CHAPTER I

GENERAL INTRODUCTION

Grain legumes are the most important group of crops after the cereals. Legumes (Leguminosae or Fabaceae) are the third largest family of flowering plants with 20,000 estimated number of species (Cronk *et al*., 2006), of which 37 species are mainly distributed across Africa, Asia and Australia. They play a crucial role in human nutrition, besides being a source of fodder for cattle. They are nutritious vegetables as they contain lots of vitamins and minerals as well as dietary fiber and protein.

Pigeonpea (*Cajanus cajan*) is a chief grain legume crop with a genome size of 85Mbp and 11 pairs of chromosomes. They cover a significant amount of the protein requirement for humans, especially in the developing countries. It is a multipurpose crop grown as a single crop or intercropping with cereals on many farms. It has useful soil ameliorating properties and is crucial in crop diversification. Pigeonpea plants have the potential to produce fair grain yield under nutrient-deprived soil and low moisture conditions, so it can be considered as a sustainable crop. It is also a restorer of soil nitrogen fertility and the deep roots of pigeonpea take up phosphorous, thus making it available to other crops (Jharna Srivastava, 2013).

Due to the importance of pigeonpea, the crop is often grown under various suboptimal field conditions. Those stressful environmental conditions severely affect the growth and productivity of plants. Under stress conditions, plants trigger a number of responses such as changes in gene expression, cell metabolism, growth rates and crop yields. Resistance or sensitivity of plants to stress conditions depends on the species, genotype, development age of plants, and the type, severity, and duration of the stress factors. Stresses are of two types, biotic/living (arising from living organisms that can cause disease or damage) or abiotic/non-living (arising from an excess or deficient in the environment). Both biotic and abiotic stresses can reduce the productivity of plant by 65 to 87%, depending on the crop.

Biotic stress is the damage caused to the pigeonpea plants by various living organisms, such as fungi, bacteria, pests, viruses and several insects. Bacteria such as *Pseudomonas syringae* cause infection to pods, leaves, seeds and whole plant. Fungi such as *Fusarium udum* cause more diseases in plants than any other biotic stress factor (Datta *et al.,* 2004). Insects such as *Odontotermes* species cause severe physical damage to plants (Sharma *et al.,* 2009). Insects can also act as a vector for viruses and bacteria from infected plants to healthy plants. The most important insects feeding on pigeonpea pods and seeds are pod sucking bugs, pod borer (*Maruca vitrata*), seed boring caterpillars and pod flies (*Helicoverpa armigera*) (Shanower *et al.,* 1999; Lateef, 1992).

Abiotic stress is the adverse impact of non-living physical and chemical factors on the living organisms in a precise environment. This stress also includes varied stresses such as low and extreme temperatures, excess light and scarcity, waterlogging, wounding, exposure to ozone and UV-B radiations, salt shock and sodicity (Dita *et al.,* 2006).

Almost 71% of the earth surface is occupied by saline water. The high salinity is a common phenomenon for the barren and waterless regions. The addition of salt to water decreases both osmotic potential, accumulation of Na^+ and Cl^- ions and availability of water to roots which ultimately leads to osmotic stress. Salinity means the presence of various salts such as NaCl, sulphates and bicarbonates of magnesium and calcium in soil. Sodium chloride is the dominant salt found in saline areas. It is predicted that 30% of land will become unusable for agriculture in 25 years because of soil salinization (Kolodyazhnaya *et al.,* 2009).

The excess accumulation of salts in soil or water is known as salinization. It can be caused by usual processes such as weathering of minerals, dust, and precipitation and by the movement of salt to the land surface with groundwater. Salts are carried inland from the ocean by wind and rainfall and have accumulated over long periods of time. Salts can be carried from a salt laden water table to the soil surface by capillary action which then will accumulate as water evaporates. And it also can be caused by artificial processes such as the use of saline water for irrigation, aquaculture activities, salting of icy roads and application of fertilizer containing potassium, which form a naturally occurring salt namely sylvite.

Drought and salinity are two main climatic factors which determine plant yield, distribution and reduced soil water potential (Groppa and Benavides, 2008). They can be expressed by causing metabolic, physiological, molecular and morphological changes in plants which may affect both plant growth and its harvest. More than 10% of arable land is affected by both salinity and drought. More than 50% of decrease in crop yield is attributed to fast increasing salinity and drought on a global scale (Bartels and Sunkar, 2005; Bray *et al.,* 2000).

Crop yields are low in the subcontinent as its cultivation is decreasing in semi-arid regions mainly due to depletion of ground water sources. Salinity imposes high solute concentration and water deficient conditions both in soil and plants. High amounts of salt taken up by a plant can lead to cell desiccation, as elevated levels of salt outside a plant cell will cause water to leave the cell. The biochemical mechanism such as osmotic adjustment helps plants to adapt in dry and saline conditions.

Drought is the prolonged deficiency of surface or underground water to plants. The other climatic conditions that can lead to dehydration in plants include high salt concentration, low temperature, and loss of turgor pressure. The reasons that affect plant responses to drought stresses are the period of water deficiency, rate of onset and acclimatization of plant. The ability of the pigeonpea to withstand severe drought conditions is due to its deep rooting system (Flower and Ludlow, 1987) and thickened cell wall.

Stress resistance mechanisms can be grouped into two general categories;

- Avoidance mechanisms (eg., succulent photosynthetic stem of *Saguaro cactus*, a drought tolerant species) are adaptations, and evolutional improvements, and make plants active before exposure to stress;

- Tolerance mechanisms (eg., black spruce shows freezing tolerance due to osmotic adjustment) which alter the plant physiology thereby acclimating themselves to an suboptimal condition and permit the plant to withstand stress.

The hormones namely abscisic acid, salicylic acid, jasmonic acid and ethylene and secondary messengers help in the adjustment of plant stress responses. The plant stress resistance response involves transcriptional activation of a number of defense-related genes and enzymes, opening of ion channels, modification of protein phosphorylation status, and primary and secondary metabolism.

Stress-induced activation of some hydrolytic enzymes results in the creation of conditions that will promote growth and reproduction of pathogens. On the other hand, the structural reinforcement of plant cell walls make it more tolerant to passage of microbes and enzymatic deterioration. Polygalacturonase-inhibiting proteins (PGIPs) inhibits a specific subclass of necrotrophic pathogen cell wall degrading enzymes called polygalacturonidases (PGs). Free acids, benzoic acid (BA) and salicylic acid (SA) accumulate and encode defense related secondary metabolites such as lignins. Plants produce pathogenesis (PRPs) related proteins such as osmotin which contribute to defense by generating secondary signal molecules. Ethylene is necessary for mediating resistance against both fungal and necrotrophic pathogens. Reactive oxygen species (ROS) such as hydrogen peroxide are used as signal molecules in plant defense responses to pathogen infection.

The numerous changes such as attenuated growth, activation and expression of genes, transient increase in ABA and anti-oxidant levels and suppression of energy consuming pathways take place during the adaptation of plant to salinity and drought.

The anti-porters play a vital role in the plasma membrane and tonoplast for the exchange of Na^+ ions from the cytoplasm to vacuole and H^+ ions from vacuole to cytoplasm. The accumulation of polyamines (Chattopadhyay *et al.,* 1997 & 2002; Cona *et al.,* 2006; Angelini *et al.,* 2010) and compatible osmolytes such as iminoacid proline, glycine and betaine can protect proteins and membrane structures from regeneration of ROS and the resultant injuries (Verslues *et al.,* 2006; Delauney and Verma, 1993).

Tissue culture is an important component of integrated approach towards crop improvement against these stress related problems. The use of plant tissue culture method has led to the development of better varieties, faster use of valuable germplasms and unique individual ecotypes. Plant tissue culture and molecular biology facilitate handling of plants for gene manipulations and speed up screening and selection processes. Thus, it has substantially enhanced the efficiency of plant breeding. The regeneration of whole plants through tissue culture has made it possible to incorporate foreign genes into host plant cells and to recover transgenic pigeonpea plants.

Introduction of foreign genes into crop plants can be carried out by physical and biological methods. Physical methods include direct transformation with polyethylene glycol, microinjection, electroporation and the use of biolistic gun gene-delivery system. The biological method of *Agrobacterium* mediated gene transfer is the most commonly used and a less expensive method. It does not require protoplast culture; foreign genes are incorporated into the host genome at single site; and regeneration techniques for different explants are readily available.

The successful exploitation of cellular and molecular genetic techniques in crop improvement is possible only if plants can be effectively regenerated from cell or tissue cultures. This may occur either through somatic embryogenesis or shoot bud differentiation (organogenesis). Organogenesis means the *de novo* production of plant organs such as shoot buds, long shoots and roots from differentiated tissues, by first induction of localized meristematic activity followed by the formation of new shoots. Regeneration through multiple shoot induction under the action of BAP from a single explant is more important for transformation through *Agrobacterium* as it gives rise to multiple transformants.

Pigeonpeas are notoriously recalcitrant to *in vitro* culture and much effort was put forth for developing and standardizing efficient *in vitro* regeneration experiments. Due to its constant prevalence in regeneration frequency and plant turnover per explant, the regeneration through organogenesis is one of the most favourable pathway in genetic transformation studies. Among the various explant tissues, embryonic structures and cotyledons are highly active for the regeneration and induction of multiple shoot buds. The successful regeneration of various pigeonpea species has been achieved by modification of certain parameters such as explants, genotype and composition of media.

The main aim of the present study is to obtain salt tolerant pigeonpea. The objectives are: 1) to standardize pigeonpea genotypes for regeneration using three different explants; 2) to develop methods of *Agrobacterium*-mediated gene transformation of embryonic tissues with two different genes; 3) to over-express a *Vigna aconitifolia P5CSF129A* gene in transgenic pigeonpea to produce salt tolerant plants.