# CHAPTER 1

# INTRODUCTION

Recent advances in the techniques and applications of plant tissue culture have created unprecedented opportunities for the genetic manipulation of plants. The potential impact of these novel and powerful biotechnologies on the genetic improvement of crop plants has generated considerable interest.

The ability to culture plant cell and organs on chemically defined media and to regenerate plants from them offer possibilities of introducing desirable genetic modifications which are otherwise difficult to be accomplished by traditional plant breeding methods (Larkin and Scowcroft, 1981, 1983) Besides, excision of plant parts from a complex and organised level of whole plant and culturing them *in vitro* makes them more vulnerable to chromosomal and gene mutations and hence, affords possibilities to exploit genetic variabilities of plant for the selection of biochemical variants or mutants. Such variants and mutants resistant to antibiotics, base and amino-acid analogues, chlorate, cycloheximide, abscisic acid, colchicine, (Flick, 1983; Maliga, 1984), fungal toxins (Sacristan, 1986), heribicides (Hughes, 1983, Chaleff, 1986) and environmental stresses (Rains *et al* 1986; Chandler and Thorpe, 1986; Tal 1983, 1990, 1992) have been isolated. Out of this wide range of variants or mutants, selection of cell lines resistant to environmental stresses like drought, salinity, mineral toxicity, high and low temprature, have emerged as an approach to immediate agronomic utility.

Salinity in soil or the water resources is an ever - increasing problem in arid and semi-arid region of the world today. The total area affected by excess of salts is about 25% of the world's potentially arable land (Raghava Ram and Nabors, 1985). In India, 7 million ha of land is salt affected and about 40% is found in the Indo-Gangetic plains of Punjab, Haryana and U.P. (Abrol and Gill, 1984). Moreover, loss of land to salt accumulation through irrigated agriculture in our country has been estimated to be at least several hundred $km^2$ a year (Flowers *et al.*, 1977). The increasing demands of the expanding population for food and energy necessitate the increase of arable land by exploiting marginal areas such as arid and semi-arid lands.

The evaluation of the prospects for increasing the quality and quantity of the world's food supply, while applying the energetically least costly methodologies, would leave us with two options, i.e. to change the environment to suit the plant or to modify the plants to suit the adverse environment (Epstein *et al.*, 1980; Rains *et al.*, 1986). The former approach includes reclamation of salt affeted soils (Bernstein, 1975) through leaching and cation exchange techniques involving heavy expenditure, greater involvement of engineering and management practices. Even though, salt build-up can be partially controlled by agricultural engineering

and / or land management (Epstein *et al.*,1980). Moreover, in developing countries where expanding industralization is inevitable, the biological approach has to be advocated which is promising and energy efficient (Shannon, 1984). Biological manipulation of the plant involves identification of plant genotypes capable of increased tolerance to salt and incorporation of such traits into economically useful crop plants, the majority of which are non-tolerant or salt sensitive. One of the major pre-requisites for this approach is the availability of genetic variability. In some species, genetic diversity of salt-resistance occurs quite extensively among their cultivars (Epstein *et al.,* 1980, Norlyn, 1980). In species in which such variation is limited or lacking, genes can be transferred from their wild salt resistant relatives (Norlyn, 1980). Conventional methods of breeding are being employed on a modest scale to develop salt-tolerant cultivars and progress has been made in a few crops (Akbar, 1986; Srivastava and Jana, 1984), because these methods were extremely time - consuming and tedious. The traditional breeding methods was to be supplemented with plant tissue culture techniques, which have many advantages over those traditionally used for plant breeding (Dracup, 1991). Tissue culture provides an alternative mean for induction, selection and characterization of salt-tolerant variants and regeneration of plants from these variant cells to develop new germplasm of salt-tolerant plants which would be incorporated into thrust worthy crop improvement programme.

Salt-tolerant cell lines have been isolated in a wide range of plant species (Rains *et al.*, 1986; Chandler and Thorpe, 1986; Tal, 1983, 1990, 1992), including a few grain-legumes, i.e. *Glycine max* (Jia - Ping *et al.*, 1981), *Pisum sativum* (Gosal and Bajaj, 1984), *Cicer arietinum* (Gosal and Bajaj, 1984; Pandey and Ganapathy, 1984) and *Vigna radiata* (Gosal and Bajaj, 1984; Kumar and Sharma, 1989a). In most of these studies, NaCl as a single salt has been used to induce salt stress (Sabbah and Tal, 1990). NaCl selection is likely to produce genotypes with resistance to $Na^+$ or $Cl^-$ ions but not to other toxic ions contributing to salinity in certain agricultural situation (Rains *et al.*, 1986). Thus, a salt mixture should be employed for selecting the variant cell lines and thereby be more closely simulating the agriculture situation.

Almost all cases of cell culture selection of NaCl-tolerant cell lines have relied on spontaneously occurring variations (McCoy, 1987b). Where mutagenesis was employed, it was found not to increase the recovery rate of NaCl-tolerant cell lines (Kochha *et al.*, 1982). Although salt-tolerant cell lines are easily selected, but there have been only a few cases where plants have been regenerated (Chandler and Thorpe, 1986; Tal 1990, 1992). In legumes, except a forage legume, *Medicago sativa* (Winicov, 1991), there is no report on regeneration of plants from salt tolerant cell lines (Tal, 1992). However, there have been only a few cases where *in vitro* selection resulted in heritable NaCl tolerance expressed at the whole plant level (Nabors *et al.*, 1980, 1982; Nabors and Dykes, 1985; Vajrabhaya *et al.,* 1989; Winicov, 1991; Sumaryati

et al., 1992). In other cases, resistance has been shown to be retained in callus derived from plants regenerated from salt-resistant cells (Tyagi *et al.,* 1981). In most of the cases, plant regenerated from salt-tolerant cell lines have not demonstrated increased NaCl tolerance (Smith and McComb, 1983; Chandler and Vasil, 1984; McCoy, 1987 b). Since selection has been performed at the cellular level, it is important to know whether salt tolerance in culture is reflected at the whole plant level and whether salt tolerance expressed by intact plants is seen at the level of callus cultures. These questions are not adequately resolved in the literature.

Cell cultures, which lack the differentiation and structural integrity of higher plants, can also be an ideal system to assess the physiological effects of salt and / or water stress at the cellular level (Lerner, 1985). A comparison of various physiological and biochemical processes of salt-tolerant and salt-sensitive cell lines will help in elucidating the mechanism of salt tolerance which has not been studied in details in most of the cases except in tobacco (Binzel *et al.*, 1985 1987 a,b; Hasegawa et al., 1986; Iraki *et al.*, 1989, LaRosa *et al.*, 1989; Schnapp *et al.*, 1990; Kononowicz *et al.,* 1990a,b,1992).

The present investigations were carried in one of the most important grain-legume of India, i.e. *Vigna radiata* (L.) Wilczek or mungbean as commonly known. It is an important pulse crop and various cultivated forms are grown in many tropical and sub-tropical agricultural areas of the world as mung bean seed is rich in protein and is consumed as human food in various forms. *Vigna radiata* has been classified as sensitive to moderately sensitive to salinity, as 50 mol $m^{-3}$ NaCl is known to bring about 60% reduction in vegetative growth and seed yield (Salim and Pitman, 1988). There have been very few reports in literature about its responses to salinity and most of them are confined to germination and vegetative growth (Sharma *et al.*, 1971; Huq and Larher, 1983; Salim and Pitman, 1983). Moreover, the perusal of literature on *in-vitro* selection for salt tolerance reveals that except two preliminary reports (Gosal and Bajaj, 1984; Kumar and Sharma, 1989a), no much work seems to have been done on *Vigna radiata*. These studies are also incomplete because neither the mechanism of salt tolerance nor the plants have been regenerated from selected salt-tolerant cell lines. The research of the present thesis encompass attempts to develop genotypes of mungbean (*Vigna radiata*) tolerant to salinity through *in vitro* screening and selection of callus cultures. The following were the objectives of the present investigation.

1) To standardize culture conditions for efficient plant regeneration in *Vigna radiata*.

2) To develop procedure for selection of callus lines resistant to salt stress.

3) To explore the mechanism of salt tolerance by physiological and biochemical analysis of salt-tolerant cell lines.

(1) Ion analysis ($Na^+$, $K^+$, $Cl^-$, $SO^{2-}_4$).

(2) Metabolites analysis (reducing sugars, sucrose, protein, proline and amino-nitrogen).

(3) Enzymes (NR, GDH, GOGAT) analysis.

(4) Qualitative analysis of proteins.

4) Co-tolerance of selected lines for other environmental stresses.

5) To regenerate salt-tolerant plants.