1. INTRODUCTION

Green revolution is one of the greatest triumphant stories in India which had resulted in increased food grain production from 50.8MT (1950-51) to 199MT (1996-97). This is a four-fold increase while the population had increased only three fold. In 1798, Thomas Malthus published his famous essay on population where he had expressed his concern about the human capacity necessary to achieve a balance between population growth and increase in food production when the global population is about to reach 930 million. At present the population of India alone exceeds one billion and it is expected to touch 1.3 billion by 2025. There are growing apprehensions about the Malthusian prediction coming true in the early decades of the 21st century (Paroda, 2000; Swaminathan, 1999).

On the basis of current consumption pattern, the estimated total requirement of food grains is 225MT for 2001 and it is expected to reach 360MT by 2025. Within this period, the requirement of grain legumes would rise from 18.5 to 24 (low yield) – 26 (high yield) MT and thus the demand would increase by 3.3% annually. This will require a 75% increase in the production of pulses by 2030. Hence the present productivity of 0.6 tonnes per hectare will have to be raised to 0.99 tonnes per hectare. The main qualities of pulses are that, are rich in protein sources, enrich soil fertility, improve soil structure and are ideal for mixed/inter/rotation and multiple cropping. Globally, India occupies first
position in area (25604 mha) and production (14.8 MT) of pulses but 118th position in productivity (650 kg/ha). The total area under cultivation of pulses remained virtually stagnant (23.19 mha) with a stable production of 14.8 MT over the last four decades. There had been a decrease in the area of pulses cultivation, probably due to increase in irrigation facilities, resulting in a shift from pulses to cereals. The per capita availability of pulses has also declined from 64 g/capita/day (1951-56) to less than 40 g/capita/day as against the FAO/WHO's recommendation of 80 g/capita/day (Asthana and Chaturvedi, 1999). However 55 g/capita/day requirement of pulses may be the realistic target.

Cowpea [Vigna unguiculata (L.) Walp., syn. Vigna sinensis (L.) Savi ex Hassk] known also as southern pea (or) blackeye pea is one of the world's most important food crops and ranks fifth in area among the edible legumes. In India where about 70% of its people live in rural environment as marginal farmers or landless labour (Pental, 1998), cowpea is considered as 'Poor Man's Meat'. Although the seed proteins are somewhat deficient in the sulfur-containing amino acids such as methionine, cysteine and tryptophan, it is valued as a major source of essential amino acids in tropical diets, which otherwise is predominated by starchy cereals, roots and tubers. In the USA, Australia and several countries of Southeast Asia, Central and South America, cowpea has important horticultural, agronomic and industrial uses.

In India, cowpea occupies an area of 3.08 mha with an annual production of 2.01 MT. The productivity of cowpea has drastically reduced especially in Tamil Nadu and Northern India. About 70% of the loss was due to viral diseases and pests. Cowpea suffers seriously from pests such as pod borers (Maruca testulalis), pod bugs (Clavigralla tomentosicolis), cowpea weevils
(Callosobruchus maculatus), aphids and flower thrips. In the past two decades, a number of high yielding varieties with improved nutritional quality and with relatively high resistance to some diseases and pests have been identified by screening cowpea accessions (selections) and breeding desirable traits from different genotypes. However, due to difficulties in obtaining fertile progeny after interspecific crosses between cultivated and wild Vigna species, conventional screening/breeding methods have not been able to overcome the major breeding constraints (Kononowicz et al., 1997). There is scope for increasing the production without endangering the ecological assets as it is a welcome trend towards sustainable agriculture. This should form a solid foundation for an 'evergreen revolution' that would take farmers into the new millennium with hope.

Gene manipulation and plant genetic transformation techniques might facilitate progress in this regard. In industrialized countries where biotechnology has been successfully applied in cereals and other crops, cowpea received very little attention as it was considered a 'minor' crop. MNCs (Multi National Corporations) and NGOs (Non Government Organizations) do not take much interest in transfer of technologies to pulses. However several attempts are in progress especially in the field of plant protection against insects such as transfer of genes encoding Bacillus thuringiensis endo-toxin, protease inhibitor, α-amylase inhibitor and lectins (PHA) into cowpea. Deficiency in sulfur-containing amino acids could also be alleviated by introducing 2s Brazil nut albumin, Zein or Nodulin genes (Monti et al., 1997).

In India notable achievements have been obtained in the cloning of novel genes. Dr. K. Veluthambi et al., have sequenced genomes of a local isolate of YMV and have succeeded in constructing a coat protein gene imparting viral
resistance (Pental, 1998). A novel insecticidal gene (Bt61-Cry V) from an Indian isolate of *B. thuringiensis* has also been cloned and characterized (Tuteja, 1996). These genes need to be genetically engineered into plants by transformation techniques. Owing to the production/breeding constraints, there is an urgent need to establish a routine genetic transformation protocol for cowpea improvement. To-date, no reliable transformation system is available for cowpea. Earlier attempts resulted in cowpea calluses (or) chimaeric plantlets (Kononowicz *et al.*, 1997) but mature transgenic plants could not be obtained.

In recent years, attention has been focused on the development of cowpea regeneration systems amenable for gene transfer. Regeneration through tissue culture is a potent tool in crop improvement and genetic engineering is a powerful technique to introduce desired genes into plants. Of the different methods to introduce genes, *Agrobacterium*-mediated gene transfer has been more successful in pulses (Trick and Finer, 1998; Trinh *et al.*, 1998). In the light of these considerations, an integrated effort has been made to achieve the following objectives:

- A repeatable protocol to regenerate plants through direct organogenesis and somatic embryogenesis
- A reliable transformation protocol employing different reporter genes and an efficient selection procedure using nptII (kanamycin resistance), hpt (hygromycin resistance) and bar (phosphinothricin resistance) genes as selectable markers
- Screening and identifying important cowpea cultivar(s) of India amenable for regeneration and transformation