants also showed increased proline levels.

Employing the embryo selective system technique of Bright et al. (1979), Kueh and Bright (1981) obtained Hordeum vulgare plants resistant to hydroxyproline by screening M₂ (the progeny of plants obtained after seed mutagenesis) embryos. The mutants accumulated free proline in the leaves upto three times the normal level and one of these mutants was less inhibited by low concentrations of NaCl than the parent cultivar (Kueh and Bright, 1982). These results are

particularly noteworthy as barley is the species for which other workers (Hanson et al., 1979; Tully et al., 1979) have provided evidence that proline accumulation is a symptom of water stress injury, although the type of stress imposed, and the level of proline accumulated were very different.

Cella et al. (1982) investigated the mechanism of azetidine-2-carboxylic acid resistance in a selected carrot cell line. Two main alterations were observed in the resistant cells: an overproduction of proline and of some other amino acids and a reduced rate of uptake either of azetidine-2-carboxylic acid or of other amino acids tested. The latter phenomenon appeared to be a secondary effect due to the increased internal amino acid concentration which inhibited the activity of the amino acid carrier(s). An increased total free amino acid content has also been observed in cell lines of carrot (Cella and Iadarola, 1983) and potato (Jacobsen et al., 1985; Jacobsen, 1986) resistant to amino acid analogues.

Cella and Iadarola (1983) selected four 5-methyl-tryptophan resistant cell lines from carrot suspension cultures (three were induced by uv and one was spontaneous) that were also found to be cross resistant to azetidine-2-carboxylic acid. The tryptophan and proline levels were found to be roughly proportional to the degree of resistance of the lines of the two analogues. The fact that all the

lines resistant to 5-methyltryptophan overproduced proline suggested that the latter feature might be a direct consequence of the increased pool of free tryptophan. Further, the four lines tested showed a rate of tryptophan uptake similar to that of the parental line. On the contrary uptake of proline and azetidine-2-carboxylic acid by 5MT-11 line was 23 per cent and 10 per cent of the parental line, respectively indicating that resistance to 5-methyltryptophan was due to the presence of an altered anthranilate synthetase and not due to the altered permeability of the analogue and these two amino acids (tryptophan and proline) were transported by two distinct carriers.

Riccardi et al. (1983) isolated mutants of Spirulina platensis and of Daucus carota resistant to azetidine-2-carboxylic acid. The overproduction of amino acid was the result of resistance to the analogue as reported earlier by Riccardi et al. (1981) and Cella et al. (1982). When the resistant lines were tested on NaCl supplemented medium, a positive correlation was found between proline overproduction and osmotolerance. In carrot lines proline overproduction was not strictly proportional to NaCl tolerance insofar as cells characterized by differences in proline overproduction showed similar osmotolerance, suggesting that other factors could be involved. Dix et al. (1984) selected HPR105A, hydroxyproline resistant cell line

of Nicotiana sylvestris which contained proline almost 100 and 4 times of those found in callus and shoots respectively of the sensitive Nicotiana sylvestris line. Moreover, the resistant cell line showed slightly better growth in 1 per cent NaCl medium. Dix et al. (1984) have also reported that three cell lines of Nicotiana plumbaginifolia resistant to 10 mM hydroxyproline did accumulate proline but without any improved resistance to NaCl as compared to sensitive cell line.

van Swaaij et al. (1986) have reported the selection of large number of hydroxyproline-resistant cell lines from the suspension cultures of a diploid potato (Solanum tuberosum). Ninety per cent of the variant cell lines contained more proline than wild type and their total amino acid content was increased 2.2 to 6.8 times the normal cell line. The variant cell lines H4a and H4b4 were found to be cross resistant to certain other proline analogues, analogues of lysine and tyrosine and grew better on a media supplemented with the inhibitory concentration of lysine plus threonine. Moreover, variant cell lines H2, H4a, H4b4 and H6 exhibited better tolerance to NaCl than wild type and one variant cell line (H4a) was successfully regenerated into plants. The leaves of these regenerated plants showed an increased frost tolerance and had a higher leaf proline content. The callus initiated from the leaves of the regenerated clones was more

resistant to hydroxyproline than wild type callus. This led them to suggest that the variant trait might be due to a mutation.

Unified concept of osmoregulation and/or stress resistance in plants

A large number of different researchers have reported that resistance to a particular environmental stress is paralleled by increased resistance to other stresses (Maximov, 1929; Scarth, 1941; Levitt and Siminovitch, 1940). Pisek and Larcher (1954) have reported that several uniform and broad leaved evergreens exhibited parallel trends in drought and freezing stress. Similarly, a parallelism between freezing resistance and heat resistance of twelve varieties of two Avena species has been observed (Coffman, 1957). Likewise Parker (1972) reported that seasonal trends in heat resistance of various species of conifers and broad leaved evergreens resembled those for freezing resistance.

Reports are available which indicate that conditioning plants to a particular environmental stress resulted in increased resistance to other stresses or the resistance to a particular stress could be elicited by any one of the several hardening stimuli. Rosa (1921) observed that increase in cold hardiness of cabbage elicited by low temperature could also be obtained by insufficient watering or introduction of salt solutions to the soil. Such observation has led to the

consideration that cold acclimation resulted in a parallel increase in tolerance to freezing, drought and osmotic stress (Siminovitch and Briggs, 1953). For example, cold acclimation, of barley plants increased their drought resistance (Levitt et al., 1960) while water stress increased the cold hardiness of red osier dogwood (Chen et al., 1977; Chen and Li, 1977, 1978).

Physiological changes that occurred during acclimation to one environmental stress could be elicited by acclimation to another stress (Maximov, 1929; Scarth, 1941; Levitt, 1958, 1972), further strengthens the consideration of a unified concept of stress resistance.

All these observations led Levitt (1972) to speculate that plant might possess a general resistance to several or even all environmental stresses. Consideration of a general resistance to environmental stress has influenced many workers to advocate and pursue selection for resistance to other stresses. For example, ability to withstand plasmolysis and subsequent deplasmolysis has been used to select for cold hardiness (Scarth and Levitt, 1937; Siminovitch and Briggs, 1953; Levitt, 1972).

It would be erroneous or misleading to accept that resistance to one environmental stress automatically imparts resistance to several other stresses. For example, the

relation between heat and frost tolerance occurred only periodically in most investigated species and was even totally absent in others (Lange, 1967). Likewise, certain similarities between frost, heat and desiccation resistance did not always change in parallel (Santarius, 1973). Further, increase in cold hardiness resulting from drought hardening was found to be much less than obtained by cold acclimation (Levitt, 1972).

Finally, apart from this, some people maintain that some genes or set of genes are responsible for all types of stress conditions because reports are available which indicate that many of the putative organic osmoregulatory compounds (osmotica) isolated from plant, microbial and animal sources are indeed biologically active as osmoprotective compounds in bacteria (Strom et al., 1983; Le Rudulier et al., 1984). Further, it would be still fair to say that the evidence for a direct biological role of these compounds in plants and animals is very thin and strong experimental evidences are needed to remedify the situation.

MATERIAL AND METHODS

Material

The present investigations were conducted on mungbean (Vigna radiata (L.) Wilczek var. radiata) cv. K851. The seeds were procured from the Department of Plant Breeding of this University.

Chemicals

The chemicals used for the preparation of media were of analytical grade obtained from B.D.H. (India) or E. Merck (India). Growth regulators, thioproline (L-thiazolidine-4-carboxylic acid), L-proline and chemicals used for enzyme assays were obtained from Sigma (USA), except Folin and Ciocalteu's Phenol reagent which was obtained from SISCO (India).

Preparation of media

All glasswares, double distilled water and media were sterilized by autoclaving at 1.1 kg/cm^2 pressure for 15 minutes at 121°C . Media were stored at room temperature and usually utilized within 20 days of preparation. All stock solutions were kept under refrigeration conditions. Sterilization and transfer of calli were done under aseptic conditions using Klenzaid's Laminar Flow Chamber.

Establishment of callus cultures

The seeds were surface sterilized by immersing in 0.2 per cent mercuric chloride solution with 4-5 drops of

detergent (Teepol) for 8 to 10 minutes followed by 7-8 washings with sterilized double distilled water under aseptic conditions. The sterilized seeds were transferred onto a medium containing 0.5 per cent sucrose and 0.8 per cent agar and incubated under 5000 lux of continuous white fluorescent light in a culture room maintained at $27^{\circ}\pm 1^{\circ}\text{C}$.

After 3-4 days, when the roots were 2-3 mm long, their tips were cut and transferred aseptically to 100 ml Erlenmeyer flasks containing callus initiation medium. These flasks were then stored in dark in a B.O.D. incubator maintained at $27\pm 1^{\circ}\text{C}$ for 7-12 days. Once the callus initiation had taken place in dark, these flasks were further incubated for 10-15 days in the culture room under identical light and temperature conditions used for the germination of seeds. The calli so formed were maintained on callus initiation medium by subculturing at an interval of 25-30 days under the above mentioned light and temperature conditions.

Composition of the modified PC-L2 (Phillips and Collins, 1979) basal medium

Inorganics

<u>Major components</u>	<u>mg/litre</u>
NH_4NO_3	1,000.0
KNO_3	2,100.0
KH_2PO_4	325.0
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	85.0

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	600.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	435.0
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (EDTA)	25.0

Minor components

H_3BO_3	5.0
KI	1.0
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	15.0
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	5.0
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.1
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.4
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.1

Organics

Thiamine HCl	2.0
Pyridoxine HCl	0.5
DL-cysteine HCl	25.0
Myo-inositol	250.0
Sucrose	25,000.0

Growth regulators

α -naphthaleneacetic acid (NAA)	0.5
2,4-dichlorophenoxyacetic acid (2,4-D)	0.5
6-benzylaminopurine (BAP)	1
Agar	8,000
pH adjusted to	5.8

Growth studies

The growth rate of the callus was measured in terms of fresh and dry weights. A known amount of callus was inoculated into 100 ml flasks containing modified PC-L2 basal medium in 20 replications. Subsequently, after every 8 days, the callus from any 5 of these flasks was taken out and its fresh weight determined. It was subsequently oven dried at 80°C for 48 h in order to determine dry weight. These observations were continued upto 32nd day.

Screening for NaCl and thioproline resistant callus lines

Mutant cells resistant to NaCl and thioproline were screened separately from the same callus cultures of Vigna radiata. No physical or chemical mutagen was employed for their isolation.

The actively growing fresh calli were exposed to increasing concentrations of NaCl (0, 25, 50, 100, 150, 200, 250, 300 and 350 mM) in order to find out the appropriate concentration which completely inhibited the growth of normal calli. The inhibitory concentration thus worked out was used for selection of mutant callus line resistant to this concentration of NaCl.

In a similar way, calli were also exposed to increasing concentrations of thioproline (0, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00 and 3.50 mM) so that inhibitory concentration of thioproline could be worked out and subsequently used for the

selection of mutant callus line resistant to that particular concentration of thioproline (L-thiazolidine-4-carboxylic acid).

Isolation of NaCl and thioproline resistant callus lines

The actively growing calli were placed on modified PC-L2 medium, containing the inhibitory concentration of NaCl. Simultaneously calli were also inoculated on a medium devoid of NaCl to serve as control. The clones which appeared on the inhibitory concentration were picked up and tested for stability of the altered response. For this purpose, variant clones were allowed to grow away from NaCl for three transfers over a period of 75 days. After this, they were again transferred to a fresh medium containing inhibitory concentration of NaCl in which they had been isolated. The clones which grew satisfactorily on the medium supplemented with the inhibitory concentration of NaCl, after passing through 3-4 generation of callus growth on normal medium, were selected for further studies as mutant callus lines.

A similar procedure was followed for the isolation of thioproline resistant callus line.

Alleviation of NaCl growth inhibition by L-proline

The normal calli were exposed to increasing concentrations of L-proline (20, 25, 33.33, 50 and 100 mM) in the presence of the inhibitory concentration of NaCl (300 mM) and thereby, concentration of exogenous L-proline required to restore normal growth of the callus was worked out.

Alleviation of thioproline growth inhibition by L-proline

Similarly, by exposing the thioproline stressed normal calli to increasing concentrations of L-proline (0.225, 0.375, 1.125, 2.250, 67.50 mM), the concentration of L-proline required to support normal growth of the callus under inhibitory concentration of thioproline was worked out.

Biochemical characterization

Periodical sampling was done to characterize sensitive, NaCl resistant and thioproline resistant callus lines with respect to free proline content, total soluble carbohydrates, total soluble proteins and certain stress related enzymes like peroxidase, malate dehydrogenase and catalase. Likewise, the normal calli grown under inhibitory concentration of either NaCl or thioproline but alleviated by exogenous proline were also characterized for these biochemical parameters.

Estimation of free proline

It was estimated according to the method of Bates et al. (1973).

(a) Reagents

- A) Acid-ninhydrin solution: Prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid, with agitation, until dissolved. Kept cool (stored at 4°C) and utilized within 24 hours.
- B) 3 per cent sulphosalicylic acid prepared in double distilled water.

C) Glacial acetic acid

D) Toluene

b) Procedure

The pooled fresh callus samples from 5 to 6 culture flasks were washed with double distilled water and from this 200 mg of callus was homogenized in 5 ml of 3 per cent aqueous sulphosalicyclic acid and centrifuged at 5000 rpm for 15 minutes to get a clean supernatant.

The residue was reextracted with 5 ml of 3 per cent sulphosalicyclic acid and centrifuged. The supernatants were pooled and volume made to 10 ml. To 2 ml of this supernatant, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added and the mixture was heated in a boiling water bath for an hour, to develop colour. The reaction was then terminated by placing the tube in an ice bath. The reaction mixture was then extracted with 5 ml of toluene and shaken vigorously on cyclomixer for 10 minutes. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve (prepared with graded concentration of purified proline) and expressed as μ moles of free proline per gram fresh weight of callus.

Extraction of total soluble carbohydrates

Total soluble carbohydrates were extracted by the method of Barnett and Naylor (1969). 0.1 g of the pooled fresh sample tissues were homogenized in 5 ml of 80 per cent ethanol (V/v) refluxed for 15 minutes in water bath at 60°C and centrifuged at 5000 rpm for 15 minutes. The supernatant was kept and residue was again refluxed twice with 80 per cent ethanol and centrifuged. The supernatants were pooled together and volume was made to 15 ml. This sample was used for soluble carbohydrate estimation.

Estimation of total soluble carbohydrates

It was estimated by the method of Dimler et al. (1952).

(a) Reagent

0.4 g anthrone in 100 ml of concentrated sulphuric acid.

(b) Procedure

A known amount of ethanol extract was evaporated to dryness in a test tube. On cooling, the residue was dissolved in 1 ml of distilled water, added to it 4 ml of anthrone reagent and heated in boiling water bath for 10 minutes. After cooling, optical density was read at 620 nm against reagent blank. The amount of total soluble carbohydrates was determined from a standard curve (prepared with graded concentration of purified glucose) and expressed as milligrams of total soluble carbohydrates per gram fresh weight of callus.

Estimation of total soluble proteins

The total soluble proteins were estimated according to the method of Lowry et al. (1951).

(a) Reagents

- A 2 per cent Na_2CO_3 in 0.1N NaOH
- B 0.5 per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ prepared in 1 per cent sodium potassium tartarate
- C Alkaline copper solution: 50 ml of reagent A was mixed with 1 ml of reagent B and the solution was discarded after one day.
- D Diluted Folin and Ciocalteu's phenol reagent: Folin and Ciocalteu's phenol reagent was diluted with double distilled water (about 2 fold) to make it 1N.

(b) Procedure

To 1 ml of properly diluted enzyme extract 5 ml of freshly prepared reagent C was added. The solutions were thoroughly mixed and allowed to stand for 10 minutes at room temperature. After this, 0.5 ml of reagent D was added very rapidly and mixed immediately. The solutions were allowed to stand for 30 minutes at room temperature and then optical density was read at 620 nm in Spectronic-20. The amount of protein was determined from standard curve drawn by taking bovine serum albumin as the standard protein.

Enzymatic Studies

Preparation of enzyme extract: Calli from 5 to 6 culture flasks were pooled and washed with double distilled water. From this 1 g of callus was weighed and subjected to grinding in chilled pestle and mortar. The homogenate was then centrifuged at 10,000 rpm for 30 minutes at -5°C in the International Refrigerated Centrifuge (IEC). The supernatant was used for the estimation of enzyme activity.

Extraction buffer

Estimation

- | | |
|-------------------------------------|-------------------------------------|
| i) 0.1M Tris-HCl (pH 7.4) | Peroxidase and malate dehydrogenase |
| ii) 0.01M Phosphate buffer (pH 7.0) | Catalase and total soluble proteins |

Units of enzyme activity

- (i) Total activity: It was expressed as the activity per gram fresh weight of callus.
- (ii) Specific activity: It was expressed as total activity per milligram of soluble proteins.
- (iii) Percentage change: It was expressed as the change over 0th day observation.

Methods of enzyme activity

Peroxidase (EC 1.11.1.7)

Enzyme activity was assayed by the method of Seever's et al. (1971).

(a) Reagents

- A) 0.5M Sodium acetate buffer (pH 4.5)
- B) 0.25 per cent O-dianisidine prepared in methanol
- C) 0.2M H_2O_2

(b) Procedure

0.1 ml of properly diluted enzyme extract was incubated with 1.8 ml of 0.5M sodium acetate buffer pH 4.5, 0.1 ml of O-dianisidine (0.25% in methanol) and 0.2 ml of 0.2M H_2O_2 . The activity of peroxidase was measured spectrophotometrically as change in absorbance at 470 nm due to the oxidation of O-dianisidine in the presence of H_2O_2 and enzyme. One unit of enzyme activity was defined as the 0.1 change in optical density per minute.

Malate dehydrogenase (EC 1.1.1.37)

The assay was done by the method of Davies (1969).

(a) Reagents

- A) 0.6M sodium phosphate buffer (pH 7.4)
- B) NADH (1 mg per ml of double distilled water)
- C) 3 mM cis-oxaloacetic acid (OAA) prepared in 0.6 per cent EDTA.

(b) Procedure

The reaction mixture containing 0.8 ml of sodium phosphate buffer, 0.4 ml of NADH solution (freshly prepared) and 0.1 ml of properly diluted enzyme extract was taken in a

cuvette and allowed to equilibrate for 5 minutes at 30°C and then the reaction was initiated by adding 0.2 ml of freshly prepared OAA. The decrease in absorbance at 340 nm was noted in an enzymeter (Calbiometer-340, calbiochem, USA). This decrease was followed every 15 seconds for 3 minutes. The decrease in absorbance per minute was calculated from the linear phase of initial reaction velocity. One unit of enzyme activity was defined as 0.1 change in optical density per minute.

Catalase (EC 1.11.1.6)

Enzyme activity was assayed according to the method of Sinha (1972).

(a) Reagents

- A) Dichromate acetic acid reagent prepared by mixing a 5 per cent solution of $K_2Cr_2O_7$ with glacial acetic acid (1:3, by volume)
- B) 0.2M H_2O_2
- C) 0.01M phosphate buffer (pH 7.0)

(b) Procedure

The assay mixture contained 4 ml of 0.2M H_2O_2 solution and 5 ml of phosphate buffer in a 50 ml Erlenmeyer flask. One ml of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1.0 ml portion of the reaction mixture was withdrawn and blown into 2 ml of

dichromate/acetic acid reagent at 60 second intervals. Addition of the reagent to H_2O_2 instantaneously produced an unstable blue precipitate of perchloric acid. On subsequent heating for 10 minutes in a boiling water bath, the colour of the solution changed to stable green due to the formation of chromic acetate. After cooling at room temperature the volume of the reaction mixture was made to 3 ml and the optical density was measured at 570 nm in Spectronic-20. The hydrogen peroxide contents of the withdrawn samples were determined from the standard curve (prepared with graded concentration of H_2O_2) and thus enzyme activity was expressed as μ mole of H_2O_2 consumed/minute (1 enzyme unit).

Estimation of potassium (K^+) and sodium (Na^+)

Fifty mg of dried and well ground callus material was taken in a 50 ml digestion flask to which 3 ml of a mixture of concentrated H_2SO_4 and $HClO_4$ in the ratio of 9:1 (V/v) were added. The flasks were heated gently over a heating plate for 5-10 minutes until the solution became colourless. The digest was cooled and diluted appropriately by adding double distilled water. Potassium and sodium were estimated from the final acid digest extract on Elico digital flamephotometer model CL-22D. Prior to the detection of K^+ and Na^+ of callus digest, the instrument was calibrated with standard solutions of KCl and NaCl containing 40 ppm and 60 ppm of K^+ and Na^+ respectively. The K^+ and Na^+ contents in

callus were determined based on the standard curve in ppm and expressed as millimoles per gram of callus dry weight.

Estimation of total calcium

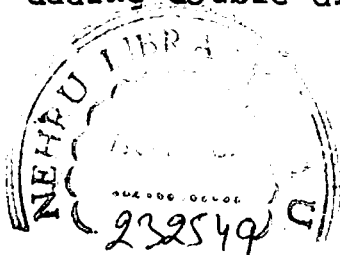
The total calcium was estimated according to the method of Cheng and Bray (1951).

(a) Reagents

- A) Aqua regia prepared by mixing 3 parts of conc. HCl with 1 part of conc. HNO_3
- B) 4N NaOH prepared by dissolving 160 g of NaOH in 1 litre of distilled water
- C) Ammonium purpurate indicator prepared by mixing thoroughly ammonium purpurate and powdered potassium sulphate in the ratio of 1:200 (W/w)
- D) 0.01N Ethylenediaminetetracetate solution prepared by dissolving 2.0 g of disodium dihydrogen ethylenediaminetetracetate and 0.05 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in water and diluted to a volume of 1 litre.

(b) Procedure

Fifty mg (sometime the concentration had to be changed but the proportions were maintained) of dried and well ground callus material was taken in a 50 ml digestion flask to which 2 ml of reagent A was added. The flask was heated gently on a hot plate for about 10 minutes until the solution became colourless. The digest was cooled and diluted appropriately by adding double distilled water. To 10 ml of



this acid-digest extract, 0.25 ml of reagent B and approximately 50 mg of reagent C were added and subsequently titrated against reagent D using a 10 ml microburette. The colour changed from orange red to purple. When close to the end point, reagent D was added at the rate of about a drop every 5 to 10 seconds, as the colour change was not instantaneous. A blank containing reagents B, C and a drop or two of D aided in distinguishing the end point.

The amount of total calcium in callus samples was calculated from the following formula and finally expressed in millimoles per gram of callus dry weight.

Milliequivalents per
litre of calcium

$$= \frac{(\text{ml. of reagent D used} \times \text{normality of reagent D} \times 1000)}{(\text{ml of sample aliquot taken})}$$

Estimation of chloride

Preparation of extract: Fifty mg (sometimes concentration had to be changed but the proportions were maintained) of ground callus material was taken in 50 ml flask to which 20 ml of $\text{HNO}_3\text{-KNO}_3$ solution (mixed in the ratio of 1:1 of 1N HNO_3 and 1N KNO_3) was added. The solution was stirred for half an hour and then filtered. To the coloured filtrates Darco G-60 was added and then again filtered. The colourless filtrate so obtained was diluted appropriately and employed for estimating total chlorides using the method of Reitemier (1943).

(a) Reagents

- A) 5 per cent solution of potassium chromate prepared by dissolving 5 g of potassium chromate in distilled water and added a saturated solution of silver nitrate until a slight permanent red precipitate was produced, filtered and diluted to 100 ml
- B) 0.01N AgNO_3 solution prepared by dissolving 1.699 g of AgNO_3 in water and diluted to 1 litre. The solution was checked by titration against pure sodium chloride and kept in a brown bottle away from light
- C) 1N Sodium hydroxide
- D) 1N H_2SO_4

(b) Procedure

To the appropriate volume of the extract, 1 ml of reagent A was added and subsequently titrated against reagent B. Prior to the titration, the pH of the sample extract was adjusted to 7 to 10 with reagent C or reagent D depending upon the pH of the original extract. The end point of the titration was reddish brown in colour. AgNO_3 titrant was standardized and reagent blank value was estimated by the titration method outlined above.

The amount of total chloride in callus samples was calculated from the following formula and finally expressed in millimoles per gram of callus dry weight.

$$\text{Milliequivalents per litre of chloride} = \frac{(X-Y) \times N \times 1000}{\text{ml of sample aliquot taken}}$$

Where

X = ml titration for sample,

Y = ml titration for blank, and

N = normality of AgNO_3 .

Determination of osmotic potential

Osmotic potential of callus was measured by psychrometric technique using a model 5100-B vapour pressure osmometer (Wescor Inc., Logan, Utah, U.S.A.). The callus sap was taken out by pressing it with a flat glass rod. Then a filter disc was dipped in the sap and placed immediately in the chamber and sealed. The osmotic potential readings were automatically displayed on the digital meter after about 2 minutes and were recorded. The osmometer was calibrated by using standard solution of sodium chloride.

EXPERIMENTAL RESULTS

Growth behaviour of callus

Effect of NaCl/thioproline stress on the growth of sensitive callus cultures

With increasing concentrations of NaCl (0-350 mM) and thioproline (0-3.50 mM), a gradual decrease in fresh and dry weights of callus was observed (Tables 1 to 4). The inhibitions in callus growth at 300 mM NaCl and 3.00 mM thioproline concentrations were comparable to the ones produced at 350 mM NaCl and 3.50 mM thioproline concentrations, respectively. But stimulation of callus growth was observed at 25 mM and 50 mM with NaCl and 0.50 mM and 0.75 mM with thioproline. Moreover, rate of accumulation of dry weight was comparatively more than the rate of fresh weight production under NaCl as well as thioproline stress conditions.

Selection of NaCl resistant and thioproline resistant callus lines

The fresh callus was grown on modified PC-L2 (Phillips and Collins, 1979) basal medium but containing inhibitory concentration of NaCl (300 mM)/thiopline (3.00 mM). The clones which appeared after 25-30 days of growth were picked up and tested for the stability of the altered response by allowing them to grow away from NaCl/thiopline for three transfers over a period of 75 days. Then the resistant clones were transferred to a fresh medium containing inhibitory concentration of NaCl/thiopline where they grew satisfactorily.

Table 1. Fresh weights* of sensitive callus cultures of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)			
	0	8	16	24
0	151 $\pm$ 10	523 $\pm$ 43 (246.36)	1710 $\pm$ 69 (1032.45)	3802 $\pm$ 98 (2417.88)
25	155 $\pm$ 12	618 $\pm$ 12 (298.71)	2436 $\pm$ 76 (1471.61)	3004 $\pm$ 109 (1838.06)
50	122 $\pm$ 10	370 $\pm$ 28 (203.28)	1943 $\pm$ 97 (1492.62)	3423 $\pm$ 82 (2705.74)
100	140 $\pm$ 10	364 $\pm$ 37 (160.00)	1379 $\pm$ 81 (885.00)	2323 $\pm$ 103 (1559.29)
150	155 $\pm$ 13	392 $\pm$ 49 (152.90)	1203 $\pm$ 57 (676.13)	2653 $\pm$ 118 (1611.61)
200	121 $\pm$ 4	299 $\pm$ 28 (147.11)	612 $\pm$ 32 (405.79)	679 $\pm$ 41 (461.16)
250	127 $\pm$ 4	211 $\pm$ 4 (66.14)	393 $\pm$ 19 (209.45)	555 $\pm$ 29 (337.00)
300	114 $\pm$ 9	121 $\pm$ 5 (6.14)	133 $\pm$ 11 (16.67)	221 $\pm$ 15 (93.86)
350	118 $\pm$ 10	125 $\pm$ 8 (5.93)	136 $\pm$ 8 (15.25)	227 $\pm$ 19 (92.37)

* Fresh weights are expressed in milligrams and are the average of five replicates $\pm$ standard error.

Figures in parentheses represent per cent increase in fresh weight over 0th day.

Table 2. Dry weights* of sensitive callus cultures of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)				
	0	8	16	24	32
0	9±0.60	57±4 (533.33)	142±24 (1477.78)	296±22 (3188.89)	388±11 (4211.11)
25	9±0.70	67±2 (644.44)	202±10 (2144.44)	267±4 (2866.67)	391±5 (4244.44)
50	7±0.60	42±3 (500.00)	177±9 (2428.57)	256±13 (3557.14)	407±27 (5714.29)
100	8±0.60	42±9 (425.00)	135±18 (1587.50)	204±17 (2450.00)	379±28 (4627.50)
150	9±0.80	49±8 (444.44)	111±20 (1133.33)	242±9 (2588.89)	363±23 (3933.33)
200	7±0.50	39±3 (457.14)	89±5 (1171.43)	108±5 (1442.86)	181±22 (2485.71)
250	8±0.50	27±3 (237.50)	64±5 (700.00)	95±13 (1087.50)	103±5 (1187.50)
300	6±0.40	7±0.30 (16.67)	8±0.40 (33.33)	13±0.90 (116.67)	15±0.80 (150.00)
350	7±0.60	8±0.50 (14.29)	9±0.60 (28.57)	15±0.50 (114.29)	17±0.60 (142.86)

* Dry weights are expressed in milligrams and are the average of five replicates ± standard error.

Figures in parentheses represent per cent increase in dry weight over 0th day.

Table 3. Fresh weights of sensitive callus cultures of *Vigna radiata* grown on a medium containing increasing concentrations of thiopropine

Thiopropine (mM)	Culture period (days)				
	0	8	16	24	32
0	120 $\pm$ 15	819 $\pm$ 35 (582.50)	2684 $\pm$ 131 (2136.67)	4250 $\pm$ 183 (3441.67)	5484 $\pm$ 89 (4470.00)
0.50	101 $\pm$ 12	708 $\pm$ 12 (600.90)	3165 $\pm$ 128 (3033.66)	4640 $\pm$ 137 (4494.06)	6085 $\pm$ 194 (5924.75)
0.75	81 $\pm$ 7	763 $\pm$ 19 (841.98)	2491 $\pm$ 89 (2975.31)	3655 $\pm$ 101 (4412.35)	4919 $\pm$ 60 (5972.84)
1.00	94 $\pm$ 11	515 $\pm$ 17 (447.87)	1169 $\pm$ 42 (1143.62)	3722 $\pm$ 124 (3859.58)	4370 $\pm$ 129 (4548.94)
1.50	111 $\pm$ 12	335 $\pm$ 51 (201.80)	1290 $\pm$ 35 (1062.16)	2455 $\pm$ 126 (2111.71)	3668 $\pm$ 76 (3204.50)
2.00	122 $\pm$ 12	336 $\pm$ 42 (175.41)	426 $\pm$ 74 (249.18)	932 $\pm$ 29 (655.74)	1952 $\pm$ 54 (1500.00)
2.50	90 $\pm$ 9	234 $\pm$ 31 (160.00)	350 $\pm$ 29 (288.89)	580 $\pm$ 37 (544.44)	663 $\pm$ 64 (636.67)
3.00	102 $\pm$ 7	232 $\pm$ 21 (127.45)	269 $\pm$ 11 (163.73)	290 $\pm$ 29 (184.31)	411 $\pm$ 13 (302.94)
3.50	109 $\pm$ 13	245 $\pm$ 18 (124.77)	285 $\pm$ 15 (161.47)	309 $\pm$ 23 (183.49)	435 $\pm$ 17 (299.08)

*Fresh weights are expressed in milligrams and are the average of five replicates $\pm$ standard error.

Figures in parentheses represent per cent increase in fresh weight over 0th day.

Table 4. Dry weights* of sensitive callus cultures of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	Culture period (days)			
	0	8	16	24
0	7±0.80	79±3 (1028.57)	211±9 (2914.29)	306±39 (4271.43)
0.50	6±0.70	60±1 (900.00)	223±14 (3616.67)	326±5 (5333.33)
0.75	5±0.40	67±2 (1240.00)	201±12 (3920.00)	249±26 (4880.00)
1.00	6±0.60	48±2 (700.00)	97±6 (1516.67)	248±16 (4033.33)
1.50	7±0.60	41±3 (485.71)	101±8 (1342.86)	169±5 (2314.29)
2.00	7±0.70	26±1 (271.43)	37±2 (428.57)	72±3 (928.57)
2.50	5±0.30	19±1 (280.00)	28±2 (460.00)	49±3 (880.00)
3.00	6±0.40	20±2 (233.33)	25±1 (316.67)	26±1 (333.33)
3.50	7±0.30	23±2 (228.57)	29±3 (314.29)	30±2 (328.57)
				390±11 (5471.43)
				357±14 (5850.00)
				337±4 (6640.00)
				302±21 (4933.33)
				259±15 (3600.00)
				144±13 (1957.14)
				53±4 (960.00)
				32±2 (433.33)
				37±4 (428.57)

* Dry weights are expressed in milligrams and are the average of five replicates ± standard error.

Figures in parentheses represent per cent increase in dry weight over 0th day.

These callus lines which showed resistance to NaCl/thioprolin were termed as mutant/variant/resistant callus lines whereas callus which was unable to grow at the inhibitory or selective concentration of NaCl/thioprolin was referred to as sensitive callus line.

Growth pattern of NaCl resistant and thioprolin resistant callus lines

The sodium chloride resistant and thioprolin resistant callus lines grew faster on normal medium than on NaCl (300 mM) and thioprolin (3.00 mM) supplemented medium respectively (Tables 5 and 6). NaCl resistant callus line showed more increase in fresh and dry weights on normal medium except on 32nd day when the dry weight accumulation was greater than in NaCl resistant or sensitive callus lines grown on normal medium (Table 5). The thioprolin resistant callus line accumulated more fresh and dry weights on normal medium than on thioprolin supplemented medium throughout the growth period (Table 6). Moreover, the growth of NaCl resistant and thioprolin resistant callus lines grown under the inhibitory concentration of NaCl (300 mM) and thioprolin (3.00 mM) respectively was comparable with the sensitive callus line grown on normal medium.

Alleviation of NaCl growth inhibition by exogenous proline

The sensitive callus line grown on normal and NaCl (300 mM) supplemented media showed alleviation in fresh and

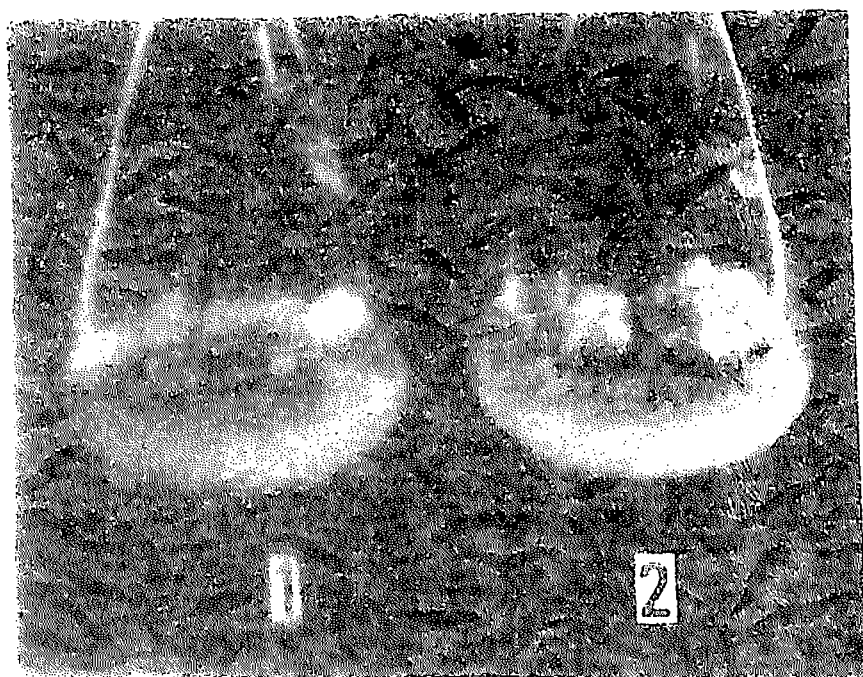


Plate A:

- 1) 15-days old sensitive callus line of Vigna radiata cv. K851 grown on normal medium.
- 2) 15-days old NaCl resistant callus line of Vigna radiata cv. K851 grown on NaCl (300 mM) supplemented medium.

Plate B:

- 1) 15-days old sensitive callus line of Vigna radiata cv. K851 grown on normal medium.
- 2) 15-days old thioproline resistant callus line of Vigna radiata cv. K851 grown on thioproline (3.00 mM) supplemented medium.

Table 5. Growth pattern (fresh and dry weights)* of sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC		SRC ⁿ		SRC ^s	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0	92±4	6±0.20	87±4	5±0.30	83±6	5±0.30
8	416±19 (352.17)	46±1 (666.67)	1572±89 (1706.90)	118±7 (2260.00)	405±14 (387.95)	55±2 (1000.00)
16	1612±43 (1652.17)	138±8 (2200.00)	2371±112 (2625.29)	207±3 (4040.00)	1533±69 (1746.99)	155±3 (3000.00)
24	2438±98 (2550.00)	199±17 (3216.67)	4469±100 (5036.78)	345±2 (6800.00)	2034±76 (2350.60)	235±13 (4600.00)
32	4398±109 (4680.43)	362±23 (5933.33)	6004±184 (6801.15)	365±11 (7200.00)	3964±112 (4675.90)	377±5 (7440.00)

* in milligrams and are average of five replicates ± standard error.

Figures in parentheses represent per cent increase in fresh weight/dry weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

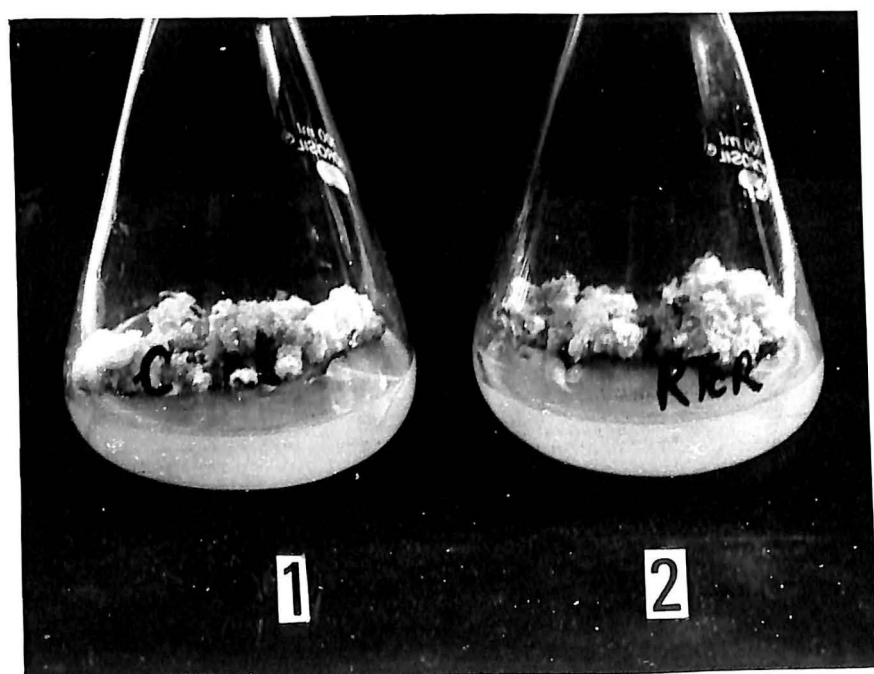
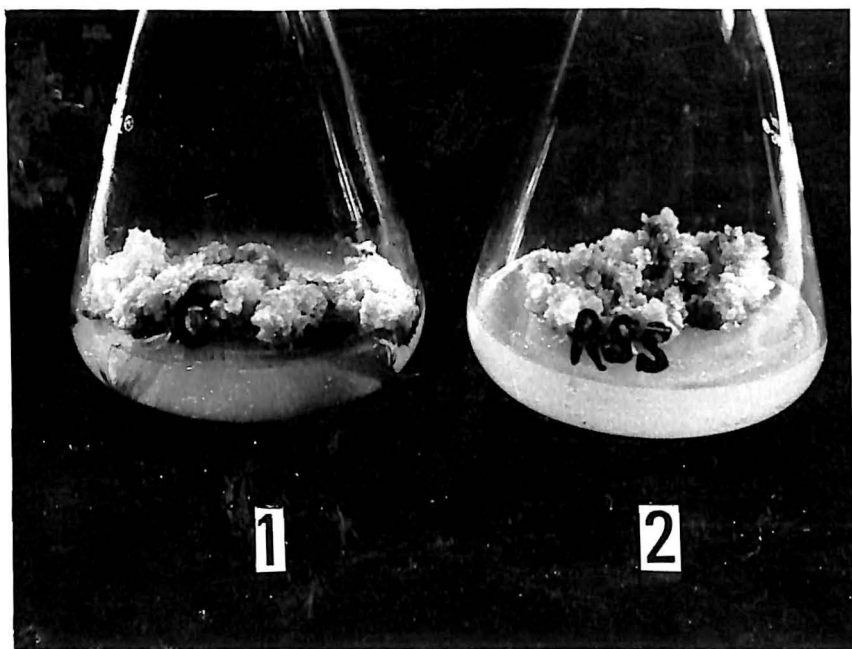


Table 5. Growth pattern (fresh and dry weights)* of sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC		SRC ⁿ		SRC ^s	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0	92 $\pm$ 4	6 $\pm$ 0.20	87 $\pm$ 4	5 $\pm$ 0.30	83 $\pm$ 6	5 $\pm$ 0.30
8	416 $\pm$ 19 (352.17)	46 $\pm$ 1 (666.67)	1572 $\pm$ 89 (1706.90)	118 $\pm$ 7 (2260.00)	405 $\pm$ 14 (387.95)	55 $\pm$ 2 (1000.00)
16	1612 $\pm$ 43 (1652.17)	138 $\pm$ 8 (2200.00)	2371 $\pm$ 112 (2625.29)	207 $\pm$ 3 (4040.00)	1533 $\pm$ 69 (1746.99)	155 $\pm$ 3 (3000.00)
24	2438 $\pm$ 98 (2550.00)	199 $\pm$ 17 (3216.67)	4469 $\pm$ 100 (5036.78)	345 $\pm$ 2 (6800.00)	2034 $\pm$ 76 (2350.60)	235 $\pm$ 13 (4600.00)
32	4398 $\pm$ 109 (4680.43)	362 $\pm$ 23 (5933.33)	6004 $\pm$ 184 (6801.15)	365 $\pm$ 11 (7200.00)	3964 $\pm$ 112 (4675.90)	377 $\pm$ 5 (7440.00)

* in milligrams and are average of five replicates $\pm$ standard error.

Figures in parentheses represent per cent increase in fresh weight/dry weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 6. Growth pattern (fresh and dry weights)* of sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC		TcRC ⁿ		TcRC ^{tc}	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0	92±4	6±0.20	101±5	6±0.20	116±5	7±0.30
8	416±19 (352.17)	46±1 (666.67)	1216±53 (1103.96)	100±2 (1566.67)	714±13 (515.52)	70±3 (900.00)
16	1612±43 (1652.17)	138±8 (2200.00)	2456±108 (2331.68)	207±6 (3350.00)	1915±52 (1550.86)	169±7 (2314.29)
24	2438.98 (2550.00)	199±17 (3216.67)	4499±63 (4354.46)	330±8 (5400.00)	3152±123 (2617.24)	271±5 (3771.43)
32	4398±109 (4680.43)	362±23 (5933.33)	5879±114 (5720.79)	383±7 (6283.33)	4805±178 (4042.24)	379±7 (5314.29)

* in milligrams and are average of five replicates ± standard error.

Figures in parentheses represent per cent increase in fresh/dry weight over 0th day.

SC: Sensitive callus line grown on normal medium.

TcRCⁿ: Thioproline resistant callus line grown on normal medium.

TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

dry weights upon exposing them to increasing concentrations of exogenous proline (Tables 7 and 8). However, maximum alleviation in callus growth inhibition was obtained at 33.33 mM exogenous proline concentration. But at higher proline concentration (50 mM), inhibition in callus growth was observed which became more pronounced at still higher concentration i.e. 100 mM (Tables 7 and 8).

Alleviation of thioproline growth inhibition by exogenous proline

Similarly, sensitive callus line grown on normal and thioproline (3.00 mM) supplemented media also showed alleviation in fresh and dry weights upon exposing them to increasing concentrations of exogenous proline (Tables 9 and 10). However, alleviation in callus growth inhibition produced at 1.125 mM and 2.250 mM exogenous proline concentrations was highest than at other concentrations of exogenous proline. But at higher concentration of exogenous proline (67.50 mM), the callus growth was inhibited both under normal as well as under thioproline stress conditions.

K⁺ content

Effect of NaCl/thioproline stress on K⁺ levels in sensitive callus cultures

With the increase in NaCl (0-300 mM) and thioproline (0-3.0 mM) concentrations, levels of K⁺ declined in sensitive callus cultures in comparison to the control

Table 7. Fresh weights* of sensitive callus cultures of *Vigna radiata* grown on a medium containing inhibitory concentration of NaCl (300 mM) and exogenously applied proline

Proline (mM)	Culture period (days)									
	0	8		16		24		32		
		SC	SC ^s	SC	SC ^s	SC	SC ^s	SC	SC ^s	
20	114±7	715±30 (527.19)	659±30 (478.07)	2351±8 (1962.28)	1204±97 (956.14)	3964±39 (3377.19)	2525±75 (2114.91)	5260±48 (4514.04)	4230±131 (3610.53)	
25	104±6	927±33 (791.35)	644±36 (519.23)	2715±47 (2510.58)	1195±29 (1049.04)	4376±120 (4107.69)	2442±64 (2248.08)	5588±22 (5273.08)	3774±127 (3528.85)	
33.33	100±5	1009±12 (909.00)	987±34 (887.00)	3082±40 (2982.00)	1383±40 (1283.00)	4811±127 (4711.00)	1846±73 (1746.00)	5778±95 (5678.00)	4653±131 (4553.00)	
50	118±5	427±10 (261.86)	676±12 (472.88)	1488±27 (1161.02)	1208±21 (923.73)	2545±102 (2056.78)	1168±84 (889.83)	4246±137 (3498.31)	2857±103 (2321.19)	
100	127±5	411±6 (223.62)	429±7 (237.80)	1245±23 (880.31)	767±12 (503.94)	2204±65 (1635.43)	958±24 (654.33)	3617±41 (2748.03)	1351±58 (963.78)	

* Fresh weights are expressed in milligrams and are the average of five replicates ± standard error.

Figures in parentheses represent per cent increase in fresh weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SC^s: Sensitive callus line grown on NaCl (300 mM) supplemented medium.

Table 8. Dry weights* of sensitive callus cultures of *Vigna radiata* grown on a medium containing inhibitory concentration of NaCl (300 mM) and exogenously applied proline

Proline (mM)	Culture period (days)									
	0		8		16		24		32	
	SC	SC ^s	SC	SC ^s	SC	SC ^s	SC	SC ^s	SC	SC ^s
20	6±0.30	74±3 (1133.33)	80±2 (1233.33)	222±4 (3600.00)	147±13 (2350.00)	339±7 (5550.00)	281±12 (4583.33)	397±4 (6516.67)	417±18 (6850.00)	
25	6±0.30	91±3 (1416.67)	78±4 (1200.00)	206±3 (3333.33)	136±4 (2166.67)	338±5 (5533.33)	273±9 (4450.00)	417±5 (6850.00)	400±8 (6566.67)	
33.33	6±0.40	94±5 (1466.67)	108±3 (1700.00)	212±2 (3433.33)	180±13 (2900.00)	354±14 (5800.00)	229±7 (3716.67)	387±8 (6350.00)	463±12 (7616.67)	
50	7±0.30	48±2 (585.71)	79±1 (1028.57)	117±4 (1571.43)	143±1 (1942.86)	204±9 (2814.29)	163±8 (2228.57)	324±10 (4528.57)	363±13 (5085.71)	
100	7±0.40	44±0.70 (528.57)	55±4 (687.71)	99±3 (1314.29)	125±6 (1685.71)	168±13 (2300.00)	151±3 (2057.14)	262±2 (3642.86)	183±9 (2514.29)	

* Dry weights are expressed in milligrams and are the average of five replicates $\pm$ standard error.

Figures in parentheses represent per cent increase in dry weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SC^S: Sensitive callus line grown on NaCl (300 mM) supplemented medium.

Table 9. Fresh weights* of sensitive callus cultures of Vigna radiata grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)									
	0		8		16		24		32	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	129 ⁺⁴ (506.20)	782 ⁺²² (583 ⁺⁵ (351.94)	2630 ⁺¹⁰³ (1938.76)	804 ⁺²¹ (523.26)	4245 ⁺⁸⁷ (3190.70)	1007 ⁺¹² (680.62)	5293 ⁺⁵⁷ (4003.10)	2166 ⁺⁸² (1579.07)		
0.375	128 ⁺⁶	867 ⁺¹² (577.34)	2792 ⁺¹²⁰ (2081.25)	926 ⁺³⁹ (623.44)	4452 ⁺¹⁵⁷ (3378.13)	2301 ⁺¹⁶ (1697.66)	5438 ⁺⁸⁷ (4148.44)	3345 ⁺³¹ (2513.28)		
1.125	128 ⁺⁷	886 ⁺¹⁴ (592.19)	2977 ⁺⁹⁹ (2225.78)	1947 ⁺²⁵ (1421.09)	4920 ⁺¹⁴⁴ (3743.75)	3031 ⁺⁴⁸ (2267.97)	5455 ⁺⁹⁰ (4161.72)	4272 ⁺¹¹² (3237.50)		
2.250	127 ⁺⁶	820 ⁺¹⁸ (545.67)	2850 ⁺⁸⁶ (2144.09)	1941 ⁺¹¹¹ (1428.35)	5130 ⁺¹⁴⁹ (3939.37)	3017 ⁺⁷⁵ (2275.59)	5494 ⁺¹⁸ (4225.98)	4220 ⁺¹¹² (3222.83)		
67.50	92 ⁺³	399 ⁺⁹ (333.70)	967 ⁺²³ (951.09)	587 ⁺¹⁹ (538.04)	1858 ⁺³⁹ (1919.57)	725 ⁺¹⁸ (688.04)	2996 ⁺³⁷ (3156.52)	1242 ⁺¹²¹ (1250.00)		

* Fresh weights are expressed in milligrams and are the average of five replicates ± standard error.

Figures in parentheses represent per cent increase in fresh weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

Table 10. Dry weights* of sensitive callus cultures of Vigna radiata grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)									
	0		8		16		24		32	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	8±0.30	79±5 (887.50)	51±2 (537.50)	227±7 (2737.50)	63±2 (687.50)	309±7 (3762.50)	137±8 (1612.50)	377±9 (4612.50)	173±10 (2062.50)	
0.375	8±0.30	85±2 (962.50)	76±3 (850.00)	208±5 (2500.00)	140±8 (1650.00)	331±7 (4037.50)	181±6 (2162.50)	387±11 (4737.50)	274±23 (3325.00)	
1.125	8±0.40	81±2 (912.50)	106±6 (1225.00)	219±3 (2637.50)	175±10 (2087.50)	357±11 (4362.50)	254±23 (3075.00)	374±5 (4575.00)	391±29 (4787.50)	
2.250	8±0.40	80±3 (900.00)	100±3 (1150.00)	229±4 (2762.50)	166±13 (1975.00)	369±8 (4512.50)	252±24 (3050.00)	378±4 (4625.00)	385±21 (4712.50)	
67.50	6±0.40	42±2 (600.00)	48±2 (700.00)	85±2 (1316.87)	105±7 (1650.00)	152±4 (2433.33)	123±4 (1950.00)	230±8 (3733.33)	145±5 (2316.67)	

* Dry weights are expressed in milligrams and are the average of five replicates ± standard error.

Figures in parentheses represent per cent increase in dry weight over 0th day.

SC: Sensitive callus line grown on normal medium.

SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

Table 11. Potassium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)				
	0	8	16	24	32
0	1.208	0.315	0.881	1.121	1.202
25	1.208	0.343	0.407	0.753	0.817
50	1.208	0.240	0.335	0.426	0.880
100	1.208	0.181	0.212	0.322	0.308
150	1.208	0.238	0.187	0.335	0.301
200	1.208	0.179	0.269	0.187	0.214
250	1.208	0.209	0.192	0.176	0.167
300	1.208	0.154	0.136	0.149	0.162

Table 12. Potassium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	Culture period (days)			
	0	8	16	24
0	1.218	0.464	0.962	1.282
0.50	1.218	0.436	0.817	0.964
0.75	1.218	0.962	0.827	1.122
1.00	1.218	0.472	0.399	0.963
1.50	1.218	0.381	0.464	0.857
2.00	1.218	0.447	0.365	0.621
2.50	1.218	0.302	0.335	0.349
3.00	1.218	0.180	0.292	0.247

(Tables 11 and 12). Also a lag period of 8 days in growth was observed for K^+ uptake.

K^+ levels in NaCl resistant and thioproline resistant callus lines

Sodium chloride resistant and thioproline resistant callus lines maintained low levels of K^+ when grown under their respective stress conditions than under normal condition (Tables 13 and 15). When sensitive and NaCl resistant callus lines were exposed to increasing concentrations of NaCl, levels of K^+ declined continuously in sensitive callus line but NaCl resistant callus line showed decreasing trend only from 150 mM NaCl concentration. Further, overall decrease in K^+ levels was more in sensitive callus line than in NaCl resistant callus line at identical levels of NaCl stress (Table 14). A similar response was observed upon exposing sensitive and thioproline resistant callus lines to increasing concentrations of thioproline. However, decrease in the levels of K^+ in thioproline resistant callus line commenced from 1.00 mM and went on declining constantly with further increase in thioproline concentration but the sensitive callus line showed decreasing trend right from lowest concentration of thioproline (Table 16).

K^+ levels in exogenous proline alleviated nonstressed and NaCl/thioproline stressed sensitive callus cultures

The sensitive callus line grown on normal and NaCl

Table 13. Potassium content (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC	SRC ⁿ	SRC ^s
0	1.208	1.430	0.410
8	0.315	0.528	0.389
16	0.881	0.873	0.459
24	1.121	1.218	0.417
32	1.202	1.628	0.392

SC: Sensitive callus line grown on normal medium.
 SRCⁿ: NaCl resistant callus line grown on normal medium.
 SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 14. Potassium content*(m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	SC	SRC
0	0.881	0.873
25	0.407	0.814
50	0.335	0.935
100	0.212	0.871
150	0.187	0.763
200	0.269	0.694
250	0.192	0.538
300	0.136	0.459

* After 16 days of growth.
 SC: Sensitive callus line.
 SRC: NaCl resistant callus line.

Table 15. Potassium content (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC	TcRC ⁿ	TcRC ^{tc}
0	1.218	1.222	0.485
8	0.464	1.667	0.487
16	0.962	1.002	0.525
24	1.282	1.497	0.501
32	1.202	1.387	0.473

SC: Sensitive callus line grown on normal medium.
TcRCⁿ: Thioproline resistant callus line grown on normal medium.
TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 16. Potassium content* (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	SC	TcRC
0	0.962	1.002
0.50	0.817	0.988
0.75	0.827	1.024
1.00	0.399	0.813
1.50	0.464	0.809
2.00	0.365	0.723
2.50	0.335	0.634
3.00	0.292	0.525

* After 16 days of growth.

SC: Sensitive callus line.

TcRC: Thioproline resistant callus line.

(300 mM) supplemented media showed increase in K^+ levels upto optimum concentration of exogenous proline (33.33 mM) followed by a decline at higher concentration of exogenous proline (50 mM) and the decrease was further pronounced at still higher concentration of exogenous proline i.e. 100 mM (Table 17). A similar trend was visible in thioproline stressed sensitive callus line alleviated by exogenous proline. However, the levels of K^+ were high and comparable at 1.125 mM and 2.250 mM and low at 67.50 mM exogenous proline concentrations (Table 18).

Na^+ content

Effect of NaCl/thioproline stress on the Na^+ levels in sensitive callus cultures

The levels of Na^+ increased in sensitive callus line with the increase in NaCl concentration in the medium (Table 19) but increase in thioproline stress did not result in any specific change in the levels of Na^+ (Table 20).

Na^+ levels in NaCl resistant and thioproline resistant callus lines

NaCl resistant and thioproline resistant callus lines behaved differently with respect to Na^+ levels when grown under inhibitory concentrations of NaCl and thioproline, respectively. NaCl resistant callus line maintained higher levels of Na^+ when grown under inhibitory concentration of NaCl stress (Table 21), whereas, low levels of Na^+ were

Table 17. Potassium content (m mole g⁻¹ dry weight) in sensitive callus culture of *Vigna radiata* grown on a medium containing inhibitory concentration of NaCl (300 mM) and exogenously applied proline

Proline (mM)	Culture period (days)							
	8		16		24		32	
	SC	SC ^s	SC	SC ^s	SC	SC ^s	SC	SC ^s
20	1.378	0.256	1.483	0.353	1.764	0.410	1.859	0.417
25	2.306	0.705	2.162	1.872	2.639	1.607	2.954	1.378
33.33	2.628	0.962	2.003	2.115	2.676	1.582	3.254	0.997
50	0.744	0.509	0.793	0.641	0.809	0.569	0.705	0.564
100	0.425	0.278	0.311	0.272	0.485	0.260	0.327	0.384
SC:	Sensitive callus line grown on normal medium.							
SC ^s :	Sensitive callus line grown on NaCl (300 mM) supplemented medium.							

Table 18. Potassium content (m mole g⁻¹ dry weight) in sensitive callus culture of *Vigna radiata* grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)					
	8		16		24	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	0.591	0.632	0.988	0.589	1.436	0.992
0.375	0.687	0.850	1.043	1.081	1.703	0.865
1.125	0.940	1.274	1.362	1.198	2.173	2.510
2.250	0.975	0.937	1.393	1.200	2.007	2.272
67.50	0.318	0.406	0.224	0.567	0.432	0.496
					0.349	0.481

SC: Sensitive callus line grown on normal medium.

SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

Table 19. Sodium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)			
	0	8	16	24
0	0.145	0.110	0.133	0.124
25	0.145	0.425	0.427	0.602
50	0.145	0.620	0.584	1.000
100	0.145	0.803	1.000	1.344
150	0.145	1.250	1.125	1.225
200	0.145	1.342	1.281	1.250
250	0.145	1.401	1.250	1.480
300	0.145	1.490	1.483	1.582
				1.520

Table 20. Sodium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	Culture period (days)				
	0	8	16	24	32
0	0.240	0.154	0.223	0.256	0.290
0.50	0.240	0.132	0.160	0.194	0.191
0.75	0.240	0.165	0.162	0.203	0.200
1.00	0.240	0.140	0.153	0.192	0.153
1.50	0.240	0.242	0.162	0.163	0.161
2.00	0.240	0.338	0.121	0.204	0.183
2.50	0.240	0.360	0.144	0.210	0.227
3.00	0.240	0.621	0.163	0.384	0.352

Table 21. Sodium content (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC	SRC ⁿ	SRC ^s
0	0.145	0.167	2.390
8	0.110	0.111	1.100
16	0.133	0.135	1.984
24	0.124	0.150	2.252
32	0.110	0.175	2.550

SC: Sensitive callus line grown on normal medium.
 SRCⁿ: NaCl resistant callus line grown on normal medium.
 SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

maintained in thioproline resistant callus line grown under inhibitory concentration of thioproline, which were in turn comparable to thioproline resistant and sensitive callus lines grown on normal medium (Table 23). When NaCl resistant and sensitive callus lines were exposed to increasing concentrations of NaCl, both these lines showed increase in Na^+ levels but in NaCl resistant callus line overall increase was more than sensitive callus line, also grown under identical levels of salinity (Table 22). Thioproline resistant callus line when grown under increasing levels of thioproline stress, did not exhibit any specific change in the levels of Na^+ in comparison to sensitive callus line also grown under identical levels of thioproline stress (Table 24).

Na^+ levels in exogenous proline alleviated nonstressed and NaCl/thioproline stressed sensitive callus cultures

With increasing concentrations of exogenous proline, levels of Na^+ decreased in sensitive callus line grown on normal and NaCl supplemented media and thus low levels of Na^+ were maintained upto optimum concentration of exogenous proline. However, levels of Na^+ increased at supraoptimal concentrations (50 mM and more) of exogenous proline (Table 25). On the other hand, application of either low or high concentrations of exogenous proline in thioproline stressed sensitive callus line did not result in any specific

Table 22. Sodium content* (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	SC	SRC
0	0.133	0.135
25	0.427	0.512
50	0.584	0.627
100	1.000	1.200
150	1.125	1.369
200	1.281	1.587
250	1.250	1.792
300	1.483	1.984

* After 16 days of growth.

SC: Sensitive callus line.

SRC: NaCl resistant callus line.

Table 23. Sodium content (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC	TcRC ⁿ	TcRC ^{tc}
0	0.240	0.210	0.219
8	0.154	0.150	0.100
16	0.223	0.194	0.167
24	0.256	0.185	0.204
32	0.290	0.228	0.237

SC: Sensitive callus line grown on normal medium.
TcRCⁿ: Thioproline resistant callus line grown on normal medium.
TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 24. Sodium content* (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	SC	TcRC
0	0.223	0.194
0.50	0.160	0.178
0.75	0.162	0.197
1.00	0.153	0.208
1.50	0.162	0.199
2.00	0.121	0.218
2.50	0.144	0.226
3.00	0.163	0.237

* After 16 days of growth.

SC: Sensitive callus line.

TcRC: Thioproline resistant callus line.

Table 25. Sodium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing inhibitory concentration of NaCl (300 mM) and exogenously applied proline

Proline (mM)	Culture period (days)							
	8		16		24		32	
	SC	SC ^s	SC	SC ^s	SC	SC ^s	SC	SC ^s
20	0.275	1.350	0.246	1.450	0.197	1.750	0.278	2.006
25	0.100	0.965	0.095	1.158	0.100	2.050	0.110	1.952
33.33	0.176	0.821	0.103	0.949	0.113	0.608	0.089	0.702
50	0.429	1.450	0.475	1.637	0.407	2.151	0.503	2.754
100	0.588	1.852	0.601	2.279	0.395	2.846	0.624	2.752

SC: Sensitive callus line grown on normal medium.

SC^s: Sensitive callus line grown on NaCl (300 mM) supplemented medium.

Table 26. Sodium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)							
	8		16		24		32	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	0.165	0.178	0.181	0.201	0.173	0.228	0.239	0.250
0.375	0.172	0.185	0.145	0.180	0.145	0.189	0.170	0.200
1.125	0.164	0.117	0.112	0.124	0.110	0.145	0.134	0.210
2.250	0.129	0.100	0.092	0.096	0.112	0.122	0.103	0.196
67.50	0.278	0.283	0.290	0.277	0.275	0.253	0.276	0.275

SC: Sensitive callus line grown on normal medium.
 SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

alteration in the levels of Na^+ (Table 26).

Cl^- content

Effect of NaCl/thioproline stress on the Cl^- levels in sensitive callus cultures

The levels of Cl^- increased continuously in sensitive callus cultures upon exposing them to increasing levels of NaCl stress (Table 27). But the sensitive callus cultures when subjected to increasing levels of thioproline stress, did not show any specific variation in the Cl^- levels (Table 28).

Cl^- levels in NaCl resistant and thioproline resistant callus lines

Sodium chloride resistant callus line maintained higher levels of Cl^- when grown under NaCl stress than under nonstress condition (Table 29), whereas, thioproline resistant callus line did not show any change in the levels of Cl^- when grown under thioproline stress and levels were comparable to the thioproline resistant and sensitive callus lines grown on thioproline free medium (Table 31). The sensitive as well as NaCl resistant callus lines showed increase in the levels of Cl^- with the increase in NaCl concentration in the medium but NaCl resistant callus line showed more increase in Cl^- levels than sensitive callus line when grown under identical levels of NaCl stress (Table 30). Contrary to this, increase in thioproline stress did not result in any specific change in the levels of Cl^- in thioproline resistant or sensitive callus lines (Table 32).

Table 27. Chloride content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)				
	0	8	16	24	32
0	0.185	0.200	0.225	0.192	0.210
25	0.185	0.667	0.695	0.586	0.619
50	0.185	0.734	1.046	0.977	0.882
100	0.185	0.945	0.892	1.217	0.901
150	0.185	1.167	1.453	1.439	1.467
200	0.185	1.334	1.927	1.862	1.834
250	0.185	1.527	2.245	2.300	2.245
300	0.185	1.938	2.170	2.478	2.520

Table 28. Chloride content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of thiopropoline

Thiopropoline (mM)	Culture period (days)				
	0	8	16	24	32
0	0.142	0.129	0.110	0.131	0.137
0.50	0.142	0.197	0.174	0.148	0.126
0.75	0.142	0.322	0.219	0.144	0.167
1.00	0.142	0.284	0.209	0.185	0.149
1.50	0.142	0.357	0.198	0.194	0.189
2.00	0.142	0.331	0.214	0.222	0.208
2.50	0.142	0.402	0.242	0.239	0.229
3.00	0.142	0.383	0.327	0.304	0.287

Table 29. Chloride content (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC	SRC ⁿ	SRC ^s
0	0.185	0.190	2.592
8	0.200	0.185	2.075
16	0.225	0.238	2.567
24	0.192	0.213	2.700
32	0.210	0.197	2.638

SC: Sensitive callus line grown on normal medium.
 SRCⁿ: NaCl resistant callus line grown on normal medium.
 SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 30. Chloride content*(m mole g⁻¹ dry weight) in sensitive and resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	SC	SRC
0	0.225	0.238
25	0.695	0.793
50	1.046	1.202
100	0.892	1.531
150	1.453	1.847
200	1.927	2.160
250	2.245	2.341
300	2.170	2.567

* After 16 days of growth.

SC: Sensitive callus line.

SRC: NaCl resistant callus line.

Table 31. Chloride content (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC	TcRC ⁿ	TcRC ^{tc}
0	0.142	0.169	0.190
8	0.129	0.206	0.210
16	0.110	0.128	0.275
24	0.131	0.187	0.188
32	0.137	0.156	0.195

SC: Sensitive callus line grown on normal medium.
TcRCⁿ: Thioproline resistant callus line grown on normal medium.
TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 32. Chloride content* in sensitive and thioproline resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	SC	TcRC
0	0.110	0.128
0.50	0.174	0.106
0.75	0.219	0.195
1.00	0.209	0.224
1.50	0.198	0.217
2.00	0.214	0.249
2.50	0.242	0.338
3.00	0.327	0.275

* After 16 days of growth.

SC: Sensitive callus line.

TcRC: Thioproline resistant callus line.

Cl⁻ levels in exogenous proline alleviated nonstressed and NaCl/thioprolin stressed sensitive callus cultures

The levels of Cl⁻ decreased in nonstressed and NaCl stressed sensitive callus cultures when exposed to increasing concentrations of exogenous proline. Low levels of Cl⁻ were maintained upto optimum concentration of exogenous proline (33.33 mM) but at higher concentration (50 mM) of exogenous proline, the levels of Cl⁻ increased in the sensitive callus cultures and this increase was further pronounced at still higher concentration (100 mM) of exogenous proline (Table 33). On the other hand, low and high concentrations of exogenous proline did not result in any specific change in the levels of Cl⁻ in thioprolin stressed sensitive callus line (Table 34).

Ca⁺ content

The present data indicated that the levels of Ca⁺⁺ were maintained constant in sensitive, NaCl resistant and thioprolin resistant callus lines grown under stress and nonstress conditions (Tables 35 to 40). Likewise application of exogenous proline in nonstressed, NaCl stressed and thioprolin stressed sensitive callus cultures also did not result in any change in the levels of Ca⁺⁺ (Tables 41 and 42).

Free proline content

Proline levels in NaCl resistant and thioprolin resistant callus lines

The proline levels were low and comparable in sensitive and NaCl resistant callus lines grown on normal medium.

Table 33. Chloride content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing inhibitory concentration of NaCl (300 mM) and exogenously applied proline

Proline (mM)	Culture period (days)							
	8		16		24		32	
	SC	SC ^S	SC	SC ^S	SC	SC ^S	SC	SC ^S
20	0.416	1.720	0.395	1.800	0.468	1.520	0.410	2.100
25	0.347	0.986	0.402	1.102	0.375	0.729	0.328	1.053
33.33	0.225	0.749	0.238	0.856	0.200	0.991	0.177	0.742
50	0.892	1.925	1.210	2.740	0.796	2.438	0.985	2.578
100	1.127	2.374	0.945	2.500	1.150	2.610	1.132	2.413

SC: Sensitive callus line grown on normal medium.

SC^S: Sensitive callus line grown on NaCl (300 mM) supplemented medium.

Table 34. Chloride content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)							
	8		16		24		32	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	0.184	0.122	0.260	0.192	0.150	0.226	0.232	0.216
0.375	0.103	0.137	0.272	0.145	0.133	0.112	0.234	0.209
1.125	0.122	0.110	0.187	0.170	0.101	0.125	0.145	0.136
2.250	0.156	0.125	0.296	0.205	0.166	0.199	0.255	0.191
67.50	0.203	0.148	0.264	0.178	0.182	0.240	0.270	0.200

SC: Sensitive callus line grown on normal medium.

SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

Table 35. Calcium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	Culture period (days)				
	0	8	16	24	32
0	0.152	0.172	0.165	0.179	0.190
25	0.152	0.185	0.162	0.194	0.182
50	0.152	0.160	0.178	0.200	0.196
100	0.152	0.178	0.192	0.185	0.170
150	0.152	0.145	0.184	0.192	0.184
200	0.152	0.190	0.180	0.188	0.177
250	0.152	0.185	0.159	0.195	0.189
300	0.152	0.180	0.170	0.167	0.200

Table 36. Calcium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	Culture period (days)				
	0	8	16	24	32
0	0.156	0.172	0.168	0.180	0.195
0.50	0.156	0.160	0.180	0.182	0.163
0.75	0.156	0.142	0.195	0.155	0.160
1.00	0.156	0.192	0.200	0.190	0.194
1.50	0.156	0.164	0.173	0.175	0.187
2.00	0.156	0.170	0.156	0.175	0.200
2.50	0.156	0.185	0.178	0.188	0.180
3.00	0.156	0.197	0.185	0.190	0.195

Table 37. Calcium content (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC	SRC ⁿ	SRC ^s
0	0.152	0.168	0.177
8	0.172	0.163	0.194
16	0.165	0.173	0.212
24	0.179	0.192	0.190
32	0.190	0.185	0.200

SC: Sensitive callus line grown on normal medium.
 SRCⁿ: NaCl resistant callus line grown on normal medium.
 SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 38. Calcium content* (m mole g⁻¹ dry weight) in sensitive and NaCl resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of NaCl

NaCl (mM)	SC	SRC
0	0.165	0.173
25	0.162	0.182
50	0.178	0.175
100	0.192	0.185
150	0.184	0.190
200	0.180	0.185
250	0.159	0.200
300	0.170	0.209

* After 16 days of growth.

SC: Sensitive callus line.

SRC: NaCl resistant callus line.

Table 39. Calcium content (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC	TcRC ⁿ	TcRC ^{tc}
0	0.156	0.185	0.180
8	0.172	0.160	0.196
16	0.168	0.184	0.192
24	0.180	0.175	0.200
32	0.175	0.178	0.200

SC:	Sensitive callus line grown on normal medium.
TcRC ⁿ :	Thioproline resistant callus line grown on normal medium.
TcRC ^{tc} :	Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 40. Calcium content* (m mole g⁻¹ dry weight) in sensitive and thioproline resistant callus lines of Vigna radiata grown on a medium containing increasing concentrations of thioproline

Thioproline (mM)	SC	TcRC
0	0.168	0.184
0.50	0.180	0.198
0.75	0.195	0.175
1.00	0.200	0.192
1.50	0.173	0.200
2.00	0.156	0.192
2.50	0.178	0.202
3.00	0.185	0.196

* After 16 days of growth.
SC: Sensitive callus line.
TcRC: Thioproline resistant callus line.

Table 42. Calcium content (m mole g⁻¹ dry weight) in sensitive callus culture of Vigna radiata grown on a medium containing inhibitory concentration of thioproline (3.00 mM) and exogenously applied proline

Proline (mM)	Culture period (days)					
	8		16		24	
	SC	SC ^{tc}	SC	SC ^{tc}	SC	SC ^{tc}
0.225	0.165	0.185	0.183	0.170	0.172	0.195
0.375	0.152	0.174	0.185	0.194	0.188	0.190
1.125	0.175	0.190	0.185	0.200	0.186	0.187
2.250	0.184	0.182	0.192	0.197	0.200	0.196
67.50	0.171	0.178	0.180	0.186	0.182	0.194

SC: Sensitive callus line grown on normal medium.

SC^{tc}: Sensitive callus line grown on thioproline (3.00 mM) supplemented medium.

However, NaCl resistant callus line grown on normal medium showed slightly more increase in the levels of proline on 10th day as compared to sensitive callus line also grown on normal medium (Fig. 1a). But NaCl resistant callus line when grown under NaCl (300 mM) stress showed more accumulation of proline as compared to sensitive and NaCl resistant callus lines grown on normal medium (Table 43 and Fig. 1a).

Higher levels of proline were observed in the thioproline resistant callus line when grown either in the presence of thioproline (3.00 mM) or in its absence. Though the overall levels of endogenous proline in thioproline resistant callus line were comparable under thioproline stress and nonstress conditions but it showed slightly more increase in the levels of proline on 10th and 20th day when grown under thioproline stress. Apart from this considerable decrease in the levels of proline was also observed in NaCl resistant and thioproline resistant callus lines on 30th day as compared to 10th/20th or both the days of growth period (Table 43 and Fig. 1a).

Endogenous proline levels in exogenous proline alleviated nonstressed and NaCl/thioproline stressed sensitive callus cultures

A large increase in proline levels was observed in sensitive callus line when grown in the presence of NaCl (300 mM) and exogenous proline (33.33 mM). Sensitive callus line when grown in the presence of only exogenous proline

Table 43. Proline content (μ mole g^{-1} fresh weight) in sensitive, NaCl resistant and thioproline resistant callus lines on Vigna radiata

Culture period (days)	SC			SRC ⁿ			SRC ^s			TcRC ⁿ			TcRC ^{tc}		
	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content
0	2.29	-	4.55	-	4.55	-	4.55	-	12.38	-	12.38	-	12.38	-	-
10	3.58	56.33	8.52	87.25	9.76	114.51	15.07	21.73	18.03	45.64					
20	3.74	63.32	4.65	2.20	14.93	228.13	13.72	10.82	20.46	65.27					
30	2.41	5.24	5.26	15.60	12.48	174.29	9.28	-25.04	10.37	-16.24					

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

TcRCⁿ: Thioproline resistant callus line grown on normal medium.

TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

(33.33 mM), also showed increase in the proline content but the levels were comparatively lower than the sensitive callus line grown under NaCl and exogenous proline. However, in both the cases proline levels were exceptionally high on 10th and 20th day in comparison to 30th day of their growth period (Table 44 and Fig. 1b).

Sensitive callus line when grown in the presence of thioproline (3.00 mM) and exogenous proline (1.125 mM) also showed higher levels of endogenous proline but these levels were comparable to those obtained in the presence of only exogenous proline (1.125 mM or 33.33 mM). In this case too a considerable decrease in the levels of endogenous proline was observed on 30th day in comparison to 10th and 20th day of growth period (Table 44 and Fig. 1b).

Osmotic potential

The osmotic potential of NaCl resistant and thioproline resistant callus lines became more negative on NaCl and thioproline supplemented medium respectively than in normal medium (Table 45). The values were inordinately high on 10th and 20th day as compared to 0th and 30th day of growth period. A similar response was also observed with sensitive callus line grown in the presence of NaCl (300 mM) and exogenous proline (33.33 mM) or thioproline (3.00 mM) and exogenous proline (1.125 mM). The data also indicated that overall

Table 44. Endogenous proline content (μ mole g^{-1} fresh weight) in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioprolin (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioprolin)

Culture period (days)	P_x SC			P_{x+s} SC			P_y SC			P_{y+tc} SC		
	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	Proline content	Proline content	% change over 0th day	% change over 0th day
0	7.20	-	16.59	-	-	9.65	11.30	-	-	-	-	-
10	12.15	68.75	27.24	64.20	4.66	10.10	14.76	30.62				
20	19.87	175.97	22.15	33.51	62.90	15.72	18.97	67.88				
30	9.34	29.72	13.60	-18.02	-35.54	6.22	9.29	-17.79				

P_x SC : Sensitive callus line grown on proline (33.33 mM) containing normal medium.
 P_{x+s} SC : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
 P_y SC : Sensitive callus line grown on proline (1.125 mM) containing normal medium.
 P_{y+tc} SC : Sensitive callus line grown on proline (1.125 mM) and thioprolin (3.00 mM) supplemented medium.

Fig. 1 Free proline content in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

- (a) SC: Sensitive callus line grown on normal medium.
- SRCⁿ: NaCl resistant callus line grown on normal medium.
- SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- TcRCⁿ: Thioproline resistant callus line grown on normal medium.
- TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.
- (b) SC^{p_x}: Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^{p_x+s}: Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^{p_y}: Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^{p_y+tc}: Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

FREE PROLINE CONTENT

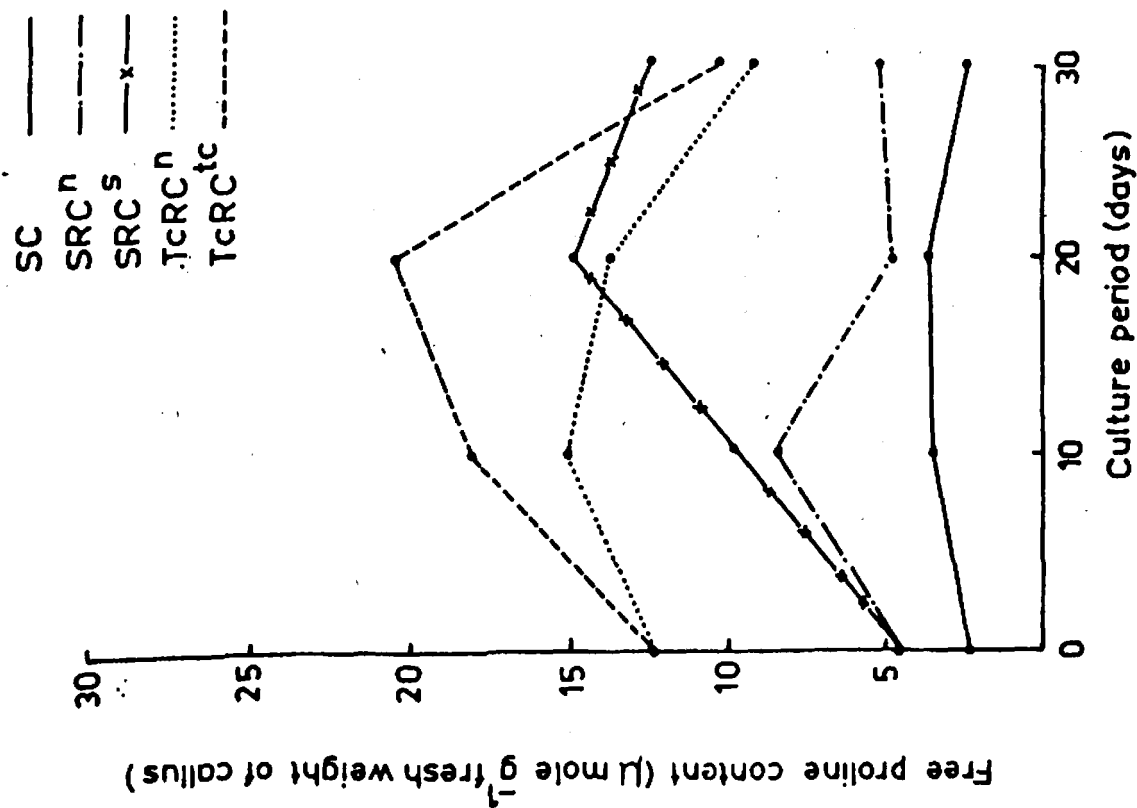


Fig. 1 a.

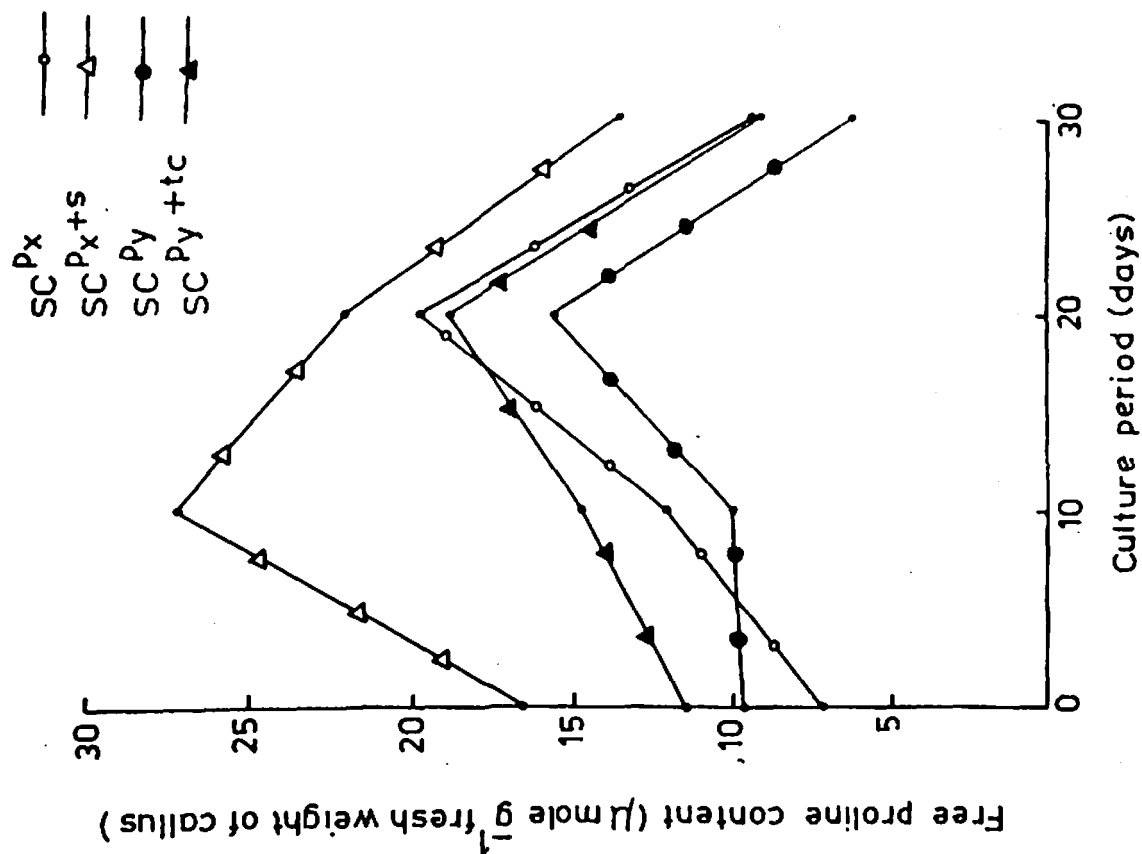


Fig. 1 b.

Table 45. Osmotic potential (-bar) of Vigna radiata callus culture

Culture period (days)	SC	SRC ⁿ	SRC ^s	TcRC ⁿ	TcRC ^{tc}	P _{SC} ^x	P _{SC} ^{x+s}	P _{SC} ^y	P _{SC} ^{y+tc}
0	9.12	7.55	7.55	7.60	7.60	9.40	8.37	7.90	8.78
10	10.23	9.70	15.32	9.67	13.58	8.78	23.7	8.00	14.93
20	9.00	7.10	19.93	12.20	16.59	12.14	26.08	9.72	17.60
30	8.70	8.02	10.57	8.61	8.03	10.45	11.56	6.25	11.72

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

TcRCⁿ: Thioproline resistant callus line grown on normal medium.

TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

P_{SC}^x: Sensitive callus line grown on proline (33.33 mM) containing normal medium.

P_{SC}^{x+s}: Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.

P_{SC}^y: Sensitive callus line grown on proline (1.125 mM) containing normal medium.

P_{SC}^{y+tc}: Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

osmotic potential of sensitive callus line grown on NaCl (300 mM) and proline (33.33 mM) supplemented medium was higher on 10th and 20th day than sensitive callus line grown on thioproline (3.00 mM) and proline (1.125 mM) containing medium or NaCl resistant callus line grown on NaCl (300 mM) supplemented medium or thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium (Table 45).

Total soluble carbohydrate content

Sodium chloride resistant and thioproline resistant callus lines showed more accumulation of soluble carbohydrates under nonstress condition than under NaCl and thioproline stress conditions, respectively. Moreover, the rates of soluble carbohydrate accumulation in these resistant callus lines grown under normal condition were comparable with the sensitive callus line also grown on normal medium (Fig. 2a). On the whole NaCl resistant, thioproline resistant and sensitive callus lines showed more accumulation of soluble carbohydrates on 10th and 20th day as compared to 30th day of growth period (Table 46 and Fig. 2a).

Application of exogenous proline in NaCl/thioproline stressed sensitive callus line did not result in any specific change in the levels of soluble carbohydrates as compared to sensitive callus line grown on proline free normal medium (Table 47 and Fig. 2b).

Table 46. Total soluble carbohydrate content (mg g^{-1} fresh weight) in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC			SRC ⁿ			SRC ^s			TcRC ⁿ			TcRC ^{tc}		
	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount
0	9.48	-	4.88	-	4.88	-	4.88	-	6.09	-	6.09	-	6.09	-	-
10	17.23	81.75	22.65	364.14	10.16	108.197	19.83	225.62	9.47	55.50					
20	15.00	58.23	16.04	228.69	13.23	171.11	12.50	105.25	14.18	132.84					
30	11.06	16.67	10.89	123.16	5.38	10.25	10.70	75.70	8.75	43.68					

SC: Sensitive callus line grown on normal medium.
 SRCⁿ: NaCl resistant callus line grown on normal medium.
 SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
 TcRCⁿ: Thioproline resistant callus line grown on normal medium.
 TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 47. Total soluble carbohydrate content (mg g^{-1} fresh weight) in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioprolone (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioprolone)

Culture period (days)	P _x		P _x + s		P _y		P _y + tc	
	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day	Total amount	% change over 0th day
0	11.98	-	9.85	-	10.25	-	9.34	-
10	12.05	0.58	19.77	100.71	13.47	31.41	15.79	69.06
20	14.39	20.12	12.65	28.43	17.04	66.24	16.92	81.16
30	9.42	-21.37	10.33	4.87	12.10	18.05	7.53	-19.38

P_x SC^x : Sensitive callus line grown on proline (33.33 mM) containing normal medium.

P_x + s SC^x : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.

P_y SC^y : Sensitive callus line grown on proline (1.125 mM) containing normal medium.

P_y + tc SC^y : Sensitive callus line grown on proline (1.125 mM) and thioprolone (3.00 mM) supplemented medium.

Fig. 2

Total soluble carbohydrate content in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

- (a) SC: Sensitive callus line grown on normal medium.
- SRCⁿ: NaCl resistant callus line grown on normal medium.
- SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- TcRCⁿ: Thioproline resistant callus line grown on normal medium.
- TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.
- (b) SC^p_x: Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^p_{x+s}: Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^p_y: Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^p_{y+tc}: Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

TOTAL SOLUBLE CARBOHYDRATE CONTENT

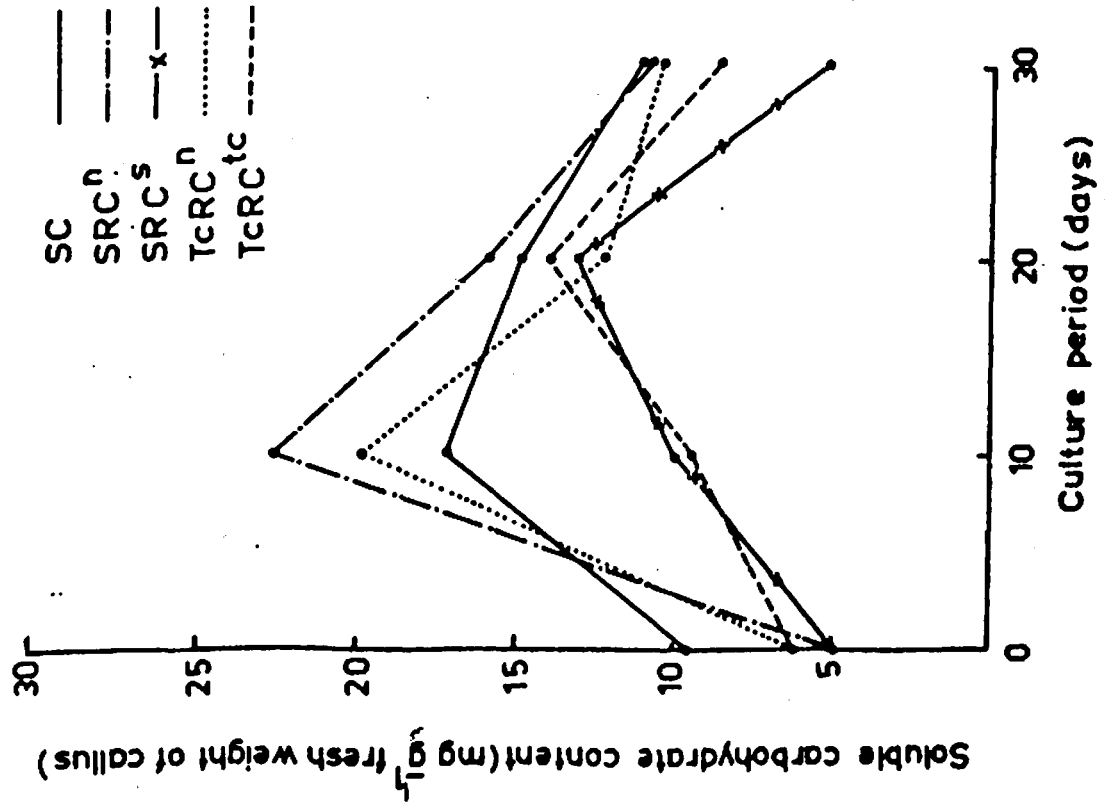


Fig. 2 a.

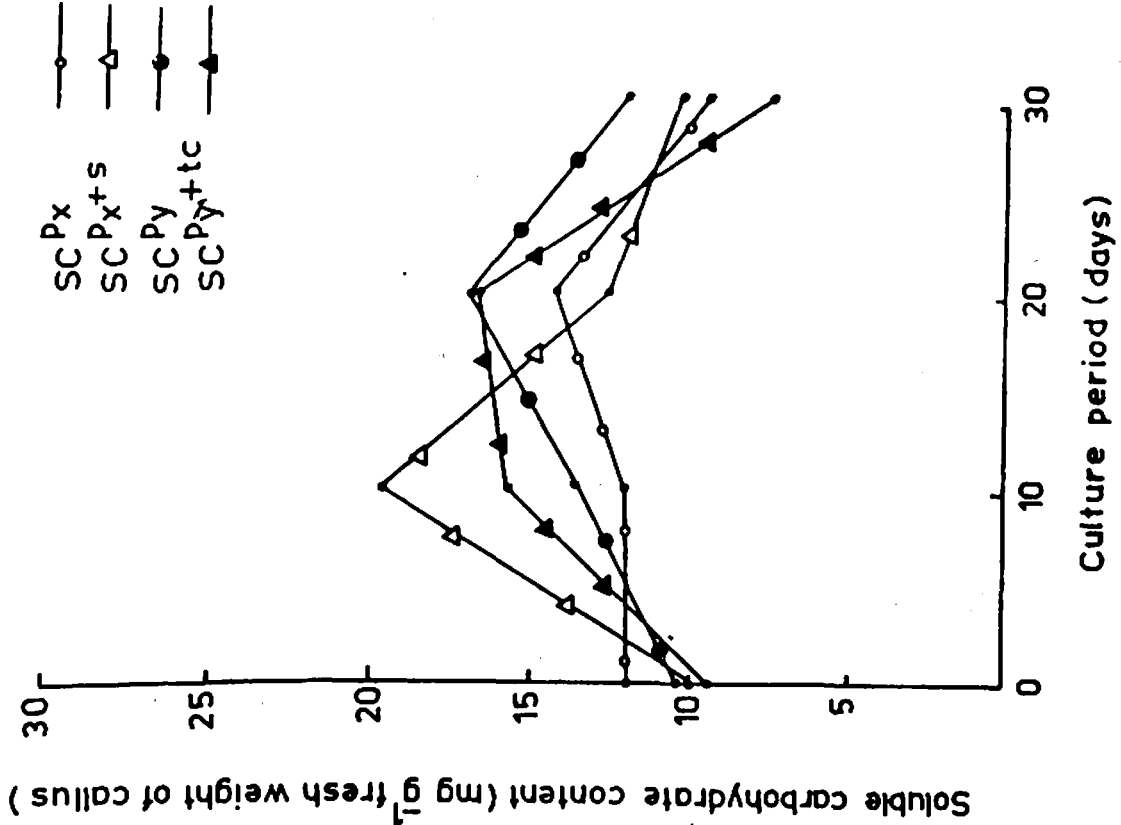


Fig. 2 b.

Total soluble protein content

The sensitive, NaCl resistant and thioproline resistant callus lines showed low but constant levels of soluble proteins when grown on normal medium. However, a marginal increase in the levels of soluble proteins was observed in NaCl resistant and thioproline resistant callus lines grown on NaCl and thioproline supplemented medium, respectively (Table 48 and Fig. 3a). But sensitive callus line grown on NaCl (300 mM) and exogenous proline (33.33 mM) supplemented medium and sensitive callus line grown on thioproline (3.00 mM) and exogenous proline (1.125 mM) containing medium showed appreciable increase in the levels of soluble proteins in comparison to sensitive callus line grown on normal medium but containing 33.33 mM or 1.125 mM concentrations of exogenous proline. However, the soluble protein levels in the latter case were low and comparable with sensitive callus line grown on normal medium (Table 49 and Fig. 3b).

Peroxidase activity

Though the overall pattern showed that peroxidase activity was high in both sensitive as well as in resistant callus lines but increase in peroxidase activity was observed on 10th and 20th day in the NaCl resistant and thioproline resistant callus lines which was more on the normal medium than on stress medium. A similar trend was also observed with their specific activities (Tables 50 and 51, Fig. 4a).

Table 48. Total soluble protein content ($\mu\text{g g}^{-1}$ fresh weight) in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC			SRC ⁿ			SRC ^s			TcRC ⁿ			TcRC ^{tc}		
	Protein content	% change over 0th day	Protein content	Protein content	% change over 0th day	Protein content	Protein content	% change over 0th day	Protein content	Protein content	% change over 0th day	Protein content	Protein content	% change over 0th day	Protein content
0	1467.46	-	1501.12	-	1501.12	-	1501.12	-	1403.89	-	1403.89	-	1403.89	-	-
10	1445.02	-15.29	1415.18	-5.73	1830.21	21.92	1545.99	10.12	1686.24	20.11					
20	1458.00	-0.64	1430.06	-4.73	1979.80	31.89	1642.47	16.99	1925.13	37.13					
30	1438.12	-1.20	1485.02	-1.07	1702.53	13.42	1522.12	8.42	1692.01	20.52					

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

TcRCⁿ: Thioproline resistant callus line grown on normal medium.

TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 49. Total soluble protein content ($\mu\text{g g}^{-1}$ fresh weight) in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioproline (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioproline)

Culture period (days)	P _x			P _x +s			P _y			P _y +tc		
	Protein content	% change over 0th day	SC	Protein content	% change over 0th day	SC	Protein content	% change over 0th day	SC	Protein content	% change over 0th day	SC
0	1440.25	-		1638.70	-		1462.45	-		1831.97	-	
10	1435.68	-0.32		2038.15	24.38		1663.80	13.77		2460.73	34.32	
20	1429.46	-0.75		3050.01	86.12		1585.29	8.40		3218.40	75.68	
30	1441.00	0.05		1905.10	16.26		1494.16	2.17		2026.11	10.60	

P_x SC : Sensitive callus line grown on proline (33.33 mM) containing normal medium.
P_x+s SC : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
P_y SC : Sensitive callus line grown on proline (1.125 mM) containing normal medium.
P_y+tc SC : Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

Fig. 3

Total soluble protein content in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

- (a) SC: Sensitive callus line grown on normal medium.
- SRCⁿ: NaCl resistant callus line grown on normal medium.
- SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- TcRCⁿ: Thioproline resistant callus line grown on normal medium.
- TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.
- (b) SC^{p_x}: Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^{p_x+s}: Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^{p_y}: Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^{p_y+tc}: Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

TOTAL SOLUBLE PROTEIN CONTENT

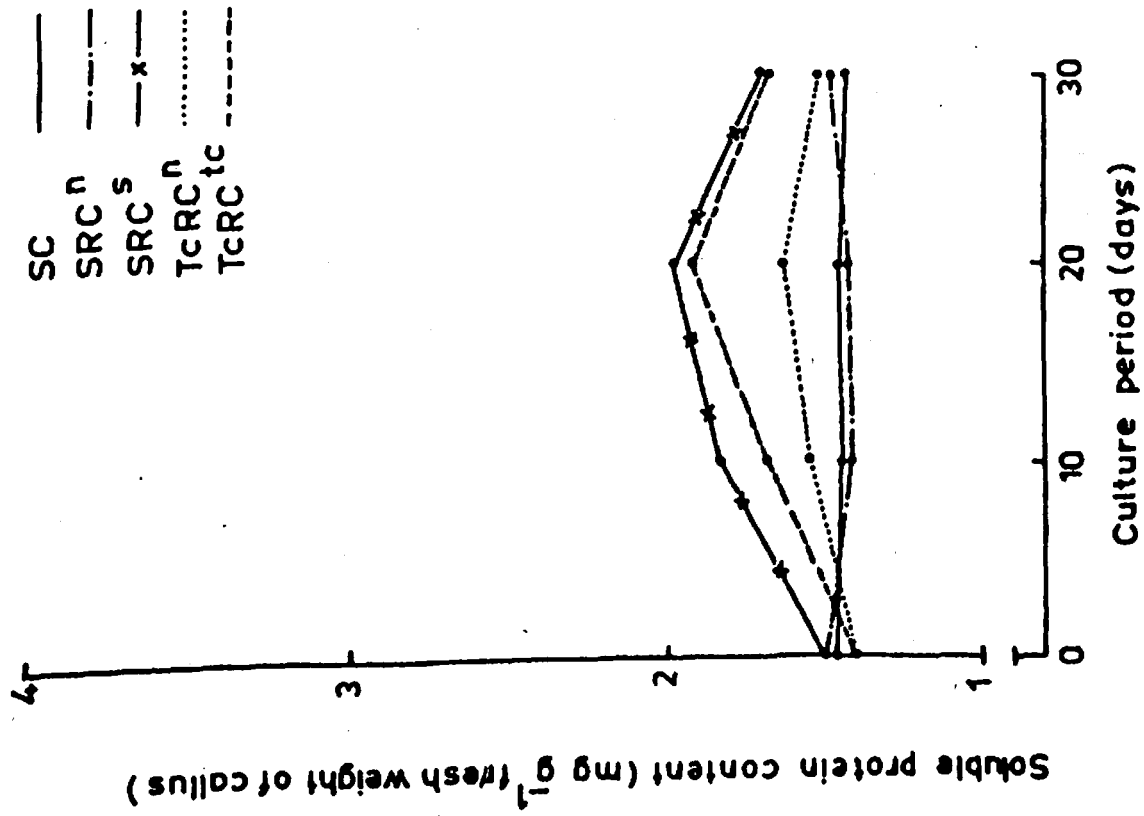


Fig. 3 a.

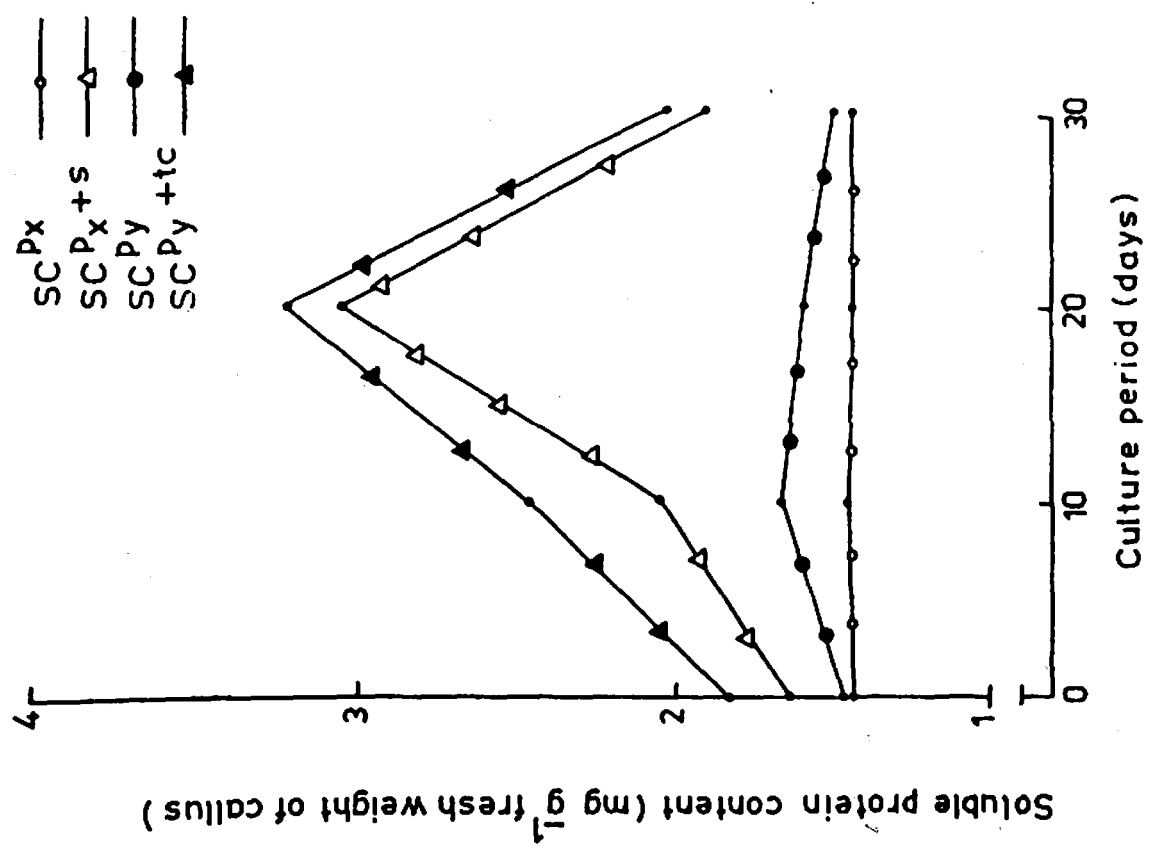


Fig.3 b.

Table 50. Peroxidase activity in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC			SRC ⁿ			SRC ^s		
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity
0	7400	-	5042.73	10400	-	6928.16	10400	-	6928.16
10	9200	24.32	6366.70	19310	85.67	13644.91	16500	58.65	9015.36
20	10321	39.47	7078.88	13800	32.69	9649.94	14000	34.62	7071.42
30	7635	3.18	5309.01	9250	-11.06	6228.87	10270	-1.25	6032.20

0.1 change in optical density per minute = 1 enzyme unit.

* per gram fresh weight of callus.

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 51. Peroxidase activity in sensitive and thiopropine resistant callus lines of Vigna radiata

Culture period (days)	SC			TcRC ⁿ			TcRC ^{tc}		
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity
0	7400	-	5042.73	10000	-	7123.07	10000	-	7123.07
10	9200	24.32	6366.70	30400	204.00	19663.78	21600	116.00	12809.56
20	10321	39.47	7078.88	18940	89.40	11531.41	20073	100.73	10426.83
30	7635	3.18	5309.01	7970	-20.3	5236.12	9285	-7.15	5487.56

0.1 change in optical density per minute = 1 enzyme unit.

* per gram fresh weight of callus.

SC: Sensitive callus line grown on normal medium.

TcRCⁿ: Thiopropine resistant callus line grown on normal medium.

TcRC^{tc}: Thiopropine resistant callus line grown on thiopropine (3.00 mM) supplemented medium.

When sensitive callus line was grown in the presence of inhibitory concentration of NaCl (300 mM) and proline (33.33 mM), peroxidase activity was maintained high throughout the growth period (maximum on 10th day) as compared to sensitive callus line grown on a normal medium containing exogenous proline (33.33 mM). On the other hand, peroxidase activity was comparable in sensitive callus line grown under the inhibitory concentration of thioproline (3.00 mM) and proline (1.125 mM) with the sensitive callus line grown on the normal medium supplemented with 1.125 mM exogenous proline. Apart from this, the specific activities were comparable with their total activity patterns (Table 52 and Fig. 4b).

Malate dehydrogenase activity

Malate dehydrogenase activity in sensitive and NaCl resistant callus lines grown on normal medium was low as compared to NaCl resistant callus line grown on NaCl supplemented medium. The latter showed inordinately high activity on 10th day followed by a decline on 20th day and went on decreasing further (Table 53 and Fig. 5a). Overall pattern of malate dehydrogenase activity in thioproline resistant callus line grown on thioproline supplemented medium was comparable with thioproline resistant and sensitive callus lines grown on normal medium but thioproline resistant callus line grown on thioproline supplemented

Table 52. Peroxidase activity in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioprolin (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioprolin)

Culture period (days)	P _x				P _x +s				P _y				P _y +tc			
	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**
0	6452	-	4479.78	10342	-	6311.10	9750	-	6666.89	9915	-	5412.21				
10	10400	61.19	7243.95	25200	143.67	12364.15	12600	29.23	7573.03	11500	15.99	4673.41				
20	9478	46.90	6630.48	19100	84.68	6262.27	13229	35.68	8344.85	12642	27.50	3928.04				
30	5600	-13.21	3886.19	11873	14.80	6232.22	10080	3.38	6746.27	7628	-23.07	3764.85				

0.1 change in optical density per minute = 1 enzyme unit.

* per gram fresh weight of callus.

** in total activity over 0th day.

P_x: Sensitive callus line grown on proline (33.33 mM) containing normal medium.

P_x+s : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.

P_y: Sensitive callus line grown on proline (1.125 mM) containing normal medium.

P_y+tc : Sensitive callus line grown on proline (1.125 mM) and thioprolin (3.00 mM) supplemented medium.

Fig. 4

Peroxidase activity in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

(a)

- SC: Sensitive callus line grown on normal medium.
- SC^n : NaCl resistant callus line grown on normal medium.
- SC^s : NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- $TcRC^n$: Thioproline resistant callus line grown on normal medium.
- $TcRC^{tc}$: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

(b)

- SC^p_x : Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^{p+x+s} : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^p_y : Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^{p_y+tc} : Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

PEROXIDASE ACTIVITY

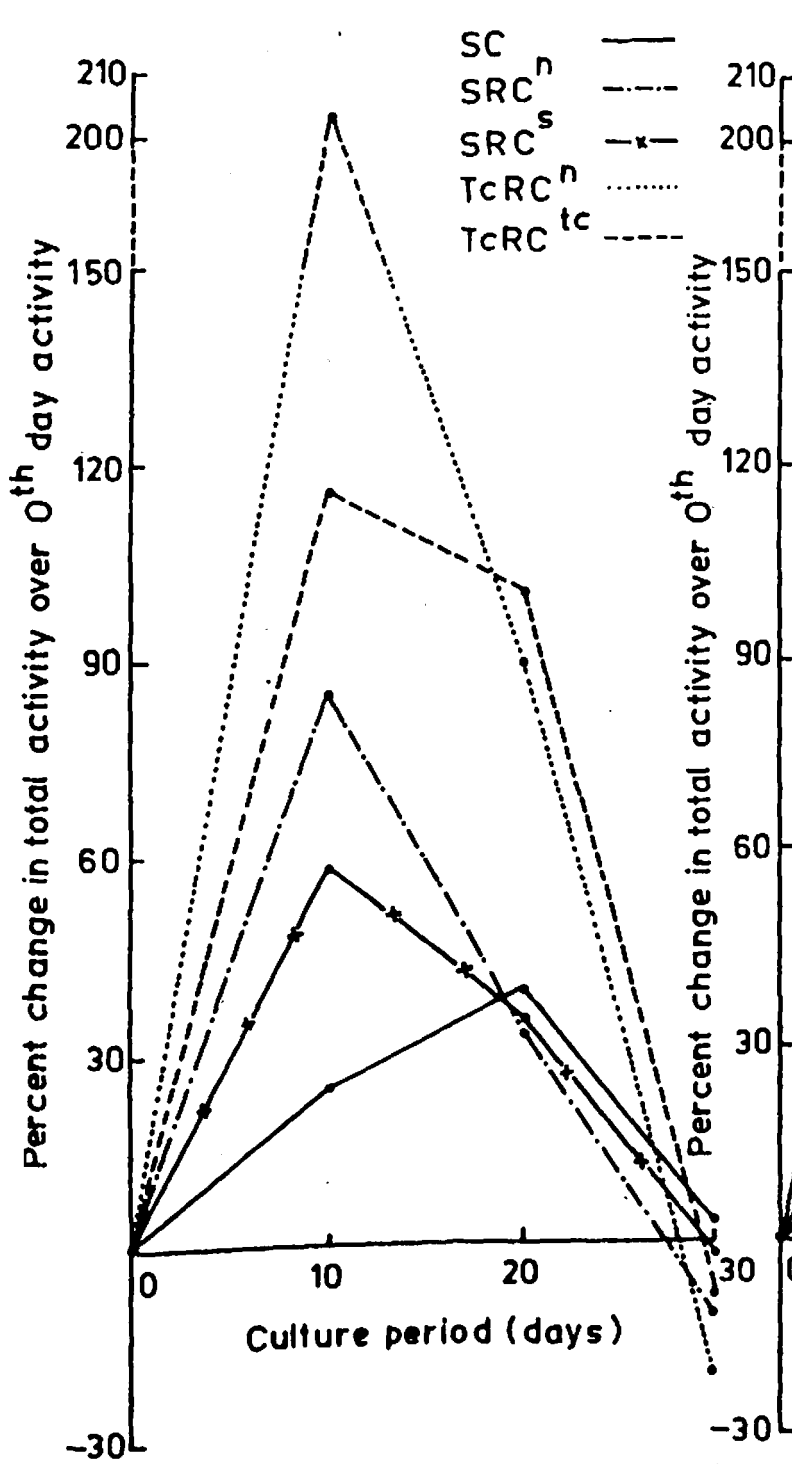


Fig. 4 a.

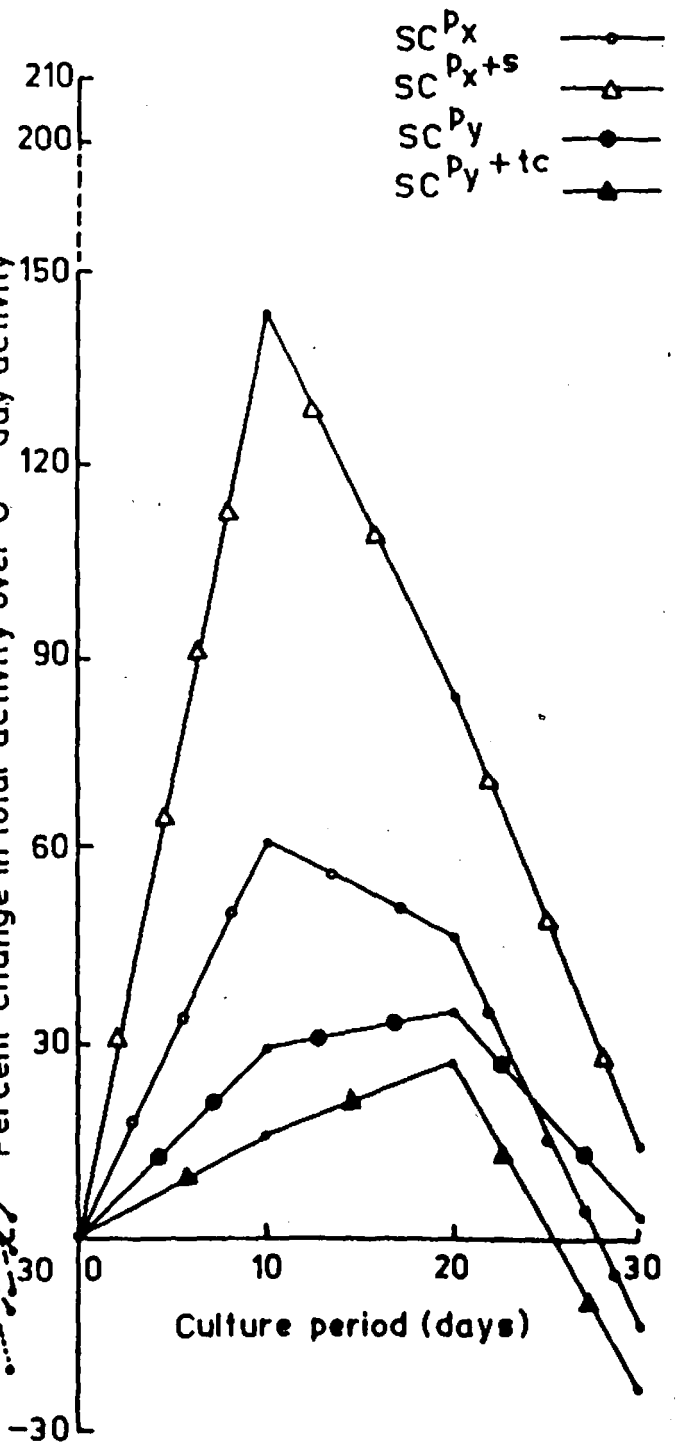


Fig. 4 b.

Table 53. Malate dehydrogenase activity in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC				SRC ⁿ				SRC ^s			
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*
0	440	-	299.84	515	-	343.08	515	-	515	-	343.08	515
10	400	-9.09	276.81	740	43.69	522.90	1449	181.36	1449	181.36	791.71	1449
20	510	15.91	349.79	480	-6.80	335.65	963	87.00	963	87.00	486.41	963
30	426	-3.18	296.22	422	-18.06	284.17	657	27.57	657	27.57	385.90	657

0.1 change in optical density per minute = 1 enzyme unit.

* per gram fresh weight of callus.

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

medium showed slightly more activity on 10th day followed by decline on 20th day (Table 54 and Fig. 5a).

Malate dehydrogenase activity in sensitive callus line grown on normal medium but supplemented with exogenous proline (33.33 mM) was low and comparable (except on 10th day) with the sensitive callus line grown on NaCl (300 mM) and exogenous proline (33.33 mM) supplemented medium (Table 55 and Fig. 5b). On the other hand, malate dehydrogenase activity in sensitive callus line grown on thioproline (3.00 mM) and proline (1.125 mM) supplemented medium was high but comparable with sensitive callus line grown on proline (1.125 mM) containing normal medium. Moreover, in this case the activity was very high on 10th day followed by a decline (Table 55 and Fig. 5b).

Moreover, overall specific activities of malate dehydrogenase in the sensitive and resistant callus lines grown under stress and nonstress conditions, were comparable with their total activity patterns (Tables 54 and 55, Figs. 5a and 5b).

Catalase activity

The present data indicated that catalase activity was low and comparable in sensitive, NaCl resistant and thioproline resistant callus lines grown on normal medium but NaCl resistant callus line grown on NaCl supplemented medium and thioproline resistant callus line grown on thioproline

Table 54. Malate dehydrogenase activity in sensitive and thioproline resistant callus lines of Vigna radiata

Culture period (days)	SC			TcRC ⁿ			TcRC ^{tc}		
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity
0	440	-	299.84	560	-	398.89	560	-	398.89
10	400	-9.09	276.81	748	33.57	483.83	930	66.07	551.52
20	510	15.91	349.79	672	20.00	409.14	685	22.32	355.82
30	426	-3.18	296.22	531	-5.18	348.86	450	-19.64	265.96

0.1 change in optical density per minute = 1 enzyme unit.

* per gram fresh weight of callus.

SC: Sensitive callus line grown on normal medium.

TcRCⁿ: Thioproline resistant callus line grown on normal medium.

TcRC^{tc}: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

Table 55. Malate dehydrogenase activity in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioproline (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioproline)

Culture period (days)	P _x				P _x +s				P _y				P _y +tc			
	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*
0	534	-	370.77	578	-	352.72	621	-	424.63	595	-	324.79				
10	724	35.58	504.29	919	59.00	450.90	1080	73.91	649.12	1145	92.44	465.31				
20	688	28.84	481.30	735	27.16	240.98	796	28.18	502.12	933	56.81	289.90				
30	502	-5.99	348.37	614	6.23	322.29	570	-8.21	381.49	628	5.55	309.95				

0.1 change in optical density per minute = 1 enzyme unit.
 * per gram fresh weight of callus.
 ** in total activity over 0th day.

P_x: Sensitive callus line grown on proline (33.33 mM) containing normal medium.

P_x+s: Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.

P_y: Sensitive callus line grown on proline (1.125 mM) containing normal medium.

P_y+tc: Sensitive callus line grown on proline (1.125 mM and thioproline (3.00 mM) supplemented medium.

Fig. 5 Malate dehydrogenase activity in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

(a)

- SC: Sensitive callus line grown on normal medium.
- SC^n : NaCl resistant callus line grown on normal medium.
- SC^s : NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- $TcRC^n$: Thioproline resistant callus line grown on normal medium.
- $TcRC^{tc}$: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

(b)

- SC^p_x : Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^{p_x+s} : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^p_y : Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^{p_y+tc} : Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

MALATE DEHYDROGENASE ACTIVITY

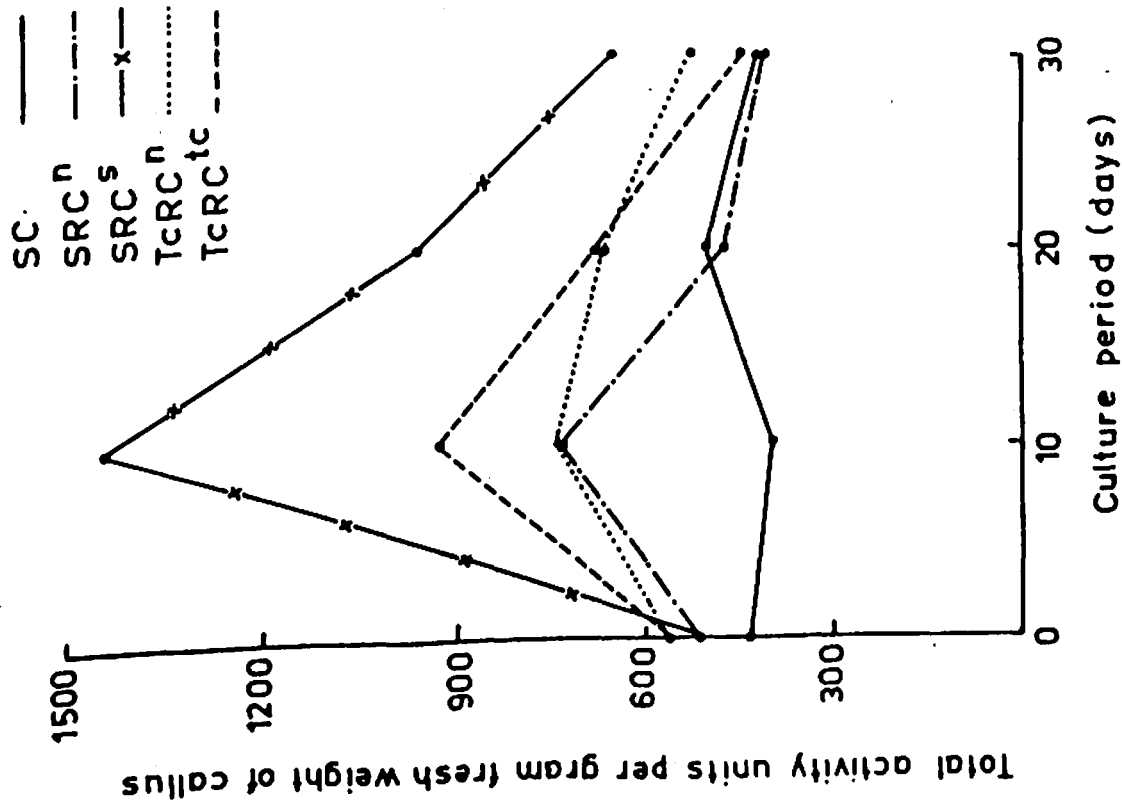


Fig. 5a.

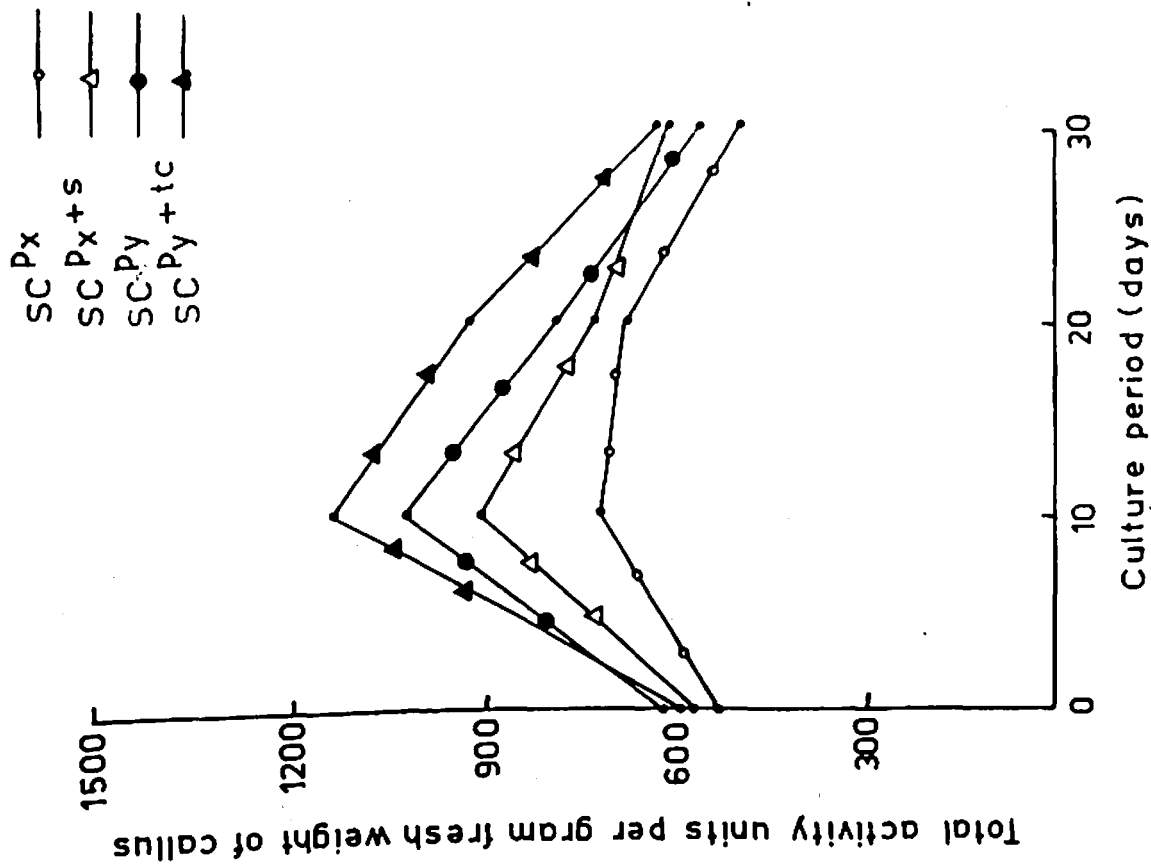


Fig. 5b.

Table 56. Catalase activity in sensitive and NaCl resistant callus lines of Vigna radiata

Culture period (days)	SC			SRC ⁿ			SRC ^s		
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity
0	85.32	-	58.14	91.75	-	61.12	91.75	-	61.12
10	95.68	12.14	66.21	90.50	-1.36	63.95	150.53	64.07	82.25
20	90.00	5.49	61.73	94.20	2.67	65.87	138.46	50.91	69.94
30	78.25	-8.29	54.41	87.00	-5.18	58.59	105.62	15.12	62.04

μ mole of H₂O₂ consumed per minute = 1 enzyme unit.

* per gram fresh weight of callus.

SC: Sensitive callus line grown on normal medium.

SRCⁿ: NaCl resistant callus line grown on normal medium.

SRC^s: NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.

Table 57. Catalase activity in sensitive and thiopropine resistant callus lines of Vigna radiata

Culture period (days)	SC			TcRC ⁿ			TcRC ^{tc}		
	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity	Total activity units*	% change in total activity over 0th day	Specific activity
0	85.32	-	58.14	106.10	-	75.58	106.10	-	75.58
10	95.68	12.14	66.21	117.00	10.27	75.68	142.72	34.51	84.64
20	90.00	5.49	61.73	124.83	17.65	76.00	178.66	68.39	92.80
30	78.25	-8.29	54.41	111.98	5.54	73.57	98.00	-7.63	57.92

μ mole of H₂O₂ consumed per minute = 1 enzyme unit.
 * per gram fresh weight of callus

SC: Sensitive callus line grown on normal medium.

TcRCⁿ: Thiopropine resistant callus line grown on normal medium.

TcRC^{tc}: Thiopropine resistant callus line grown on thiopropine (3.00 mM) supplemented medium.

Table 58. Catalase activity in sensitive callus culture of Vigna radiata grown on inhibitory concentration of NaCl (300 mM)/thioprolin (3.00 mM) and exogenously applied proline (33.33 mM with NaCl and 1.125 mM with thioprolin)

Culture period (days)	P _x			P _x + s			P _y			P _y + tc		
	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity	Total acti- vity units*	% change**	Speci- fic acti- vity
0	97.00	-	63.35	103.67	-	63.26	92.58	-	63.30	119.13	-	65.03
10	114.00	17.53	79.40	152.87	47.46	75.00	102.45	10.66	61.58	147.92	24.17	60.11
20	109.30	12.68	76.46	161.00	55.30	52.79	118.74	28.26	74.90	170.63	43.23	53.01
30	86.00-11.34		59.68	95.13	-8.24	49.93	100.00	8.01	66.93	112.27	-5.76	55.41

μ mole of H₂O₂ consumed per minute = 1 enzyme unit.

* per gram fresh weight of callus.

** in total activity over 0th day

SC^{P_x} : Sensitive callus line grown on proline (33.33 mM) containing normal medium.

P_x + s

SC^{P_x} : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.

P_y

SC^{P_y} : Sensitive callus line grown on proline (1.125 mM) containing normal medium.

P_y + tc

SC^{P_y + tc} : Sensitive callus line grown on proline (1.125 mM) and thioprolin (3.00 mM) supplemented medium.

Fig. 6

Catalase activity in sensitive, NaCl resistant and thioproline resistant callus lines of Vigna radiata cv. K851.

(a)

- SC: Sensitive callus line grown on normal medium.
- SC^n : NaCl resistant callus line grown on normal medium.
- SC^s : NaCl resistant callus line grown on NaCl (300 mM) supplemented medium.
- $TcRC^n$: Thioproline resistant callus line grown on normal medium.
- $TcRC^{tc}$: Thioproline resistant callus line grown on thioproline (3.00 mM) supplemented medium.

(b)

- SC^{p_x} : Sensitive callus line grown on proline (33.33 mM) containing normal medium.
- SC^{p_x+s} : Sensitive callus line grown on proline (33.33 mM) and NaCl (300 mM) supplemented medium.
- SC^{p_y} : Sensitive callus line grown on proline (1.125 mM) containing normal medium.
- SC^{p_y+tc} : Sensitive callus line grown on proline (1.125 mM) and thioproline (3.00 mM) supplemented medium.

CATALASE ACTIVITY

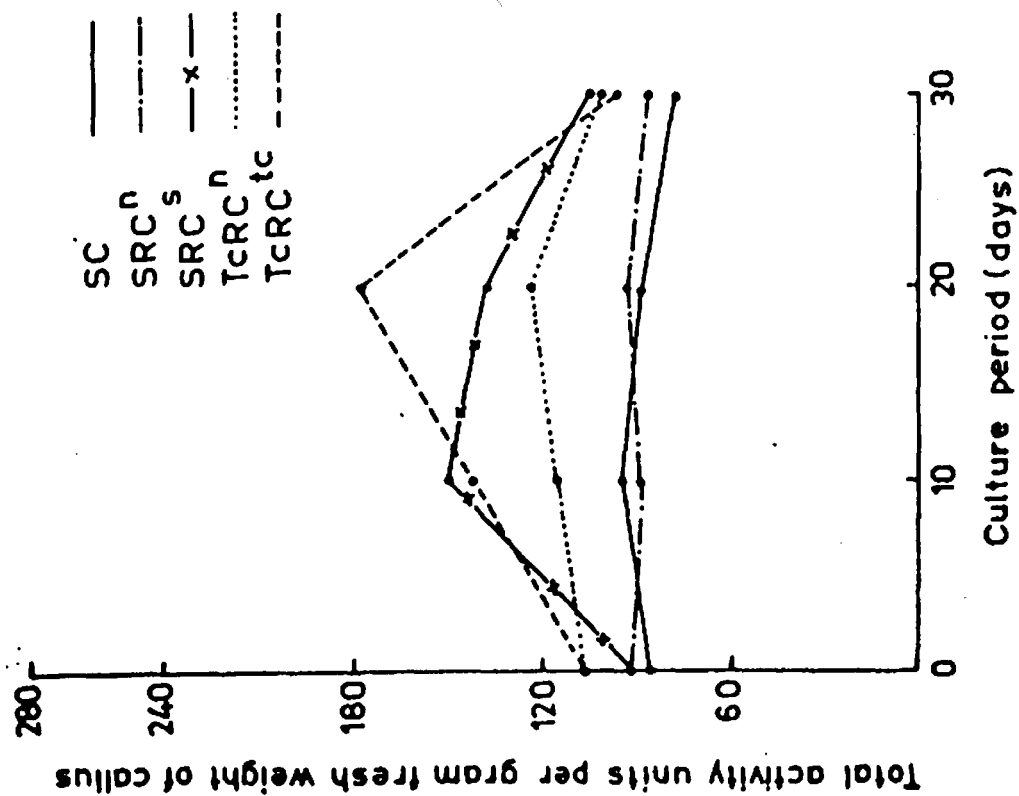


Fig. 6 a.

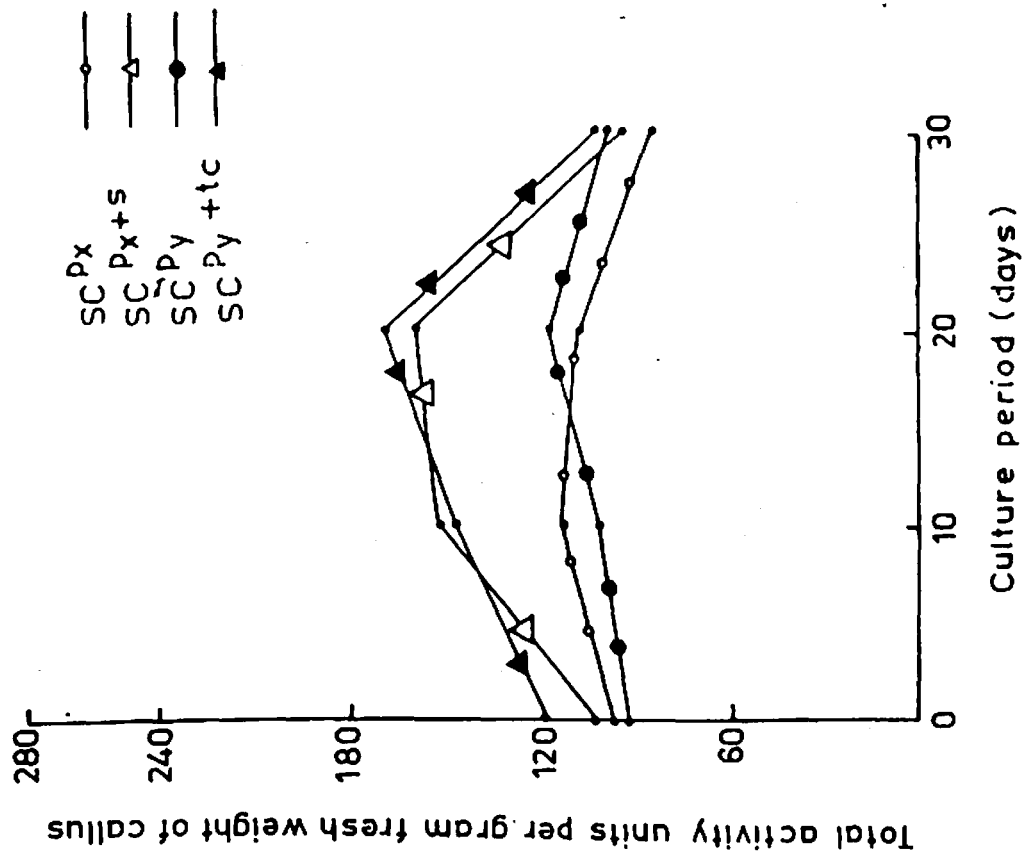


Fig. 6 b.

supplemented medium showed marginal increase in their activities. A similar pattern was also observed in their specific activities (Tables 56 and 57, Fig. 6a).

Sensitive callus line grown under inhibitory concentration of NaCl (300 mM) and exogenous proline (33.33 mM) showed marginal increase in catalase activity on 10th and 20th day especially as compared to sensitive callus line grown on proline (33.33 mM) containing normal medium. A similar trend was also seen upon comparing sensitive callus line grown on thioproline (3.00 mM) and proline (1.125 mM) supplemented medium with sensitive callus line grown on proline (1.125 mM) containing normal medium. The specific activity pattern coincided with the total activity pattern in this case too (Table 58, Fig. 6b).

DISCUSSION

Growth studies

Growth characteristics of NaCl/thioproline stressed sensitive callus line

The callus growth declined gradually with the increase in concentration of NaCl in the culture medium. There was almost complete inhibition in callus growth at 300 mM NaCl concentration and was comparable with the inhibition produced at 350 mM NaCl concentration (Tables 1 and 2). Nabors et al. (1975) in tobacco, Croughan et al. (1978) in alfalfa and Tal (1983) in tomato have reported similar growth inhibition kinetics upon exposing the cultured cells to increasing levels of sodium chloride.

At low concentrations of NaCl (25-50 mM), callus growth was stimulated in terms fresh as well as dry weight (Tables 1 and 2). Pandey and Ganapathy (1984a) in Cicer arietinum and Gosal and Bajaj (1984) in Pisum sativum too reported stimulation in callus growth at low concentrations of NaCl. From these observations, it appears that the osmotic strength of the medium was lower than the actual requirement of cells and thus by making low levels of NaCl available in the medium, the growth of cells got stimulated. Huq and Larher (1983) have also reported that stimulation in the growth of Phaseolus plants at low concentrations of NaCl may be perhaps due to osmoregulatory role of Na⁺.

Likewise, the growth of sensitive callus line was

inhibited gradually upon increasing the levels of thioproline in the medium and inhibitions produced at high concentrations of thioproline were comparable (Tables 3 and 4). It seems that thioproline incorporation takes place in the growing protein chains thereby causing cell growth inhibitions as has also been reported in hydroxyproline stressed sycamore cells (Holleman, 1967). But other possibilities of cell growth inhibition by the toxic levels of thioproline can not also be ruled out. In this case too, low concentrations (0.50-0.75 mM) of thioproline were responsible for the stimulation in callus growth (Tables 3 and 4). This may be due to the ability of nontoxic concentrations of thioproline to provide a favourable osmotic strength and to act as source of reduced nitrogen and carbon to the growing cells.

The dry matter accumulation of NaCl/thioproline stressed sensitive callus line was comparatively more than the fresh weight production. This could be due to the inability of some of the cells to retain water in presence of increasing levels of NaCl/thioproline stress and these cells, tended to accumulate high levels of NaCl/thioproline and eventually died. The accumulation of high levels of NaCl perhaps contributed towards the increase in the dry matter. The observations also reveal that the overall water content of NaCl/thioproline stressed sensitive callus line was low and accumulation of substances was high.

Growth behaviour of NaCl resistant and thioproline resistant callus lines

The growth rates of NaCl resistant and thioproline resistant callus lines grown under NaCl (300 mM) and thioproline (3.00 mM) stress respectively, were comparable with the sensitive callus line grown on normal medium. But under nonstress condition, NaCl resistant and thioproline resistant callus lines grew faster than the sensitive callus line also grown on normal medium (Tables 5 and 6). The overall growth pattern also showed that accumulation of dry matter in resistant callus lines was comparatively higher than the fresh weight production when grown on normal and NaCl/thioproline supplemented media.

These observations show that growth and metabolic activity of NaCl resistant and thioproline resistant callus lines grown under stress conditions was higher than the sensitive callus line and became still higher when grown on normal medium, indicating thereby that stable change was induced in the stress selected callus lines. Similar changes in growth pattern of stress selected cell lines in other cases have also been reported (Dix and Street, 1975; Nabors et al., 1975; Maliga, 1980, 1984).

While a halophytic mode of resistance to NaCl is presumed to have been induced in the salt selected callus line, thioproline perhaps caused feedback inhibition of proline biosynthesis. The cells that survived might have an

altered proline enzyme system that might also have enhanced tolerance through cellular adaptation to osmotic stress (Widholm, 1972a, 1972b, 1976; Croughan et al., 1978).

Alleviation of growth inhibition by exogenous proline

The growth inhibition of NaCl (300 mM) stressed sensitive callus line was alleviated upon exposing to increasing levels of exogenous proline. Maximum alleviation in callus growth inhibition was observed at 33.33 mM of exogenous proline concentration but higher concentrations proved inhibitory for growth (Tables 7 and 8). Thus in the present case, 33.33 mM was regarded as the optimum concentration of exogenous proline required to alleviate the growth inhibition of NaCl stressed sensitive callus line and concentrations higher than this were termed as supraoptimal concentrations.

When thioproline (3.00 mM) stressed sensitive callus line was exposed to the concentrations of exogenous proline used for protecting NaCl stressed sensitive callus line, no alleviation or stimulation in callus growth was observed. But upon using very low range of exogenous proline concentration, thioproline stressed sensitive callus line too showed alleviation of its growth inhibition (Tables 9 and 10). Further, alleviation at 1.125 mM and 2.250 mM exogenous proline concentration were highest and comparable. These were in turn regarded as optimum concentrations of exogenous proline

required to bring about the normal growth of thioproline stressed sensitive callus line.

These observations are in correspondence with those of Pandey and Ganapathy (1985) and Handa et al. (1986) who also reported stimulatory effects of exogenous proline in sodium chloride stressed Cicer arietinum callus cultures and polyethyleneglycol stressed cells of Lycopersicon esculentum, respectively. Thus, these results reveal that proline acts as a compatible osmotic solute or a stabilizing factor for proteins under limiting cell water conditions as suggested by Schobert and Tschesche (1978) or as a source of reduced nitrogen and carbon (Aspinall and Paleg, 1981). In the present study, it is also indicated that there may be some sort of interaction between exogenous proline and thioproline because concentrations of exogenous proline which stimulated the growth of NaCl stressed cells were found to be inhibitory for thioproline stressed cells. Further, low concentrations of exogenous proline may not be significant enough for such an interaction to occur, hence, their growth got stimulated. From the results, it appears that exogenous proline enhanced NaCl tolerance of the sensitive callus line and also alleviated the growth inhibition of nonstressed cells at low concentrations but were found to be inhibitory at higher concentrations. Since the endogenous levels of proline were low in the nonstressed sensitive callus line

(Table 43), application of low concentrations of exogenous proline resulted in rapid uptake of proline from the medium (Table 44), thereby stimulating the growth of sensitive callus line. But very high concentrations of exogenous proline may be responsible for increase in osmotic strength of the medium along with causing other changes not yet understood and thereby affected the uptake of essential nutrients due to change in membrane permeability and, consequently, resulted in retarded growth of sensitive callus line grown under normal and stress conditions.

K⁺ content in sensitive, NaCl resistant and thioproline resistant callus lines

As the levels of NaCl/thioproline increased in the medium, K⁺ content in sensitive callus line exhibited a decreasing trend. Moreover, a considerable loss in the K⁺ levels of sensitive callus line was observed during first 8 days of growth period when grown under stress or nonstress conditions. However, uptake of K⁺ picked up with further increase in the growth period (Tables 11 and 12). Watad et al. (1983) too reported that wild type Nicotiana tabacum cells lost a considerable part of their K⁺ and extent of loss increased with rise in external NaCl concentration. Pandey and Ganapathy (1984a) too observed that K⁺ content decreased with increasing salinization in callus cultures of Cicer arietinum.

Sodium chloride resistant and thioproline resistant callus lines maintained higher levels of K^+ than sensitive callus line when grown under increasing levels of stress (Tables 14 and 16) but the levels of K^+ were low when compared with sensitive or resistant callus lines grown on normal medium (Tables 13 and 15). Croughan et al. (1978) reported that as the concentration of NaCl in the nutrient medium increased, K^+ levels in salt selected and nonselected cell lines of alfalfa decreased but depression was more pronounced in the nonselected cell line. Similarly these results also correspond with those of Watad et al. (1983) who found that NaCl resistant cell line of Nicotiana tabacum, by contrast lost no K^+ upto 50 mM external NaCl concentration while at higher concentration the loss was less pronounced than in the wild type cell line.

Exogenous proline and the levels of K^+ in the nonstressed and NaCl/thioprolin stressed sensitive callus cultures

As the levels of exogenous proline increased in the medium, there was concomitant increase in the levels of K^+ in NaCl/thioprolin stressed as well as nonstressed callus cultures. But at higher concentrations of exogenous proline, the levels of K^+ decreased suddenly and continued to decline further as the concentrations of proline were increased in the medium (Tables 17 and 18). Thus stressed and nonstressed callus cultures maintained higher levels of K^+ upto optimum concentrations of exogenous proline whereas supraoptimal

concentrations of exogenous proline resulted in the loss of K^+ .

Na^+ and Cl^- contents in sensitive, NaCl resistant and thioproline resistant callus lines

The sensitive and NaCl resistant callus lines exhibited increase in Na^+ and Cl^- contents with the increase in the levels of NaCl in the medium. But NaCl resistant callus line maintained higher levels of Na^+ and Cl^- than sensitive callus line when subjected to same levels of salinity (Tables 22 and 30). Further, the levels of Na^+ and Cl^- in NaCl resistant callus line grown on normal medium were low and comparable with the sensitive callus line also grown under nonstress condition (Tables 21 and 29). Croughan et al. (1978) also observed that intracellular concentration of Na^+ in NaCl selected and nonselected cell lines of alfalfa increased with the increase in NaCl concentration in the nutrient medium without showing any significant difference in the pattern of their response. However, Cl^- content which also increased in response to external salinity was maintained at higher levels in salt selected cell line than in nonselected cell line. Likewise Watad et al. (1983) too reported increasing trends of Na^+ content in NaCl selected and nonselected cell lines of Nicotiana tabacum upon exposing them to increasing levels of salinity. Further, Na^+ levels were maintained higher in NaCl selected cell line than nonselected one. Pandey and Ganapathy (1984a) too observed that concentrations of Na^+ and Cl^- in callus cultures of Cicer arietinum increased

with increasing salinity of the medium.

Contrary to the above findings, thioproline resistant and sensitive callus lines did not show any specific change in the levels of either Na^+ or Cl^- when grown under increasing levels of thioproline stress (Tables 24 and 32). Their Na^+ and Cl^- levels corresponded to those of sensitive or NaCl resistant callus lines grown under nonstress condition (Tables 21, 23, 29 and 31).

Exogenous proline and the levels of Na^+ and Cl^- in nonstressed and NaCl/thioproline stressed sensitive callus cultures

The levels of Na^+ and Cl^- decreased in the NaCl stressed and nonstressed sensitive callus line upon increasing the levels of exogenous proline in the medium. But at supraoptimal concentrations of exogenous proline, levels of Na^+ and Cl^- suddenly increased (Tables 25 and 33). However, the application of exogenous proline did not result in any alteration in the levels of either Na^+ or Cl^- in thioproline stressed sensitive callus line (Tables 26 and 34). In this case levels of Na^+ and Cl^- were comparable with those of sensitive callus line grown under nonstress condition without proline. From these results, it appears that at low levels, exogenous proline maintains a favourable osmotic strength of the medium and allows rapid growth of the cells even in presence of NaCl. As a consequence of selective uptake, Na^+ and Cl^- levels are kept fairly low and nontoxic to the cells. However, at higher concentrations, proline itself appears to

be toxic and resulted in impaired growth. Presumably some of the cells were killed and their membranes became nonselective for NaCl uptake. It is substantiated from the fact that at high concentrations of proline there was sudden increase in the levels of Na^+ and Cl^- in the callus cultures.

Calcium content

The sensitive, NaCl resistant and thioproline resistant callus lines maintained constant levels of calcium when grown under stress and nonstress conditions (Tables 35 to 40). Similarly application of exogenous proline was unable to change the intracellular levels of Ca^{++} either in NaCl/thioproline stressed or nonstressed sensitive callus cultures (Tables 41 and 42).

Pandey and Ganapathy (1984a) too reported that levels of Ca^{++} were maintained appreciably in Cicer arietinum callus cultures at increasing levels of salinity. Epstein (1961) has also suggested that maintenance of satisfactory levels of Ca^{++} in the presence of high levels of NaCl can be identified with salinity resistance.

Endogenous proline and osmotic potential

Sodium chloride resistant callus line exhibited higher accumulation of free proline when grown under NaCl stress but the levels of proline remained low and comparable with sensitive callus line when grown on normal medium (Table 43 and Fig. 1a). The findings of Dix and Pearce (1981),

Wataad et al. (1983) and Pandey and Ganapathy (1985) are substantiated by these observations.

On the other hand, thioproline resistant callus line maintained higher levels of proline than NaCl resistant callus line when grown under thioproline stress or in its absence (Table 43 and Fig. 1a). The increased levels of proline in thioproline resistant callus line indicate that the feedback control in the biosynthetic pathway in question might be altered as has been reported for tryptophan, lysine, methionine and proline analogue resistance and oversynthesis (Widholm, 1972a, 1972b; Widholm, 1976).

The sensitive callus line when grown in the presence of NaCl (300 mM) and exogenous proline (33.33 mM) accumulated higher levels of endogenous proline than the sensitive callus line grown on proline (33.33 mM) containing normal medium indicating thereby that sensitive callus line when grown on NaCl and proline supplemented medium showed uptake of exogenous proline and/or synthesis of proline in the cells (Table 44 and Fig. 1b). But when sensitive callus line was grown on a medium containing thioproline (3.00 mM) and exogenous proline (1.125 mM), levels of endogenous proline were slightly higher though comparable with the sensitive callus line grown on proline (1.125 mM) containing normal medium (Table 44 and Fig. 1b). This indicated that sensitive callus line grown under thioproline stress and exogenous proline exhibited mainly

uptake of exogenous proline with little endogenous synthesis if at all.

The osmotic potential became more negative in NaCl resistant and thioproline resistant callus lines when grown under stress conditions than in the absence of stress. Similarly sensitive callus line grown in presence of inhibitory concentration of NaCl (300 mM)/thioproline (3.00 mM) and exogenous proline (33.33 mM with NaCl and 1.125 mM with thioproline) also showed similar trend, however, values in this case were more negative (Table 45). These findings when compared with the levels of endogenous proline under stress conditions reveal a positive correlation of high proline accumulation with the more negative osmotic potential. Similar observations have been made by Handa et al. (1986), who attempted to correlate osmotic potential, turgor potential with endogenous levels of proline in cultured tomato cells.

Soluble carbohydrates

Thioproline resistant and sodium chloride resistant callus lines accumulated low levels of soluble carbohydrates when grown under thioproline and NaCl stress conditions, respectively. But on normal medium, these resistant callus lines exhibited higher rates of soluble carbohydrate accumulation which were in turn comparable with the sensitive callus line also grown under nonstress condition (Table 46 and Fig. 2a). However, the soluble carbohydrate content

remained almost unchanged when sensitive callus line was grown in the presence of inhibitory concentration of NaCl (300 mM)/thioprolin (3.00 mM) and optimum concentrations of exogenous proline i.e. 33.33 mM with NaCl and 1.125 mM with thioprolin (Table 47 and Fig. 2b).

From these observations, it appears that NaCl resistant and thioprolin resistant callus lines when grown under stress conditions showed more consumption of soluble carbohydrates indicating thereby that stress tolerance of these resistant callus lines was at the cost of higher expenditure of metabolic energy or due to efficient conversion of energy sources to growth and development in stress environment (Croughan et al., 1981). Stavarek and Rains (1984a) too reported that NaCl selected cell line of alfalfa was found to have a greater energy use efficiency and to maintain higher levels of respiration than the nonselected line when these lines were exposed to salinity.

Soluble proteins and enzyme activities

The levels of soluble proteins were low and comparable in NaCl resistant, thioprolin resistant and sensitive callus lines when grown on normal medium, however, a marginal increase was observed in the resistant callus lines when grown under stress conditions (Table 48 and Fig. 3a). There was an appreciable increase in the levels of soluble proteins in sensitive callus line grown on NaCl (300 mM)/thioprolin (3.00 mM) and proline (33.33 mM with NaCl and 1.125 mM with

thioprolin) containing media (Table 49 and Fig. 3b).

These findings reveal that under stress conditions the suppressed genes are perhaps switched on which is reflected in terms of increase in the levels of soluble proteins. These newly synthesized proteins may be involved in antistress activity. Similarly, large increase in the levels of soluble proteins of the sensitive callus line grown in presence of inhibitory concentration of NaCl/thioprolin and optimum concentrations of exogenous proline indicated that additional genes became active whose products were, in turn, utilized in the transport of exogenous proline into the cell cytoplasm. However, other possibilities of such a large increase in soluble proteins cannot be ruled out.

Sodium chloride resistant and thioprolin resistant callus lines exhibited slightly more increase in peroxidase activity on normal medium than on stress medium (Tables 50, 51 and Fig. 4a). The peroxidase activity was higher in sensitive callus line grown under the inhibitory concentration of NaCl (300 mM) and optimum concentration of proline (33.33 mM) than the sensitive callus line grown on proline (33.33 mM) containing normal medium. But the activity of peroxidase was comparable in sensitive callus line grown under the inhibitory concentration of thioprolin (3.00 mM) and exogenous proline (1.125 mM) with the sensitive callus line grown on proline (1.125 mM) containing normal medium (Table 52 and Fig. 4b).

Malate dehydrogenase activity of NaCl resistant callus line was high on NaCl supplemented medium as compared to that on normal medium. The activity of this enzyme was also low in sensitive callus line grown under normal condition (Table 53, Fig. 5a). Thioproline resistant callus line showed low and comparable malate dehydrogenase activity when grown under thioproline stress or nonstress conditions, however, its activity was slightly more than the sensitive callus line grown on normal medium (Table 54, Fig. 5a). The sensitive callus line grown in presence of NaCl (300 mM)/thioproline (3.00 mM) and exogenous proline (33.33 mM with NaCl and 1.125 mM with thioproline) exhibited higher malate dehydrogenase activity on 10th day as compared to the other days of growth period whereas sensitive callus line grown in the presence of exogenous proline (33.33 mM) showed comparatively lower activity of malate dehydrogenase than the sensitive callus grown in presence of low concentration of exogenous proline i.e. 1.125 mM (Table 55 and Fig. 5b).

Sodium chloride resistant and thioproline resistant callus lines grown under stress conditions exhibited marginal increase in catalase activity as compared to sensitive, NaCl resistant callus lines grown on normal medium (Tables 56 and 57, Fig. 6a). The sensitive callus line grown in presence of NaCl (300 mM)/thioproline (3.00 mM) and exogenous proline (33.33 mM with NaCl and 1.125 mM with thioproline) exhibited

marginal increase in catalase activity as compared to the sensitive callus grown on proline (33.33 mM or 1.125 mM) containing normal medium (Table 58, Fig. 6b).

Thus the enzymatic studies reveal that the total and specific activity patterns of peroxidase and catalase were comparable in NaCl resistant and thioproline resistant callus lines grown under stress and nonstress conditions. But malate dehydrogenase activity pattern exhibited gross differences in the two selected callus lines. Further, it appears that malate dehydrogenase activity showed some relation with the phenomenon of osmoregulation as compared to peroxidase and catalase activities.

From these observations, it is quite evident that like other cases of cultured cells (Dix and Street, 1975; Chaleff and Parsons, 1978; Croughan et al., 1978; Hibberd and Green, 1982; Watad et al., 1983; Nabors and Dykes, 1985) it is possible to screen/develop cell lines endured with resistance to stress conditions. Although it is not possible to conclude whether the variability for stress tolerance pre-existed in the explants or arose de novo during cultures, there is more likelihood that variant cells pre-existed in the explants. Cell variability has been amply demonstrated by D'Amato (1952) and Partenen (1963) using differentiated tissues. Since then this phenomenon has been found to be of common occurrence

(D'Amato, 1977; Skirvin, 1978; Bayliss, 1980; Orton, 1980a). Because of the lack of regenerated plants and the inheritance pattern of the traits selected, it is not possible to pinpoint the genetic basis of salt tolerance as reported in many other instances (Widholm, 1976; Croughan et al., 1978, 1981; Nabors et al., 1980; Nielsen et al., 1980; Dix et al., 1983; Smith and McComb, 1983), however, the stability of the selected trait during culture period indicates a stable genetic/epigenetic change manifesting in altered osmoregulation.

While comparing NaCl resistant and thioproline resistant callus lines, it becomes apparent that these two lines had similarities with respect to growth patterns, K^+ , Ca^{++} , soluble carbohydrate and soluble protein contents, osmotic potential, peroxidase and catalase activities, while they differed for Na^+ , Cl^- , proline contents and malate dehydrogenase activity.

Keeping in view these results, it becomes obvious that though the overall mechanisms of tolerance in NaCl resistant and thioproline resistant callus lines showed differences, however, these callus lines exhibited some similarities with respect to certain steps of the overall pathways of stress tolerance. Such conclusions are also substantiated with the findings of Lange (1967), Levitt (1972) and Santarius (1973).

The osmoprotective compounds such as described in

bacteria (Le Rudulier et al., 1984) may play a similar critical role in plants because molecules synthesized by plants during stress possess potent biological activity in protecting bacteria against osmotic stress. Similarly, plant tissue like pollen when subjected to dehydration often produce large amounts of these compounds (Virtanen and Kan, 1955). Moreover, the levels of putative osmoprotective compounds change in synchrony with stress. This implies that plants and bacteria may perhaps be sharing a common mechanism of osmoprotection. In bacteria, gene conferring osmotic tolerance (Osm gene) has been identified, mapped and subsequently cloned (Le Rudulier and Valentine, 1982; Le Rudulier et al., 1984). But so far we have no information whether such type of gene or genes exist in the plants. It is, therefore, imperative to know about their existence in plant system so that they could be isolated from salt tolerant species and incorporated into the genome of salt sensitive species by employing recombinant DNA or molecular cloning techniques.

SUMMARY

In the present study NaCl resistant and thioproline resistant callus lines were selected from the sensitive callus cultures of Vigna radiata without employing any physical or chemical mutagen. The growth pattern, Na^+ , K^+ , Cl^- , Ca^{++} , proline, total soluble carbohydrates, total soluble proteins, osmotic potential and activities of some stress related enzymes (peroxidase, malate dehydrogenase, catalase) were studied in sensitive, NaCl resistant, thioproline resistant callus lines and NaCl/thioproline stressed sensitive callus cultures but alleviated by optimum concentrations of exogenous proline.

1. Upon increasing the levels of NaCl/thioproline stress, callus growth declined gradually.
2. The growth of the sensitive callus line was stimulated at low concentrations of NaCl (25-50 mM) and thioproline (0.50-0.75 mM).
3. NaCl and thioproline resistant callus lines grew faster on normal medium than on their respective stress media.
4. Exogenous proline alleviated the growth inhibition of NaCl/thioproline stressed sensitive callus line at lower concentrations but was found to be inhibitory at higher concentrations.

5. The concentration of exogenous proline required to get maximum recovery of growth in NaCl stressed sensitive callus line was comparatively very high than the concentration of exogenous proline required to bring maximum recovery of growth in thioproline stressed sensitive callus line.
6. Decrease in K^+ content was found to be a function of increase in NaCl or thioproline stress in sensitive callus line.
7. NaCl resistant and thioproline resistant callus lines maintained higher levels of K^+ under NaCl and thioproline stress conditions, respectively. Their levels of K^+ were also found to be greater than the sensitive callus line grown under identical stress conditions.
8. Potassium content increased continuously upto the optimum concentrations of exogenous proline but declined suddenly at supraoptimal concentrations in NaCl/thioproline stressed or nonstressed sensitive callus cultures.
9. Increase in Na^+ and Cl^- content was found to be a function of increase in NaCl stress in sensitive callus line but Na^+ and Cl^- levels remained unchanged with the increase in thioproline stress in the same callus line.

10. Na^+ and Cl^- contents were higher in NaCl resistant callus line grown under NaCl stress than NaCl resistant or sensitive callus lines grown under nonstress condition. But there was no effect on the Na^+ and Cl^- levels in thioproline resistant callus line grown under thioproline stress.
11. Na^+ and Cl^- levels declined upto optimum concentration of exogenous proline but increased at supraoptimal concentrations in NaCl stressed or nonstressed sensitive callus cultures. However, thioproline stressed sensitive callus cultures did not show any change in the level of Na^+ or Cl^- in response to the increasing concentrations of exogenous proline.
12. The sensitive, NaCl resistant and thioproline resistant callus lines maintained identical levels of Ca^{++} under stress and nonstress conditions. Similarly application of exogenous proline did not alter the levels of Ca^{++} in NaCl or thioproline stressed sensitive callus cultures.
13. The proline contents were low in sensitive and NaCl resistant callus lines grown on normal medium as compared to NaCl resistant callus line grown under NaCl stress or thioproline resistant callus line grown under normal and thioproline stressed conditions.

14. Sensitive callus line grown in the presence of proline (33.33 mM) and NaCl (300 mM) showed more accumulation of endogenous proline as compared to sensitive callus line grown on proline (33.33 mM) containing normal medium. But the endogenous proline levels in the sensitive callus line grown in the presence of proline (1.125 mM) and inhibitory concentration of thioproline (3.00 mM) were comparable with sensitive callus line grown on proline (33.33 mM) containing normal medium.
15. The rates of soluble carbohydrate accumulation were found to be higher in sensitive, NaCl resistant and thioproline resistant callus lines grown on normal medium as compared to NaCl resistant and thioproline resistant callus lines grown on NaCl and thioproline stressed media, respectively.
16. Application of optimum concentrations of exogenous proline did not alter the levels of soluble carbohydrates in nonstressed or NaCl/thioproline stressed sensitive callus cultures.
17. Sensitive, NaCl resistant and thioproline resistant callus lines grown on normal medium maintained low and constant levels of soluble proteins but NaCl resistant callus line grown under NaCl stress and thioproline resistant callus line grown under thioproline stress exhibited marginal increase in the levels of soluble proteins.

18. Sensitive callus line grown in the presence of proline (33.33 mM/1.125 mM) and inhibitory concentration of NaCl (300 mM)/thioprolin (3.00 mM) showed appreciable increase in the levels of soluble proteins as compared to same callus lines grown on normal medium but containing exogenous proline (33.33 mM or 1.125 mM).
19. The osmotic potential became more negative under stress than under nonstress conditions in sensitive and resistant callus cultures.
20. Peroxidase activity was more in resistant callus lines than sensitive callus line. Further, it was more under nonstress than under stress conditions.
21. Sensitive callus line grown in the presence of NaCl and exogenous proline showed more increase in peroxidase activity as compared to the same callus line grown in the presence of only optimum concentration of proline. However, no such differences were found upon comparing sensitive callus line grown in the presence of thioprolin and optimum concentration of proline with the sensitive callus line grown on proline containing normal medium.
22. Malate dehydrogenase activity was found to be low in sensitive, NaCl resistant, thioprolin resistant callus lines grown on normal medium and thioprolin resistant

callus line grown on thioproline supplemented medium as compared to NaCl resistant callus line grown under NaCl stress.

23. Sensitive callus line grown in the presence of NaCl (300 mM) and proline (33.33 mM) or on proline (33.33 mM) containing normal medium showed lower malate dehydrogenase activity (especially on 10th day) as compared to sensitive callus line grown on thioproline (3.00 mM) and proline (1.125 mM) or on proline (1.125 mM) containing normal medium.
24. The catalase activity was found to be low both under stress and nonstress conditions but there was marginal increase in catalase activity of NaCl resistant and thioproline resistant callus lines when grown under NaCl and thioproline stress conditions, respectively.
25. Sensitive callus line grown in the presence of proline (33.33 mM or 1.125 mM) and NaCl (300 mM)/thioproline (3.00 mM) also showed marginal increase in catalase activity as compared to sensitive callus line grown on proline (1.125 mM or 3.00 mM) containing normal medium.
26. It became apparent from these observations that stress tolerant cells could be screened under in vitro conditions in Vigna radiata and that the cell lines

resistant to NaCl stress and thioproline stress exhibited some similarities in their behaviour while differed for a few attributes.

27. Although the stress tolerant cell lines showed altered osmoregulation, the basis whether it was genetic or epigenetic could not be found out.

LITERATURE CITED

- Abel, G.H. (1969). Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans. *Crop Sci.*, 9: 697-698.
- Abel, G.H. and Mackenzie, A.J. (1964). Salt tolerance of soybean varieties (Glycine max L.Merrill) during germination and later growth. *Crop Sci.*, 4: 157-161.
- Aggarwal, R.K. (1986). Studies on in vitro and in vivo response to salinity in barley (Hordeum vulgare L.). Ph.D. Thesis, Haryana Agric. Univ., Hisar.
- Ahmad, I., Larher, F. and Stewart, G.R. (1979). Sorbitol, a compatible osmotic solute in Plantago maritima. *New Phytol.*, 82: 671.
- Ahmad, R., Gupta, S.D. and Gosh, P.D. (1986). Isolation of L-methionine resistant cell lines in Vigna sinensis L. after mutagen treatment. *Plant Cell, Tissue and Organ Culture*, 7: 135-144.
- Aslam, M., Huffaker, R.C. and Rains, D.W. (1984). Early effects of salinity on nitrate assimilation in barley seedlings. *Plant Physiol.*, 76: 321-325.
- Aspinall, D. and Paleg, L.G. (1981). Proline accumulation: physiological aspects. In: *The physiology and biochemistry of drought resistance in plants* (eds., L.G. Paleg and D. Aspinall). Academic Press, New York, pp. 205-241.
- Barnett, N.M. and Naylor, A.W. (1969). Amino acid and protein metabolism in Bermuda grass during water stress. *Plant Physiol.*, 41: 1222-1230.
- Bar-Nun, N. and Poljakoff-Mayber, A. (1977). Salinity stress and content of proline in roots of Pisum sativum and Tamarix tetraoyna. *Ann. Bot.*, 41: 173-179.

- Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.
- Bayliss, M.W. (1980). Chromosomal variation in plant tissue culture. *Intl. Rev. Cytol. Suppl.*, 11A: 113-144.
- Ben-Hayyim, G. and Kochba, J. (1982). Growth characteristics and stability of tolerance of citrus callus cells subjected to NaCl stress. *Plant Sci. Lett.*, 27: 87-94.
- Ben-Hayyim, G. and Kochba, J. (1983). Aspects of salt tolerance in a NaCl-selected stable cell line of Citrus sinensis. *Plant Physiol.*, 72: 685-690.
- Benzioni, A., Hai, C. and Vaadia, Y. (1967). Water and salt stresses, Kinetin and protein synthesis in tobacco leaves. *Plant Physiol.*, 42: 361-365.
- Benzioni, A. and Tal, M. (1978). Ion imbalance in Capsicum annum scabrous diminutive, a wilthy mutant of pepper. II. rubidium fluxes. *J. Exp. Bot.*, 29: 879-884.
- Berlin, J. (1980). Para-fluorophenylalanine resistant cell lines of tobacco. *Z.Pflanzenphysiol.*, 97: 317-324.
- Berlin, J. and Vollmer, B. (1979). Effects of -aminooxy- -phenylpropionic acid on phenylalanine metabolism in p-fluorophenylalanine sensitive and resistant tobacco cells. *Z.Naturforsch.*, 34C: 770-775.
- Berlin, J. and Widholm, J.M. (1977). Correlation between phenylalanine ammonia lyase activity and phenolic biosynthesis in p-fluorophenylalanine-sensitive and -resistant tobacco and carrot tissue cultures. *Plant Physiol.*, 59: 550-553.
- Berlin, J. and Widholm, J.M. (1978a). Amino acid uptake by amino acid analog resistant tobacco cell lines. *Z.Naturforsch.*, 33C: 634-640.

- Berlin, J. and Widholm, J.M. (1978b). Metabolism of phenylalanine and tyrosine in tobacco cell lines resistant and sensitive to p-fluorophenylalanine. *Phytochemistry*, 17: 65-68.
- Berlyn, M.B. (1980). Isolation and characterization of isonicotinic acid hydrazide-resistant mutants of *Nicotiana tabacum*. *Theor. Appl. Genet.*, 58: 19-26.
- Bernstein, L. (1963). Osmotic adjustment of plants to saline media. II. Dynamic phase. *Am. J. Bot.*, 48: 360-370.
- Bernstein, L. (1975). Effects of salinity and sodicity on plant growth. *Ann. Rev. Phytopathol.*, 13: 295-312.
- Bernstein, L. and Ayers, A.D. (1953). Salt tolerance of five varieties of carrots. *Proc. Am. Soc. Hort. Sci.*, 61: 360-366.
- Bhaskaran, S., Smith, R.H. and Schertz, K. (1983). Sodium chloride tolerant callus of sorghum bicolor(L.). *Z. Pflanzenphysiol.*, 104: 81-89.
- Binzel, M.L., Hasegawa, P.M., Bressan, R.A., Handa, S.A. and Handa, A.K. (1983). Adaptive responses of cultured tobacco cells to NaCl. *Pl. Physiol.*, 72: S136.
- Bourgin, J.P. (1978). Valine resistant plants from in vitro selected tobacco cells. *Molec. Gen. Genet.*, 161: 225-230.
- Bressan, R.A., Handa, A.K., Handa, S. & Hasegawa, P.M. (1982). Growth and water relations of cultured tomato cells after adjustment to low external water potential. *Pl. Physiol.*, 70: 1303-1309.
- Bright, S.W.J., Norbury, P.B. and Mifflin, B.J. (1979). Isolation of a recessive barley mutant resistant to S-(2-amino-ethyl)L-cysteine. *Theor. Appl. Genet.*, 55: 1-4.

- Brown, J.C. (1963). Iron chlorosis in soybeans as related to the genotype of root stock. *Soil Sci.*, 96: 387-394.
- Brown, J.W. and Hayward, H.E. (1956). Salt tolerance of alfalfa varieties. *Agron. J.*, 48: 18-20.
- Buhl, M.B. and Stewart, C.R. (1983). Effect of NaCl on proline synthesis and utilization in excised barley leaves. *Plant Physiol.*, 72: 664-667.
- Carlson, P.S. (1973). Methionine sulfoximine resistant mutants of tobacco. *Science*, 180: 1366-1368.
- Carlson, P.S., Dearing, R.D. and Floyd, B.M. (1973). Defined mutants in higher plants. In: *Genes, enzymes and populations* (ed., A.M.Srb). Plenum, New York, pp. 99-107.
- Cella, R. and Iadarola, P. (1983). Characterization of carrot cell lines resistant to 5-methyltryptophan obtained by irradiating suspension cultures with uv light. *Plant Sci. Lett.*, 29: 327-337.
- Cella, R., Parisi, B. and Nielsen, E. (1982). Characterization of a carrot cell line resistant to azetidine-2-carboxylic acid. *Plant Sci. Lett.*, 24: 125-135.
- Chaleff, R.S. and Carlson, P.S. (1975). In vitro selection for mutants of higher plants. In: *Genetic manipulation with plant material* (ed., L.Ledoux). Plenum, New York, pp. 351-364.
- Chaleff, R.S. and Parsons, M.F. (1978). Direct selection in vitro for herbicide-resistant mutants of Nicotiana tabacum. *Proc. Natl. Acad. Sci.(USA)*, 75: 5104-5107.
- Chandler, S.F. and Thorpe, T.A. (1987). Characterization of growth water relations, and proline accumulation in sodium sulfate tolerant callus of Brassica napus L. cv. Westar (Canola). *Plant Physiol.*, 84: 106-111.

- Chen, H.H. and Li, P.H. (1978). Interactions of low temperature, water stress and short days in the induction of stem frost hardiness in the red osier dogwood. *Plant Physiol.*, 62: 833-835.
- Chen, P.M. and Li, P.H. (1977). Induction of frost hardiness in stem cortical tissues of Cornus stolonifera Michx. by water stress. II. Biochemical changes. *Plant Physiol.*, 59: 240-243.
- Chen, P.M., Li, P.H. and Burke, M.J. (1977). Induction of frost hardiness in stem cortical tissues of Cornus stolonifera Michx. by water stress. I. Unfrozen water in cortical tissues and water status in plants and soil. *Plant Physiol.*, 59: 236-239.
- Chen, Y., Zahavi, E., Barak, P. and Umiel, N. (1980). Effects of salinity stresses on tobacco. I. The growth of Nicotiana tabacum callus cultures under sea water, NaCl and mannitol stresses. *Z. Pflanzenphysiol.*, 98: 141-153.
- Cheng, K.L. and Bray, R.H. (1951). Determination of calcium and magnesium in soil and plant material. *Soil Sci.*, 72: 449-458.
- Chowdhury, V.K. (1982). Induction of mutants at cellular level in mungbean (Vigna radiata). Ph.D. Thesis, Haryana Agric. Univ., Hisar.
- Chu, T.M., Aspinall, D. and Paleg, L.G. (1976). Stress metabolism. VII. Salinity and proline accumulation in barley. *Aust. J. Plant Physiol.*, 3: 219-228.
- Cleland, R. and Olson, A.C. (1968). Direct incorporation of hydroxyproline into Avena coleoptile proteins. *Biochemistry*, 7: 1745-1751.

- Clemens, J., Campbell, L.C. and Nurisjah, S. (1983). Germination, growth and mineral ion concentrations of Casuarina species under saline conditions. Aust. J. Bot., 31: 1-9.
- Coffman, F.A. (1957). Cold resistant oat varieties also resistant to heat. Science, 125: 1298-1299.
- Croughan, T.P., Stavarek, S.J. and Rains, D.W. (1978). Selection of a NaCl-tolerant line of cultured alfalfa cells. Crop Sci., 18: 959-963.
- Croughan, T.P., Stavarek, S.J. and Rains, D.W. (1981). In vitro development of salt resistant plants. Environ. Exp. Bot., 21: 317-324.
- Csonka, L.N. (1980). The role of L-proline in response to osmotic stress in Salmonella typhimurium: selection of mutant with increased osmoregulation as strains which overproduced L-proline. In: Genetic engineering of osmoregulation (eds., D.W.Rains, R.C.Valentine and A.Hollaender), Plenum, New York, pp. 35-52.
- D' Amato, F. (1952). Polyploidy in the differentiation and function of tissues and cells in plants. A critical examination of the literature. Caryologia, 4: 311-358.
- D' Amato, F. (1977). Cytogenetics of differentiation in tissues and cell cultures. In: Applied and fundamental aspects of plant cell, tissue and organ culture (eds., J.Rèinert and Y.P.S.Bajaj). Springer-Verlag, Berlin, pp. 343-357.
- Davies, D.D. (1969). Malate dehydrogenase from pea epicotyles. In: Methods in Enzymology (ed., J.M.Lowenstein). Academic Press, New York, V-XLII, pp. 148-150.
- Dimler, R.J., Schaefer, W.C., Wise, C.S. and Rist, C.E. (1952). Quantitative paper chromatography of D-glucose and its oligosaccharides. Analyt. Chemist., 24: 1411-1414.

- Dix, P.J. (1980). Environmental stress resistance, selection and plant cell cultures. In: Plant cell cultures: results and perspectives (eds., E.Sala, B.Parisi, B.Cella and O.Ciferri). Elsevier/North Holland Press, Amsterdam, pp. 183-186.
- Dix, P.J., McIysaght, V.A. and Pearce, R.S. (1984). The potential of cell cultures for the production of salt tolerant cultivars. In: Efficiency in plant breeding (eds., W.Lange, A.C.Zeven and N.G.Hogenboom). Pudoc, Wageningen, pp. 219-223.
- Dix, P.J. and Pearce, R.S. (1981). Proline accumulation in NaCl-resistant and sensitive cell lines of Nicotiana sylvestris. Z.Pflanzenphysiol., 102: 243-248.
- Dix, P.J., Plunkett, A. and Toth, I. (1983). The use of tissue culture for investigating the physiology of salt stress. In: Plant tissue culture in relation to biotechnology (eds., A.C.Cassells and J.A.Kavanagh). Royal Irish Academy, Dublin, pp. 95-104.
- Dix, P.J. and Street, H.E. (1975). Sodium chloride-resistant cultured cell lines from Nicotiana sylvestris and Capsicum annum. Plant Sci. Lett., 5: 231-237.
- Duncan, D.R. and Widholm, J.M. (1987). Proline accumulation and its implication in cold tolerance of regenerable maize callus. Plant Physiol., 83: 703-708.
- Dvorak, J., Ross, K. and Mendelinger, S. (1985). Transfer of salt tolerance from Elytrigia pontica (Podp.) Holub to wheat by the addition of an incomplete Elytrigia genome. Crop Sci., 25: 306-309.
- El-Fouly, M.M. and Jung, J. (1972). Enzyme activity in wheat seedlings grown under different NaCl salinity levels and their interaction with growth regulators. Biochem. Physiol. Pflanzen., 163: 492-498.

- El-Fouly, M.M. and Jung, J. (1981). Influence of increasing NaCl concentrations in the irrigation water on growth, mineral content and enzyme activity of wheat seedlings. *Agrochemica*, 25: 306-317.
- Epstein, E. (1961). The essential role of calcium in selective cation transport by plant cells. *Plant Physiol.*, 36: 437-444.
- Epstein, E. (1969). Mineral metabolism of halophytes. In: *Ecological aspects of the mineral metabolism of plants* (ed., I.H. Rorison). Blackwell, Oxford, pp. 345-355.
- Epstein, E. (1972). *Mineral nutrition of plants: principles and perspectives*. John-Wiley, New York.
- Epstein, E. and Jefferies, R.L. (1964). The genetic basis of selective ion transport in plants. *Ann. Rev. Plant Physiol.*, 15: 169-184.
- Epstein, E., Rains, D.W. and Elzam, O.E. (1963). Resolution of dual mechanism of potassium absorption by barley roots. *Proc. Natl. Acad. Sci. (USA)*, 49: 684-692.
- Evans, D.A. and Gamborg, O.L. (1979). Effects of para-fluorophenylalanine on ploidy levels of cell suspension cultures of Datura innoxia. *Environ. Exp. Biol.*, 19: 269-275.
- Flick, C.E., Jensen, R.A. and Evans, D.A. (1981). Isolation, protoplast culture, and plant regeneration of PFP-resistant variants of Nicotiana tabacum Su/Su. *Z. Pflanzenphysiol.*, 103: 239-245.
- Flowers, T.J., Troke, P.F. and Yeo, A.R. (1977). The mechanism of salt tolerance in halophytes. *Ann. Rev. Plant Physiol.*, 28: 89-121.

- Flowers, T.J. and Yeo, A.R. (1986). Ion relations of plant under drought and salinity. *Aust. J. Plant Physiol.*, 13: 75-91.
- Fowden, L. (1963). Amino-acid analogues and the growth of seedlings. *J. Exp. Bot.*, 14: 387-398.
- Fowden, L., Neale, S. and Tristram, H. (1963). Effect of 3,4-dehydro-DL-proline on growth and protein synthesis. *Nature*, 199: 35-38.
- Gale, J. and Boll, W.G. (1979). Growth of bean cells in suspension culture in the presence of NaCl and protein-stabilizing factors. *Can. J. Bot.*, 57: 777-782.
- Gates, C.T., Haydock, K.P. and Robins, M.F. (1970). Salt concentration and the content of phosphorous, potassium, sodium and chloride in cultivars of G.wrightii (G.javanica). *Aust. J. Exp. Agric. Anim. Husb.*, 10: 98-110.
- Gathercole, R.W.E. and Street, H.E. (1976). Isolation, stability and biochemistry of a p-fluorophenylalanine-resistant cell line of Acer pseudoplatanus L. *New Phytol.*, 77: 29-41.
- Gathercole, R.W.E. and Street, H.E. (1978). A p-fluorophenylalanine resistant cell line of sycamore with increased contents of phenylalanine, tyrosine, and phenolics. *Z.Pflanzenphysiol.*, 89: 283-287.
- Gauch, H.G. and Eaton, F. M. (1942). Effect of saline substrate on hourly levels of carbohydrates and inorganic constituents of barley plants. *Plant Physiol.*, 17: 347-365.
- Gavazzi, G., Racchi, M.N. and Tonelli, C. (1975). A mutation causing proline requirement in Zea mays. *Theor. Appl. Genet.*, 46: 339-345.

- Gerloff, G.C. (1963). Comparative mineral nutrition of plants. *Ann. Rev. Plant Physiol.*, 14: 107-124.
- Goas, G., Goas, M. and Larher, F. (1982). Accumulation of free proline and glycine betaine in Aster tripolium subjected to saline shock: a kinetic study related to light period. *Physiol. Plant.*, 55: 383-388.
- Goldner, R., Umiel, N. and Chen, Y. (1977). The growth of carrot callus cultures at various concentrations and composition of saline water. *Z. Pflanzenphysiol.*, 85: 303-317.
- Gosal, S.S. and Bajaj, Y.P.S. (1984). Isolation of sodium chloride resistant cell lines in some grain-legumes. *Indian J. Exp. Biol.*, 22: 209-214.
- Greenway, H. (1962). Plant responses to saline substrates. I. Growth and ion uptake of several varieties of Hordeum during and after sodium chloride treatment. *Aust. J. Biol. Sci.*, 15: 16-38.
- Greenway, H. (1965). Plant response to saline substrates. VII. Growth and ion uptake throughout plant development in two varieties of Hordeum vulgare. *Aust. J. Biol. Sci.*, 18: 763-779.
- Greenway, H. (1973). Salinity, plant growth and metabolism. *J. Aust. Inst. Agric. Sci.*, 39: 24-34.
- Greenway, H. and Munns, R. (1980). Mechanism of salt tolerance in nonhalophytes. *Ann. Rev. Plant Physiol.*, 31: 149-190.
- Greenway, H. and Osmond, C.B. (1972). Salt responses of enzymes from species differing in salt tolerance. *Plant Physiol.*, 49: 256-259.
- Hall, J.L., Harvey, D.M.R. and Flowers, T.J. (1978). Evidence for the cytoplasmic localization of betaine in leaf cells of Suaeda maritima. *Planta*, 140: 59-62.

- Hall, M.N. and Silhavy, T.J. (1981). Genetic analysis of the major outer membrane proteins of Escherichia coli. Ann. Rev. Genet., 15: 91-142.
- Handa, A.K., Bressan, R.A., Handa, S. and Hasegawa, P.M. (1982a). Characteristics of cultured tomato cells after prolonged exposure to medium containing polyethylene glycol. Plant Physiol., 69: 514-521.
- Handa, A.K., Bressan, R.A., Handa, S. and Hasegawa, P.M. (1982b). Tolerance to water and salt stresses in cultured cells. In: Plant Tissue Culture (ed., A. Fujiwara). Jap. Assoc. Plant Tissue Culture, Tokyo, pp. 471-474.
- Handa, A.K., Bressan, R.A., Handa, S. and Hasegawa, P.M. (1983). Clonal variation of tolerance to polyethylene glycol-induced water stress in cultured tomato cells. Pl. Physiol., 72: 645-653.
- Handa, S., Handa, A.K., Hasegawa, P.M. and Bressan, R.A. (1986). Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol., 80: 938-945.
- Hanson, A.D., Nelson, C.E., Pedersen, A.R. and Everson, E.H. (1979). Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. Crop Sci., 19: 489-493.
- Hasegawa, P.M., Bressan, R.A. and Handa, A.K. (1980). Growth characteristics of NaCl-selected and nonselected cells of Nicotiana tabacum L. Plant Cell Physiol., 21: 1347-1355.
- Hason-Porath, E. and Poljakoff-Mayber, A. (1969). The effect of salinity on the malic dehydrogenase of pea roots. Plant Physiol., 44: 1031-1034.

- Hegde, B.R. and Joshi, G.V. (1974). Mineral salt absorption in saline rice variety Kala Rata. *Plant and Soil*, 41: 421-424.
- Helal, H.M. and Mengel, K. (1979). Nitrogen metabolism of young barley plants as affected by NaCl-salinity and potassium. *Plant and Soil*, 51: 457-462.
- Hellebust, J.A. (1976). Osmoregulation. *Ann. Rev. Plant Physiol.*, 27: 485-505.
- Hewitt, E.J. and Notton, B.A. (1967). Inhibition by L-azetidine-2-carboxylic acid of induction of nitrate reductase in plants and its reversal by L-proline. *Phytochemistry*, 6: 1329-1335.
- Heyser, J.W. and Nabors, M.W. (1981). Osmotic adjustments of cultured tobacco cells (*Nicotiana tabacum* var. Samsun) grown on sodium chloride. *Plant Physiol.*, 67: 720-728.
- Hibberd, K.A. and Green, C.E. (1982). Inheritance and expression of lysine plus threonine resistance selected in maize tissue culture. *Proc. Natl. Acad. Sci.(USA)*, 79: 559-563.
- Hibberd, K.A., Walter, T., Green, C.E. and Gengenbach, B.G. (1980). Selection and characterisation of a feedback insensitive tissue culture of maize. *Planta*, 148: 183-187.
- Hodges, T.K. (1976). ATPase associated with membranes of plant cells. In: *Encyclopedia of Plant physiology*, new series, Vol. 2, Transport in plants (eds., U.Lüttge and M.G.Pitman). Springer-Verlag, Berlin, pp. 260-283.

- Holleman, J. (1967). Direct incorporation of hydroxyproline into protein of sycamore cells incubated at growth-inhibitory levels of hydroxyproline. *Proc. Natl. Acad. Sci. (USA)*, 57: 50-54.
- Huq, S.M.I. and Larher, F. (1983). Osmoregulation in higher plants: effects of NaCl salinity on non-nodulated Phaseolus vulgaris L. I. Growth and mineral content. *New Phytol.*, 93: 203-208.
- Jacobsen, E. (1986). Isolation, characterization and regeneration of a S-(2-aminoethyl) cysteine resistant cell line of dihaploid potato. *J. Plant Physiol.*, 123: 307-315.
- Jacobsen, E., Visser, R.G.F. and Wijbrandi, J. (1985). Phenylalanine and tyrosine accumulating cell lines of a dihaploid potato selected by resistance to 5-methyltryptophan. *Plant Cell Rep.*, 4: 151-154.
- Jacoby, B. (1964). Function of bean roots and stems in sodium retention. *Plant Physiol.*, 39: 445-449.
- Janardan, K.V., Murthy, R.S.P., Giriraj, K. and Panchaksharaiah, S. (1976). Salt tolerance of cotton and potential use of saline water for irrigation. *Curr. Sci.*, 45: 334-336.
- Jefferies, R.L. (1980). The role of organic solutes in osmoregulation in halophytic higher plants. In: *Genetic engineering of osmoregulation: impact on plant productivity for food, chemicals and energy* (eds., D.W.Rains, R.C.Valentine and A.Hollaender). Plenum, New York, pp. 135-154.
- Jeschke, W.D. (1979). Univalent cation selectivity and compartmentation in cereals. In: *Recent advances in the biochemistry of cereals* (eds., D.L.Laidman and R.G.Wyn Jones). Academic Press, New York, p. 63.

- Jeschke, W.D. (1984). K^+ - Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. In: Salinity tolerance in plants: strategies for crop improvement (eds., R.C. Staples and G.H. Toenniessen). John-Wiley, New York, pp. 37-66.
- Joshi, Y.C., Qadar, A., Bal, A.R. and Dwivedi, R.S. (1985). Differences between salt sensitive and salt tolerant wheat species in relation to Na and K accumulation. Indian J. Plant Physiol., 28: 81-84.
- Kahane, I. and Poljakoff-Mayber, A. (1968). Effect of substrate salinity on the ability in protein synthesis in pea roots. Plant Physiol., 43: 1115-1119.
- Kennedy, E.P. (1982). Osmotic regulation and the biosynthesis of membrane-derived Oligosaccharides in Escherichia coli. Proc. Natl. Acad. Sci.(USA), 79: 1092-1095.
- Kochba, J., Ben-Yahhim, G., Spiegel-Roy, P., Saad, S. and Neumann, H. (1982). Selection of stable salt-tolerant callus cell lines and embryos in Citrus sinensis and C. aurantium. Z.Pflanzenphysiol., 106: 111-118.
- Kochba, J., Spiegel-Roy, P. and Saad, S. (1980). Selection for tolerance to NaCl and 2,4-D in ovular callus lines of Citrus sinensis. In: Plant Cell Cultures: results and perspectives. (eds., E.Sala, B.Parisi, B.Cella and O.Ciferri). Elsevier/North Holland Press, Amsterdam, pp. 187-192.
- Kruckeberg, A.R. (1959). Ecological and genetic aspects of metallic ion uptake by plants and their possible relation to wood preservation. In: Marine boring and fouling organisms (ed., D.L.Ray). Univ. Washington, Seattle, pp. 526-536.

- Kucherenko, L.A. (1980). Tissue culture in rice improvement: experiences in USSR. In: International rice research institute: innovative approaches to rice breeding. Las Baños, Laguna, Philippines, pp. 93-102.
- Kueh, J.S.H. and Bright, S.W.J. (1981). Proline accumulation in a barley mutant resistant to trans-4-hydroxy-L-proline. *Planta*, 153: 166-171.
- Kueh, J.S.H. and Bright, S.W.J. (1982). Biochemical and genetical analysis of three proline accumulating barley mutants. *Pl. Sci. Lett.*, 27: 233-241.
- Kulieva, F.B., Shamina, Z.B. and Strogonov, B.P. (1975). Effect of high concentrations of sodium chloride on multiplication of cells of Crepis capillaris in vitro. *Fiziologiya Rastenii*, 22: 131-135.
- Kylin, A. and Quatrano, R.S. (1975). Metabolic and biochemical aspects of salt tolerance. In: Plants in saline environments. (eds., A. Poljakoff-Mayber and J. Gale). Springer-Verlag, Berlin, pp. 147-167.
- LaHaye, P.A. and Epstein, E. (1969). Salt tolerance by plants: enhancement with calcium. *Science*, 166: 395-396.
- LaHaye, P.A. and Epstein, E. (1971). Calcium and salt toleration by bean plants. *Physiol. Plant.*, 25: 213-218.
- Laimins, L.A., Rhoads, D.B. and Epstein, W. (1981). Osmotic control of Kdp operon expression in Escherichia coli. *Proc. Natl. Acad. Sci. (USA)*, 78: 464-468.
- *Lange, O.L. (1967). Investigations on the variability of heat resistance in plants. In: The cell and environmental temperature (ed., A.S. Troshin). Pergamon Press, Oxford.

- Langridge, J. (1958). An osmotic mutant of Arabidopsis thaliana. Aust. J. Biol. Sci., 11: 457-470.
- Läuchli, A. (1976). Genotypic variation in transport. In: Encyclopedia of plant physiology: New series, vol. 2 Transport in plants (eds., U. Lüttge and M. G. Pitman). Springer-Verlag, Berlin, pp. 372-393.
- Läuchli, A. and Epstein, E. (1970). Transport of potassium and rubidium in plant roots: the significance of calcium. Plant Physiol., 45: 639-641.
- Läuchli, A. and Wieneke, J. (1979). Studies on growth and distribution of Na^+ , K^+ and Cl^- in soybean varieties differing in salt tolerance. Z. Pflanzenernaehr Boden Kd., 142: 3-13.
- Lauter, D. J., Munns, D. N. and Clarkin, K. L. (1981). Salt response of chickpea as influenced by N supply. Agron. J., 73: 961-966.
- Lawyer, A. L., Berlyn, M. B. and Zelitch (1980). Isolation and characterization of glycine hydroxamate resistant cell lines of Nicotiana tabacum. Plant Physiol., 66: 334-341.
- Lerner, H. R. (1985). Adaptation to salinity at the plant cell level. Plant and Soil, 89: 3-14.
- *Le Rudulier, D. and Valentine, R. C. (1982). Genetic engineering in agriculture: Osmoregulation. Trends in Biochem. Sci., 7: 431.
- Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T. and Valentine, R. C. (1984). Molecular biology of osmoregulation. Science, 224: 1064-1068.

- Lessani, H. and Marschner, H. (1978). Relation between salt tolerance and long distance transport of sodium and chloride in various crop species. *Aust. J. Plant Physiol.*, 5: 27-37.
- *Levitt, J. (1958). Frost, drought and heat resistance. In: *Protoplasmatologia*, Vol. 8 (eds., L.V. Heilbrunn and F. Weber). Springer, Vienna.
- Levitt, J. (1972). Responses of plants in environmental stresses. Academic Press, New York.
- Levitt, J. (1980). Responses of plants to environmental stresses. Vol. II. Water, salt and other stresses. Academic Press, New York.
- Levitt, J. and Siminovitch, D. (1940). The relation between frost resistance and the physical state of the protoplasm. I. The protoplasm as a whole. *Canad. J. Res.*, 18: 550-561.
- Levitt, J., Sullivan, C.J. and Krull, E. (1960). Some problems in drought resistance. *Bull. Res. Council Israel*, Sect. D, 8: 173-180.
- Liu, M. and Yeh, H. (1982). Selection of NaCl-tolerant line through stepwise salinized sugarcane cultures. In: *Plant tissue culture* (ed., A. Fujiwara). Jap. Assoc. Plant Tissue Culture, Tokyo, pp. 477-478.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol-reagent. *J. Biol. Chem.*, 193: 265-275.
- Lubin, M. and Ennis, H.L. (1964). On the role of intracellular potassium in protein synthesis. *Biochem. Biophys. Acta.*, 80: 614-631.

- *Maddur, N. (1976). The inheritance of salt tolerance in barley. Ph.D. Thesis, Michigan State Univ., Michigan.
- Maliga, P. (1978). Resistant mutants and their use in genetic manipulation. In: Frontiers of plant tissue culture (ed., T.A. Thorpe). Intl. Assoc. Plant Tissue Culture, Calgary, pp. 381-392.
- Maliga, P. (1980). Isolation, characterization and utilization of mutant cell lines in higher plants. Int. Rev. Cytol. Suppl. 11A: 225-250.
- Maliga, P. (1984). Isolation and characterization of mutants in plant cell culture. Ann. Rev. Plant Physiol., 35: 519-542.
- Mathur, A.K., Ganapathy, P.S. and Johri, B.M. (1980). Isolation of sodium chloride tolerant plantlets of Kickxia ramosissima under in vitro conditions. Z.Pflanzenphysiol., 99: 287-294.
- Matthews, B.F., Shye, S.C.H. and Widholm, J.M. (1980). Mechanism of resistance of a selected carrot cell suspension culture to S-(2-aminoethyl)-L-cysteine. Z.Pflanzenphysiol., 96: 453-463.
- *Maximov, N.A. (1929). Internal factors of frost and drought resistance in plants. Protoplasma, 7: 259.
- McHughen, A. and Swartz, M. (1984). A tissue culture derived salt-tolerant line of flax. J. Plant Physiol., 117: 109-117.
- Melchers, G. (1972). Haploid higher plants for plant breeding. Z.Pflanzenzüchtg., 67: 19-32.
- *Morton, J. (1979). Comparative investigation of the activity of membrane-bound and adenosine triphosphatase of roots of normal and mutant (scabrous diminutive) pepper. M.Sc. Thesis, Ben Gurion University of the Negev, Beer-Sheva, Israel.

- Myers, W.M. (1960). Genetic control of physiological processes: consideration of differential ion uptake by plants. In: Radio-isotopes in the biosphere (eds., R.S. Caldecott and L.A. Snyder). Univ. Minnesota, Minneapolis, pp. 201-226.
- Nabors, M.W., Daniels, A., Nadolny, L. and Brown, C. (1975). Sodium chloride tolerant lines of tobacco cells. Plant Sci. Lett., 4: 155-159.
- Nabors, M.W. and Dykes, T.A. (1985). Tissue culture of cereal cultivars with increased salt, drought and cold tolerance. In: Biotechnology in international agricultural research (Proc. Inter-Center Seminar on IARC and Biotechnology, 23-27 April, 1984), IRRI, Manila, pp. 121-138.
- Nabors, M.W., Gibbs, S.E., Bernstein, C.S. and Meis, M.E. (1980). NaCl-tolerant tobacco plants from cultured cells. Z.Pflanzenphysiol., 97: 13-17.
- Nabors, M.W., Heyser, J.W., Dykes, T.A. and DeMott, K.J. (1983). Long-duration, high frequency plant regeneration from cereal tissue cultures. Planta, 157: 385-391.
- Nabors, M.W., Kroskey, C.S. and McHugh, D.M. (1982). Greenspots are predictors of high callus growth rates and shoot formation in normal and in salt stressed tissue cultures of oat (Avena sativa L.). Z.Pflanzenphysiol., 105: 341-349.
- Nielsen, E., Amileni, A.R., Rouchi, S. and Parisi, B. (1980). A carrot cell line resistant to azetidine-2-carboxylic acid. In: Plant cell cultures: results and perspectives (eds., F. Sala, B. Parisi, R. Cella and O. Ciferri). Elsevier/North Holland Press, Amsterdam, pp. 151-155.

- Nightingale, G.T. and Farnham, R.B. (1935-36). Effects of nutrient concentration on anatomy, metabolism and bud abscission of sweet pea. *Bot. Gaz.*, 97: 497-517.
- Nyman, L.P., Gonzales, C.J. and Arditti, J.A. (1983). In-vitro selection for salt tolerance of taro (Colocasia esculenta var. antiquorum). *Ann. Bot.*, 51: 229-236.
- Oono, K. and Sakaguchi, S. (1978). Induction of mutation in tissue culture and their use for plant breeding. II. Induction of saline resistant and copper sulphate resistant calluses and regeneration of plants. *Jap. J. Breed. (Suppl. 2)*, 28: 124-125.
- Oono, K. and Sakaguchi, S. (1980). Mutations induced by rice tissue culture. IV. Induction of saline resistant mutants. In: Abstracts 7th Symp. plant tissue and cell culture, Tsukuba, Japan, p. 7.
- Orton, T.J. (1980a). Chromosomal variability in tissue culture and regenerated plants of Hordeum. *Theor. Appl. Genet.*, 56: 101-112.
- Orton, T.J. (1980b). Comparison of salt tolerance between Hordeum vulgare and H. jubatum in whole plants and callus cultures. *Z. Pflanzenphysiol.*, 98: 105-118.
- *Osmond, C.B. (1979). Ion uptake, transport and secretion. In: *Arid-Land Ecosystems: Structure, Functioning and Management, Vol. I* (eds., R.A. Perry and D.W. Goodall). Cambridge University Press, Cambridge.
- Palmer, J.E. and Widholm, J.M. (1975). Characterization of carrot and tobacco cell cultures resistant to p-fluorophenylalanine. *Plant Physiol.*, 56: 233-238.
- Pandey, R. and Ganapathy, P.S. (1984a). Effects of sodium chloride stress on callus cultures of Cicer arietinum L. cv. BG-203: growth and ion accumulation. *J. Exp. Bot.*, 35: 1194-1199.

- Pandey, R. and Ganapathy, P.S. (1984b). Isolation of sodium chloride-tolerant callus line of Cicer arietinum L. cv. BG-203. Plant Cell Reports, 3: 45-47.
- Pandey, R. and Ganapathy, P.S. (1985). The proline enigma: NaCl-tolerant and NaCl-sensitive callus line of Cicer arietinum. Plant Sci., 40: 13-17.
- Parker, J. (1972). Protoplasmic resistance to water deficits. In: Water deficits and plant growth, Vol. 3 (ed., T.T.Kozlowski). Academic Press, New York, pp. 125-176.
- Partenen, C.R. (1963). Plant tissue culture in relation to developmental cytology. Intl. Rev. Cytol., 15: 215-243.
- Phillips, G.C. and Collins, G.B. (1979). In vitro tissue culture of selected legumes and plant regeneration from callus cultures of red clover. Crop Sci., 19: 59-64.
- *Pisek, A. and Larcher, W. (1954). Zusammenhang zwischen Austrocknungsresistenz und Frosthärte bei immergrünen Pflanzen. Protoplasma, 44: 30.
- Pitman, M.G. (1984). Transport across the root and shoot/root interactions. In: Salinity tolerance in plants: strategies for crop improvement (eds. R.C.Staples and G.H.Toenniessen). John-Wiley, New York, pp. 93-124.
- Plaut, Z. (1974). Nitrate reductase activity of wheat seedlings during exposure of and recovery from water stress and salinity. Physiol. Plant., 30: 212-217.
- Poljakoff-Mayber, A., Bar-Nun, N., Hason, E. and Heichal, O. (1981). Respiratory carbohydrate metabolism of different varieties under saline conditions. Bot. Gaz., 142: 431-437.
- Porath, E. and Poljakoff-Mayber, A. (1964). Effect of salinity on metabolic pathways in pea root tips. Israel J. Bot., 13: 115-121.

- Porath, E. and Poljakoff-Mayber, A. (1968). The effect of salinity in the growth medium on carbohydrate metabolism in pea root tips. *Plant Cell Physiol.*, 9: 195-203.
- Rains, D.W. (1972). Salt transport by plants in relation to salinity. *Ann. Rev. Plant Physiol.*, 23: 367-388.
- Rains, D.W. (1979). Salt tolerance of plants: strategies of biological systems. In: *The biosaline concept: an approach to the utilization of saline environments* (ed., A. Hollaender). Plenum, New York, pp. 47-67.
- Rains, D.W., Croughan, T.P. and Stavarek, S.J. (1980b). Selection of salt tolerant plants using tissue culture. In: *Genetic engineering of osmoregulation: impact on plant productivity for food, chemicals and energy* (eds., D.W. Rains, R.C. Valentine and A. Hollaender). Plenum, New York, pp. 279-292.
- Rains, D.W. and Epstein, E. (1967). Sodium absorption by barley roots: its mediation by mechanism '2' of alkali cation transport. *Plant Physiol.*, 42: 319-323.
- Rains, D.W., Valentine, R.C. and Hollaender, A. (eds.). (1980a). *Genetic engineering of osmoregulation: impact on plant productivity for food, chemicals and energy*. Plenum, New York.
- Ramage, R.T. (1980). Genetic methods to breed salt tolerance in plants. In: *Genetic engineering of osmoregulation* (eds., D.W. Rains, R.C. Valentine and A. Hollaender). Plenum, New York, pp. 311-318.
- Rana, R.S., Joshi, Y.C., Kadar, A. and Singh, K.N. (1976). Adaptive characteristics of salt tolerant genotypes, mineral analysis and growth characteristics. *Ann. Report, CSSRI, Karnal*, pp. 52-57.

- Ranch, J.P. and Widholm, J.M. (1983). Expression of 5-methyl-tryptophan resistance in regenerated Datura innoxia plants. In: Plant cell cultures in crop improvement (eds., S.K.Sen and K.L.Giles). Plenum, New York, pp. 381-386.
- Rangan, T.S. and Vasil, I.K. (1983). Sodium chloride tolerant embryogenic cell lines of Pennisetum americanum (L.). K. Schum. Ann. Bot., 52: 59-64.
- Ratanadilok, N., Marcarian, V. and Schmalzel, C. (1978). Salt tolerance in grain sorghum. Uni. Arizona, Tucson, Arizona. Agron. Abst. No. 160.
- Reddy, M.P. and Vora, A.B. (1985). Effect of salinity on protein metabolism in bajra (Pennisetum typhoides S and H) leaves. Indian J. Plant Physiol., 28: 190-195.
- Reddy, P.J. and Valdyanath, K. (1985). In vitro selection for salt tolerance in basmati rice. Indian J. Plant Physiol., 28: 88-91.
- Reish, B., Duke, S.H. and Bingham, E.T. (1981). Selection and characterization of ethionine-resistant alfalfa (Medicago sativa L.) cell lines. Theor. Appl. Genet., 59: 89-94.
- Reitemier, R.F. (1943). Semimicroanalysis of saline soil solutions. Indus. and Engin. Chem., Analyt. Ed., 15: 393-402.
- Riccardi, G., Cella, R., Camerino, G. and Ciferri, O. (1983). Resistance to azetidine-2-carboxylic acid and sodium chloride tolerance in carrot cell cultures and Spirulina platensis. Plant Cell Physiol., 24: 1073-1078.
- Riccardi, G., Sora, S. and Ciferri, O. (1981). Production of amino acids by analog resistant mutants of cyanobacterium Spirulina platensis. J. Bact., 147: 1002-1007.

- *Rosa, J.T. (1921). Investigation of the hardening process in vegetable plants. Miss. Agr. Exp. Sta. Res. Bull., 48: 1.
- Rosen, A. and Tal, M. (1981). Salt tolerance in the wild relatives of the cultivated tomato: responses of naked protoplasts isolated from leaves of Lycopersicon esculentum and L.peruvianum to NaCl and proline. Z.Pflanzenphysiol., 102: 91-94.
- Rush, D.W. and Epstein, E. (1981). Breeding and selection for salt tolerance by the incorporation of wild germplasm into a domestic tomato. J. Am. Soc. Hort. Sci., 106: 699-704.
- Santarius, K.A. (1973). The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. Planta, 113: 105-114.
- Scarth, G.W. (1941). Dehydration injury and resistance. Plant Physiol., 16: 171-179.
- Scarth, G.W. and Levitt, J. (1937). The frost-hardening mechanism of plant cells. Plant Physiol., 12: 51-78.
- Schobert, B. and Tschesche, H. (1978). Unusual solution properties of proline and its interaction with proteins. Biochem. Biophys. Acta, 541: 270-277.
- Scott, A.I., Mizukami, H. and Lee, S.L. (1979). Characterization of a 5-methyltryptophan resistant strain of Catharanthus roseus cultured cells. Phytochemistry, 18: 795-798.
- SeEVERS, P.M., DALY, J.K. and CATEDRAL, F.F. (1971). The role of peroxidase isoenzyme in resistance to wheat stem rust disease. Plant Physiol., 48: 353-360.

- Sharma, S.K. (1985). Accumulation and distribution of Na^+ and K^+ in rice genotypes with different sodicity resistance. Intl. Rice Res. Newsletter, 10(6): 16.
- Sharma, S.K. (1986). Mechanism of tolerance in rice varieties differing in sodicity tolerance. Plant and Soil, 93: 141-145.
- Sharma, S.K. and Garg, O.P. (1985). Salinity induced changes in plant growth and activities of glutamate dehydrogenase, aspartate and alanine aminotransferase in wheat. Indian J. Plant Physiol., 28: 407-412.
- Sharma, S. K., Joshi, Y.C. and Bal, A.R. (1984). Osmotic and ionic effects in salt sensitive and resistant wheat varieties. Indian J. Plant Physiol., 27: 153-158.
- Sheoran, I.S. and Garg, O.P. (1979). Quantitative and qualitative changes in peroxidase during germination of mungbean under salt-stress. Physiol. Plant., 46: 147-150.
- Siminovitch, D. and Briggs, D.R. (1953). Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. III. The validity of plasmolysis and desiccation tests for determining the frost hardiness of bark tissue. Plant Physiol., 28: 15-34.
- Simons, R.A., Nabors, M.W. and Lee, C.W. (1984). A model of mutant selection in plant suspension cultures. J. Plant Physiol., 116: 95-102.
- Sinha, A.K. (1972). Colorimetric assay of catalase. Analyt. Biochem., 47: 389-394.
- Skirvin, R.M. (1978). Natural and induced variation in tissue culture. Euphytica, 27: 241-266.

- Slayman, C.W. (1973). The genetic control of membrane transport. In: Current topics in membranes and transport (eds., F. Bronner and A. Kleinzeller). Academic Press, New York, 4: 1-174.
- Smith, M.K. and McComb, J.A. (1981a). Effect of NaCl on the growth of whole plants and their corresponding callus cultures. Aust. J. Plant Physiol., 8: 267-275.
- Smith, M.K. and McComb, J.A. (1981b). Use of callus cultures to detect NaCl tolerance in cultivars of three species of pasture legumes. Aust. J. Plant Physiol., 8: 437-442.
- Smith, M.K. and McComb, J.A. (1983). Selection for NaCl tolerance in cell cultures of Medicago sativa and recovery of plants from a NaCl tolerant cell line. Plant Cell Reports, 2: 126-128.
- Spiegel-Roy, P. and Ben-Hayyim, G. (1985). Selection and breeding for salinity tolerance in vitro. Plant and Soil, 89: 243-252.
- Staples, R.C. and Toenniessen, G.H. (eds.). (1984). Salinity tolerance in plants: strategies for crop improvement. John-Wiley, New York.
- Stavarek, S.J., Croughan, T.P. and Rains, D.W. (1980). Regeneration of plants from long-term cultures of alfalfa cells. Plant Sci. Lett., 19: 253-261.
- *Stavarek, S. J. and Rains, D.W. (1981). Ion uptake of salt-selected plant cells. Proc. 57th Annual Meeting Am. Soc. Plant Physiologist.
- Stavarek, S.J. and Rains, D.W. (1982). Growth and ionic characteristics of alfalfa cells exposed to different salts. Plant Physiol. (Suppl.), 69: 32, Abst. No. 177.

- Stavarek, S.J. and Rains, D.W. (1984a). Cell culture techniques: selection and physiological studies on salt tolerance. In: Salinity tolerance in plants: strategies for crop improvement (eds., R.C. Staples and G.H. Toenniessen). John-Wiley, New York, pp. 332-333.
- Stavarek, S.J. and Rains, D.W. (1984b). The development of tolerance to mineral stress. Hort. Sci., 19: 377-382.
- Stewart, G.R. and Lee, J.A. (1974). The role of proline accumulation in halophytes. Planta, 120: 279-289.
- Storey, R. and Wyn Jones, R.G. (1975). Betaine and choline levels in plants and their relationship to NaCl stress. Plant Sci. Lett., 4: 161-168.
- Strom, A.R., Le Rudulier, D., Jakowec, M.W., Bunnell, R.C. and Valentine, R.C. (1983). Osmoregulatory (Osm) genes and osmoprotective compounds. In: Genetic engineering of plants (eds., T. Kosuge, C.P. Meredith and A. Hollaender). Plenum Press, New York, pp. 39-59.
- Sung, Z.R. (1979). Relationship of indole-3-acetic acid and tryptophan concentrations in normal and 5-methyltryptophan resistant cell lines of wild carrots. Planta, 145: 339-345.
- Tal, M. (1983). Selection for stress tolerance. In: Handbook of plant cell culture, Vol. I (eds., D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada). MacMillan, New York, pp. 461-488.
- Tal, M. (1985). Genetics of salt tolerance in higher plants: theoretical and practical considerations. Plant and Soil, 89: 199-226.
- Tal, M. and Benzioni, A. (1977). Ion imbalance in Capsicum annuum scabrous diminutive, a wilthy mutant of pepper. I. Sodium fluxes. J. Exp. Bot., 28: 1337-1341.

- Tal, M., Eshel, A. and Witztum, A. (1976). Abnormal stomatal behaviour and ion imbalance in Capsicum scabrous diminutive. J. Exp. Bot., 27: 953-960.
- Tal, M., Heikin, H. and Dehan, K. (1978). Salt tolerance in the wild relatives of the cultivated tomato: responses of callus tissue of Lycopersicon esculentum, L. peruvianum and Solanum pennellii to high salinity. Z. Pflanzenphysiol., 86: 231-240.
- Tal, M. and Katz, A. (1980). Salt tolerance in the wild relatives of the cultivated tomato: The effect of proline on the growth of callus tissue of Lycopersicon esculentum and L. peruvianum under salt and water stresses. Z. Pflanzenphysiol., 98: 283-288.
- Tal, M., Witztum, A. and Shifriss, C. (1974). Abnormal stomatal behaviour and leaf anatomy in Capsicum annum scabrous diminutive, a wilthy mutant of pepper. Ann. Bot., 38: 983-988.
- Treichel, S.P., Kirst, S.O. and von Willert, D.J. (1974). NaCl induced alteration of phosphophenol pyruvate carboxylase activity in halophytes of different habitats. Z. Pflanzenphysiol., 71: 437-449.
- Tulley, R.E., Hanson, A.D. and Nelsen, C.E. (1979). Proline accumulation in water-stressed barley leaves in relation to translocation and the nitrogen budget. Pl. Physiol., 63: 518-523.
- Tyagi, A.K., Rashid, A. and Maheshwari, S.C. (1981). Sodium chloride resistant cell line from haploid Datura innoxia mill. A resistant trait carried from cell to plantlet and vice-versa in vitro. Protoplasma, 105: 327-332.

- Umiel, N., Zahavi, E. and Chen, Y. (1980). Effects of salinity stresses on tobacco. II. Short term kinetics of Na^+ and K^+ uptake by callus cultures grown on media containing NaCl. *Z. Pflanzenphysiol.*, 100: 363-367.
- Van Steveninck, R.F.M., Van Steveninck, M.E., Stelzer, R. and Lauchli, A. (1982). Studies on the distribution of Na and Cl in two species of lupin (Lupinus luteus and Lupinus angustifolius) differing in salt tolerance. *Physiol. Plant.*, 56: 465-473.
- van Swaaij, A.C., Jacobsen, E. and Feenstra, W.J. (1985). Effect of cold hardening, wilting and exogenously applied proline on leaf proline content and frost tolerance of several genotypes of *Solanum*. *Physiol. Plant.*, 64: 230-236.
- van Swaaij, A.C., Jacobsen, E., Kiel, J.A.K.W. and Feenstra, W.J. (1986). Selection, characterization and regeneration of hydroxyproline-resistant cell lines of Solanum tuberosum: Tolerance to NaCl and freezing stress. *Physiol. Plant.*, 68: 359-366.
- * Varner, J.E. (1980). The direct conversion of free hydroxyproline to proline. *Biochem. Biophys. Res. Commun.*, 70: 692-695.
- * Virtanen, A.J. and Kan, S. (1955). Free amino acids in pollen. *Acta Chem. Scand.*, 9: 1548.
- Voetberg, G. and Stewart, C.R. (1984). Steady state proline levels in salt-shocked barley leaves. *Plant Physiol.*, 76: 567-570.
- von-Hedenstrom, H. and Breckle, S.W. (1974). Obligate halophytes ? A test with tissue culture methods. *Z. Pflanzenphysiol.*, 74: 183-185.

- Waisel, Y. (ed.). (1972). Biology of halophytes. Academic Press, New York.
- Watad, A.A., Reinhold, L. and Lerner, H.R. (1983). Comparison between a stable NaCl selected Nicotiana cell line and the wild type. Plant Physiol., 73: 624-629.
- Weimberg, R. (1967). Effect of sodium chloride on the activity of a soluble malate dehydrogenase from pea seeds. J. Biol. Chem., 242: 3000-3006.
- Weimberg, R. (1970). Enzyme levels in pea seedlings grown on highly saline media. Plant Physiol., 46: 466-470.
- Weimberg, R. (1975). Effect of growth in highly salinized media on the enzymes of the photosynthetic apparatus in pea seedlings. Plant Physiol., 56: 8-12.
- Weiss, M.G. (1943). Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics, 28: 253-268.
- Widholm, J.M. (1972a). Cultured Nicotiana tabacum cells with an altered anthranilate synthetase which is less sensitive to feedback inhibition. Biochem. Biophys. Acta, 261: 52-58.
- Widholm, J.M. (1972b). Anthranilate synthetase from 5-methyl-tryptophan-susceptible and -resistant cultured Daucus carota cells. Biochem. Biophys. Acta, 279: 48-57.
- Widholm, J.M. (1974). Cultured carrot cell mutants: 5-methyl-tryptophan-resistance trait carried from cell to plant and back. Plant Sci. Lett., 3: 323-330.
- Widholm, J.M. (1976). Selection and characterization of cultured carrot and tobacco cells resistant to lysine, methionine, and proline analogs. Can. J. Bot., 54: 1523-1529.

- Winter, E. and Lauchli, A. (1982). Salt tolerance of Trifolium alexandrinum L. I. Comparison of the salt response of T.alexandrinum and T.pratense. Aust. J. Plant Physiol., 9: 221-226.
- Winter, E. and Preston, J. (1982). Salt tolerance of Trifolium alexandrinum L. IV. Ion measurements by X-ray micro-analysis in unfixed, frozen hydrated leaf cells at various stages of salt treatment. Aust. J. Plant Physiol., 9: 251-259.
- Wyn Jones, R.G. (1980). Salt tolerance. In: Physiological processes limiting plant productivity (ed., C.B.Johnson). Butterworths, London, p. 271.
- Wyn Jones, R.G., Brady, C.J. and Spiers, J. (1979). Ionic and osmotic relations in plant cells. In: Recent advances in the biochemistry of cereals (eds., D.L.Laidman and R.G.Wyn Jones). Academic Press, London, pp. 37-104.
- Wyn Jones, R.G. and Storey, R. (1978). Salt stress and comparative physiology in the gramineae. II. Glycine-betaine and proline accumulation in two salt and water-stressed barley cultivars. Aust. J. Plant Physiol., 5: 817-830.
- Wyn Jones, R.G. and Storey, R. (1981). Betaines. In: The physiology and biochemistry of drought resistance in plants (eds., L.G.Paleg and D.Aspinall). Academic Press, New York, pp. 171-204.
- Yamada, Y., Ogawa, M. and Yano, S. (1983). Tissue culture in sea water increases salt tolerance of rice plants. In: Cell and tissue culture techniques for cereal crop improvement. Sinica Press, Beijing, China, pp. 229-235.
- Yamaguchi, H. and Matsumoto, K. (1984). Induced variation of NaCl tolerance with EMS in trifoliate orange in vitro.

In: Abstracts Intl. Symp. Genetic Manipulation in Crops: 3rd Intl. Symp. Haploidy and 1st Intl. Symp. Somatic cell Genet. Crops, Oct. 22-26, 1984, Beijing, China.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. and Somero, G.N. (1982). Living with water stress: evolution of osmolyte systems. *Science*, 217: 1214-1222.

Yeo, A.R. (1983). Salinity resistance: physiologies and prices. *Physiol. Plant.*, 58: 214-222.

Yoshida, S., Ogawa, M., Suenaga, K. and HeChun, Ye (1983). Induction and selection of salt-tolerant mutant rices by tissue culture- recent progress at IRRI. In: Cell and tissue culture techniques for cereal crop improvement. Sinica Press, Beijing, China, pp. 237-254.

Zenk, M.H. (1974). Haploids in physiological and biochemical research. In: Haploids in higher plants: advances and potential (ed., K.J.Kasha). Uni. Guelph Press, Guelph, pp. 339-354.

Zhou Jia-Ping, Roth, E.J., Terzaghi, W. and Lark, K.G. (1981). Isolation of sodium dependent variants from haploid soybean cell culture. *Plant Cell Reports*, 1: 48-51.

* Original not seen.

