General Introduction
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Mungbean (*Vigna radiata* (L.) Wilczek) is the most important legume crop due to its high seed protein (25.20 – 26.95 %) and its capacity to fix atmospheric nitrogen. In India, among various pulses, mungbean stands third, having an area of 2.5 million hectares of cultivation (Singh *et al.*, 1994). Since mungbean has a very short maturity span (55-70 days), it is grown under various cropping systems, contributing to the increase of the small landholder's income and to the improvement of the soil conditions (Fernandez and Shanmugasundaram, 1988). It provides excellent source of easily-digestible protein (AVRDC, 1975). Vavilov (1935) included mungbean in the Indian center of origin of cultivated plants. Vavilov's theory was supported by other authors based on the morphological diversity (Singh *et al.*, 1974), existence of wild and weedy types (Chandel, 1984; Paroda and Thomas, 1988) and archaeological remains (Jain and Mehra, 1980) of India.

Mungbean is an erect or sub-erect annual herb. Stem is 45-120 cm. high, with a slight tendency to twine on its upper branches; leaves trifoliate; leaflets entire, rarely trilobed and ovate in outline; flowers yellow and crowded in clusters of 10-25 on long pedicels; pods 5.5-10 cm. long, thin cylindrical, almost glabrous or with short pubescence, seeds more or less globular, mostly green in colour, the surface exhibits many fine, wavy ridges which may sometimes be almost invisible; hilum flat, covered with a white rough layer. Mungbean is generally cultivated as a *kharif* crop and prefers deep and well-drained loam soil. It is not suited to clays and succeeds best with a fair amount of moisture in the soil; heavy rainfall is harmful. Mungbean seeds are used as an excellent source of vegetable protein. The cooked seeds and dal form a valuable constituent of the diet for a
considerable number of people. The seeds have a pleasant taste and are wholesome and command a higher price as compared to other pulses (Anonymous, 1976).

Mungbean is widely grown in India, Pakistan, Burma, Thailand, Philippines, China and Indonesia as well as in East and Central Africa, West Indies, USA and Australia. The total world annual production is more than $10^6$ tonnes on more than $3.4\times10^6$ ha cultivation area with an average yield of 384 kg/ha worldwide. India accounts for more than two-thirds of total world production of mungbean. However, the national average grain yield of 338 kg/ha is low compared to the world average (Jeswani and Baldev, 1990). Moreover, its production has not improved significantly during the last three decades in spite of the best efforts of plant breeders (Tiyagi and Maskoor Alam, 1992).

The various factors responsible for the reduction and uncertainty in the yield are its sensitivity to salinity, drought stresses, viral pathogens and insect pests etc. (Kim, 1994). The three diseases which altogether responsible for more than 70% of yield losses in mungbean are yellow mosaic virus, a viral disease transmitted by white flies and the fungal diseases such as powdery mildew caused by Erysiphe polygoni and Cercospora leaf spot caused by Cercospora canescens (Jeswani and Baldev, 1990). In addition, mungbean is attacked by about 30 insect species. Among them, whitefly (Bremisia tabaci Genn), jassids (Empoasca moti Pruthi and E.terminalis Distant), hairy caterpillars (Diacrisia obliqua) and pod borers (Lampides boeticus, Maruca testulalis and Heliothis armigera) and storage pest bruchids (Callosobruchus chinensis) are the most serious and cause considerable loss in yield (Chhabra and Kooner, 1980).

Chemical control measures for diseases and pests are expensive and ineffective. Moreover, the increasing use of chemicals in agriculture is affecting the quality of the products as well as representing a problem for ecosystem (Fillipone, 1993). Strategies to improve resistance against diseases and insect pests by classical breeding have met with limited success due to lack of sufficient and satisfactory level of genetic variability within germplasm. Sources of resistance for some of the diseases and insect pests were identified but they were not included in breeding programs. Moreover, most of the
resistant lines identified earlier did not maintain the same level of resistance over the

time. Thus, stable resistance is difficult to be achieved in mungbean (Jaiwal and Gulati,

1995).

Genes conferring resistance to biotic and abiotic stresses have been found in many wild

or related species, which are sexually incompatible with the cultivated ones. Recently,
some of these genes have been isolated and characterized and cloned from other plant
species and microorganisms. These genes are to be transferred to cultivated *Vigna* in

order to increase and stabilize its production and thereby to combat the shortage in food
supplies, especially vegetable protein (Jaiwal and Gulati, 1995). Breeding for desirable
characters has been contributing to the pulse production. Even with the intensive
breeding efforts, success in the improvement of pulses has not been dramatic (Rao,

1996). The main causes and constraints contributing to this have now been understood

and the breeders are expecting the assistance of biotechnologists to meet the challenges

(Lai and Chandra, 1987).

The idea that isolated plant cells had the ability to divide and grow into complete plants

was conceived early in this century by Haberlandt (1902), which laid the foundation that
led to the defined conditions for cultivation of plant cells and regenerating plants from
them. The culture of plant cell and tissues in *in vitro* has opened up new avenues for crop
improvement (Bottino, 1975; Carlson and Polacco, 1975).

Genetic improvement of legumes through biotechnological methods is still a difficult
task. However, successful regeneration of whole plants from a variety of explants has
been reported in many legumes including pigeonpea and chickpea (Rao *et al.*, 1996).

(First observation on multiple shoot regeneration without callus formation reported in pea
by Kartha *et al.* (1974). Later on, regeneration from shoot tip has been fairly standardized
for chickpea, lentil, pea and mungbean (Bajaj and Dhanju, 1979).) Plants have been
regenerated from apical meristems of chickpea, cowpea, and bean (Kartha *et al.*, 1981).
Success has been obtained by establishing various culture conditions and systems (Singh
*et al.*, 1981, Sebastian, 1983; Martins and Sandahl, 1984; Rublou and Kartha, 1985,
Allavena and Rosetti, 1986; Rao and Chopra, 1987a). Direct in vitro bud formation was also observed from cotyledon explants of pigeonpea (Mehta and Mohan Ram, 1980; George and Eapen, 1994; Mohan and Krishnamurthy, 1998), chickpea (Khan and Ghosh, 1984; Rao and Chopra, 1989; Polisetty, 1997), mothbean (Godbole et al., 1984), peanut (Kanyand et al., 1994) cowpea (Pellegrineschi, 1997), urdbean (Ignacimuthu and Franklin, 1999), and Brassica (Zhang et al., 1998) and cotyledonary node in pigeonpea (Mehta and Mohan Ram, 1980; Shiva Prakash et al., 1994), peanut (Saxena et al., 1992). Shoot formation was also observed from nodal explants of chickpea (Altaf and Ahmad, 1986; Rao and Chopra, 1989); and pea (Griga et al., 1986), cowpea (Kulothungan, 1997).

Regeneration via callus in pulses is achieved only with limited success (Vasil et al., 1979; Rao, 1996). Effects of various components of culture medium and their concentrations were evaluated to find optimal condition for callus growth and differentiation in chickpea (Singh et al., 1981, Anil et al., 1986a, b; Rao and Chopra, 1987 b, c); bengalgram, redgram, greengram and lentil (Gosal and Bajaj, 1979); pea (Kallak and Yarvekylg, 1971; Sekiya et al., 1977); french bean (Mehta, 1966; Komizorko et al., 1981; Alfanso and Capote, 1984); cowpea (Reddy and Narayana, 1971, 1973; Matsubara, 1975; Kulothungan 1997); Vicia faba (Venkateswaran, 1962; Mitchell and Gildow, 1975; Shamina and Butenko, 1977) and in Dolichos lab-lab (Kumar et al., 1972). Once the callus is initiated, it can be maintained as callus or suspension culture. Suspension cultures have been established in bean (Mehta et al., 1976), Dolichos lab-lab (Gnanam and Padmanabhan, 1979) and cowpea (Ahmed et al., 1986). Regeneration from callus culture has been reported in Cicer arietinum (Rao and Padmaja, 1996) and Pisum sativum (Faruqui et al., 1996).

Somatic embryogenesis is currently applied to a wide range of genera and species (Ammirato, 1983) as a tool to obtain rapidly a large number of elite or disease resistant plants. Furthermore, a well-defined somatic embryogenesis procedure would be useful for in vitro selection of disease resistant plants and/or genetic transformation (Merkle et al., 1990; Parrott et al., 1991). Somatic embryogenesis is the process by which the somatic cells differentiated into plants through embryological stages simulating zygotic
embryogenesis without the fusion of gametes (Rangaswamy, 1986). First observed in
carrot by Steward (1958), somatic embryogenesis has acquired much importance in
agriculture, silviculture, horticulture and industries involved in continual supply of basic
plant material of elite varieties (Rangaswamy, 1986). Since majority of the reports on
regeneration in grain legumes deal with the development of shoots from pre-existing
meristems, it is ideal to evolve a culture system capable of plant regeneration through
somatic embryogenesis (George and Eapen, 1996). Somatic embryogenesis has been
reported in *Glycine max* (Lippmann and Lippmann, 1984; Lazzeri et al., 1987), *Vigna
aconitifolia* (Bhargava and Chandra, 1983; Gill et al., 1986, Kumar et al., 1988), *Pisum
sativum* (Kysley and Jacobsen, 1987), *Vicia faba* (Griga et al., 1987) and *Vicia
narbonensis* (Pickardt and Schieder, 1987), *Vigna unguiculata* (Kulothungan et al.,
1995), *Arachis hypogaea* (Chengalrayan et al., 1997), *Glycine max* (Samoylov et al.,
1998), *Cajanus cajan* (Sreenivasu et al., 1998; Anbazhagan and Ganapathi, 1999). In
most of the species the somatic embryos developed into complete plants while in a few
others they failed to differentiate into plants as in *Vigna mungo* (Gill et al., 1987, Eapen
and George, 1990). The ontogeny of somatic embryogenesis has been studied in *Vigna
aconitifolia, Vigna mungo* and *Vigna radiata* (Eapen and George, 1990), but they failed
to induce plantlet regeneration in *Vigna radiata*. Chen et al. (1990) have observed
induction of somatic embryogenesis from interspecific hybrid embryos of *Vigna
glabrescens × V. radiata*. But till date there is no reliable protocol standardized on
somatic embryogenesis of mungbean.

Salinity affects about one billion hectares of the world’s land area i.e. approximately 60%
of the cultivated land, which includes one-third area of the irrigated soil (Epstein
et al., 1980; Chowdhury et al., 1993; Ramagopal, 1993). Efforts are being made to
overcome this problem primarily by modifying the saline environment e.g. reclamation,
drainage, use of high leaching fractions, application of soil amendments and breeding
and/or selecting salt tolerant cultivars (Chowdhury et al., 1993; Grover et al., 1993).
However, a lot to be done to make these technological modifications cost effective and
viable. Salinity has two major effects on plant metabolism. One is high ion concentration
causing a low osmotic potential of the soil solution, which in turn, effect physiological
drought symptoms in plants. The other is due to specific ionic effects; the presence of high and potentially toxic concentrations of different ions (Na⁺, K⁺, Mg²⁺, Cl⁻ and SO₄²⁻ etc.) have adverse effects on different plant metabolism (Mass and Hoffman, 1977; Epstein et al., 1980).

Many soil scientists accept that even economically feasible engineering methods can not completely eliminate salts from saline environments; at best they can only minimize their effects. For this reason and because of increasing cost of water and energy, there is increasing need for the development of new strategies (Ashraf, 1997). Improving salinity and drought tolerance of crop plants by genetic means has been an important but largely unfulfilled aim of modern agricultural development (Winicov, 1998). The decline in plant productivity due to salinity and drought necessitates the development of stress-tolerant crops. Of the various approaches available, cell culture and selection of mutants has received increasing attention in recent years and salt tolerant cell lines have been isolated from several plant species, but few plants have been recovered from NaCl tolerant cells (Tal, 1990). Cell culture systems provide a reliable and efficient alternative method for screening, selecting and characterizing salt tolerance at the cellular level (Chandler and Thorpe, 1986; Tal, 1990) and also in subsequent regenerated plants (Winicov, 1991, 1996; Patnaik and Debata, 1997a; Ochatt et al., 1999).

Legumes have long been recognized to be, in general, very sensitive to salinity because they are unable to survive even in mild salinity conditions (Mass and Hoffman, 1977). Although mungbean (*Vigna radiata*) is not very salt tolerant, it is widely grown in the subcontinent, because of its importance as pulse crop (Ashraf, 1997). Recent advances in biotechnology have offered an opportunity to develop new germplasm. The development of such technologies largely depends on efficient regeneration of sexually mature plants. The success of gene transfer techniques depends upon efficient, repeatable and rapid *in vitro* regeneration system (Jaiwal and Gulati, 1995).

There have been a few attempts to regenerate mungbean plants *via* tissue culture (Mathews, 1987; Gulati and Jaiwal, 1990). Recovery of single plants from mungbean
meristems on basal medium was reported (Goel et al. 1983; Mathews, 1987). However only single (Bajaj and Dhanju, 1979) or few (0-3) shoots (Singh et al., 1985) were produced on basal medium supplemented with cytokinin and auxin. Multiple shoot regeneration has been reported from cotyledon (Mathews, 1987; Gulati and Jaiwal, 1990; Mendoza et al., 1993) and cotyledonary node (Mathews, 1987; Gulati and Jaiwal, 1994a). Somatic embryos have failed to regenerate plants (Eapen and George, 1990). A preliminary study on selection of NaCl tolerant cell lines has been done in mungbean from root callus culture (Kumar and Sharma, 1989). The lack of an efficient regeneration system for mungbean has slowed the improvement of this species via tissue culture and plant transformation (Gulati and Jaiwal, 1990). Regeneration of plants and induction of somatic embryos are restricted to a few genotypes with low efficiency (Jaiwal and Gulati, 1995). Lack of efficient regeneration protocol has hampered the recovery of transgenic plants in mungbean (Jaiwal et al., 1998). Hence, an alternative approaches like alien gene transfer and biotechnological innovations are envisaged for genetic upgrading of this crop. Efficient regeneration protocols are prerequisites for the application of such modern gene transfer technologies to plant improvement (George, 1993).

In the light of these considerations the following objectives were made

1. Standardization of an efficient protocol for multiple shoot regeneration from various explants of mungbean

2. Scrutinization of various explants to find out their efficiency for callus induction and plantlet regeneration

3. Development of suitable liquid suspension culture system for somatic embryogenesis, and

4. Selection of NaCl tolerant cell line