Food security is a major global concern and prime importance in current scenario. It has been projected that the world’s population is expected to reach about 9.1 billion by 2050 (about 34% higher than today’s population); and to cope up with this increasing population food production must be increased by 70% (FAO, 2009). Due to advancement of human life, industrialization, and population outnumber results into the decrease of arable land. To feed the world, at present itself, is a daunting task, conditions seem to be much difficult in near future due to dynamic global environmental changes. To face these challenges, modern agriculture implementing various biotechnological advancement to increase the crop productivity and looking for the constraints which limit the crop productivity. There are many factors which limit the crop productivity and all these factors may be grouped into biotic and abiotic factors. Biotic factors involved all the living being viz. bacteria, virus, fungi and others which create infection or competition by other organisms. Plants are very often exposed to environmental stresses viz. ions deficit or excess; water deficit or excess; temperature low or high; and light deficit or excess which prevents the yield of crop plants to express in their full genetic potential. To meet the demand of food production and deterioration of arable land creates the pressing need to harness the yield of crop plants in their fullest genetic potential. In recent years several potential genes and their products were discovered which have proven their capability to enhance the crop productivity under various abiotic stress conditions. Cereals and legumes comprise the major portions of the dietary requirement and constitute the major energy and protein source. The transporter genes which have been proven potential candidates for the salt tolerance and may be utilized for the increase the yield for global food demand. In order to prove the concept, present study was conducted. Groundnut, a
major oilseed crop plants, was genetically transformed with the vacuolar membrane transporter SbNHX1 with Agrobacterium- method and transgenic lines were raised. The transgenic plants growth was monitored under containment facility and seeds were harvested. The molecular, physio-biochemical characterizations of (T1) generation plants were carried out under lab conditions and it was found that transgenic plants were performed better compared to wild type plants in terms of survival under salinity stress conditions.

1.1 Salinity

Salinity is prevalent throughout the world. Salinity is a soil condition characterized by high levels of Cl\(^-\), SO\(_4\)^{2-}, CO\(_3\)^{2-}, HCO\(_3\)\(^-\) salts of Na\(^+\), K\(^+\), Mg\(^+\)or Ca\(^{2+}\) but, NaCl is the major cause in global context and around 830 to 950 million hectares land estimated to be salt-affected (Flowers et al. 1986; Rengasamy 2002; Brady and Weil 2008; Munns and Tester 2008; Rozema and Flowers 2008; Yadav et al. 2011; Hasegawa 2013). Soils are classified as saline when the electrical conductivity (EC\(_e\)) is more than 4 dS/m which is equivalent to approximately 40mM NaCl or osmotic pressure of 0.2 MPa (Munns and Tester 2008). About 50% of salt-affected soils are sodic, where; at least 15% of the soil cation exchange capacity is contributed by Na\(^+\) (Brady and Weil 2008; Rozema and Flowers 2008). High salinity stress is the most severe among abiotic stresses, which plague food production with perhaps the greatest threat being to sustainable irrigated agriculture. Salinity adversely affects crop yield and agricultural expansion and currently affects more than 10% of arable land, which results in decline of the average yields of major crops more than 50% (Yang et al. 2009). Soil salinity may be a result of heavy irrigation, poor water management, high evaporation and previous exposure to seawater.
Irrigated lands have twice the productivity that of the rain fed region and hence contribute world’s one third of the agriculture production. But current trend of salinization of irrigated land may be a limiting factors to the global food demand reducing the yield and growing area under cultivation.

These abiotic stresses negatively affect the growth and productivity and lead to yield penalty up to 50% in major crop plants (Vinocur and Altman 2005). Salinity stress hampers plant development by adversely affecting various biochemical reactions and physiological processes such as photosynthesis, antioxidant metabolism, mineral nutrients homeostasis, osmolytes accumulation and hormonal signaling. Improving salinity and drought tolerance of crop plants by genetic means has been an important but largely unfulfilled aim of modern agricultural development. Developing resistance against the biotic stress which is dependent on monogenic traits is easier to control and engineer comparative to abiotic stresses which are multigenic in nature and genetically complex. Plant engineering strategies for abiotic stress tolerance deployed several genes involved in membrane transport, signaling and regulatory pathways or genes that encode proteins conferring stress tolerance or enzymes present in the synthesis of functional and structural metabolites (Vinocur and Altman 2005). Recent advances in specialized plant membrane transporters can be used to enhance yields of staple crops, increase nutrient content and could expand available arable land by increasing resistance to key stresses, including salinity, pathogens and various metal toxicity (Schroeder et al. 2013).
1.2 Genetic Variation for Salinity Responses

Salinity is a powerful tool for the study of adaptation and speciation (Edelist et al. 2006; 2009; Lowry et al. 2009; Flowers et al. 2010). Species evolved and adapted to saline environments are referred as halophytes (Flowers et al. 1977; 1986) while those are sensitive to salinity and could not thrive well termed as glycophytes (Greenway and Munns 1980). Halophytes generally found in the extremes of various habitats and exhibit a range of salinity tolerance with some species having maximum growth only at high salt concentrations, while others survive equivalent to seawater concentrations (Flowers et al. 1977; Greenway and Munns 1980; Munns and Tester 2008). A more functional definition describes halophytes as plants which are survive to reproduce and complete the life cycle in salt concentration more than 200 mM or greater regardless of their natural environments (Flowers and Colmer 2008; Munns and Tester 2008; Flowers et al. 2010). In contrast, glycophytes, which includes most crop plants and constitutes around 99% of world flora, are salt sensitive. Glycophytes growth and biomass accumulation are severely affected at salt concentrations above nutrient optima, and many cannot survive at 200 mM of NaCl (Greenway and Munns 1980; Flowers et al. 1986; Flowers and Colmer 2008; Munns and Tester 2008). Salinity may be a factor for the speciation in different taxonomic groups (Flowers et al. 2010) with notable examples is Helianthus sp. (Edelist et al. 2009), members of the Triticeae (Huang et al. 2008; Nevo and Chen 2010) and Oryza sp. (Lin et al. 2004). Plants respond to salinity for enhancing salt tolerance by regulating developmental, physiological and metabolic processes and these responses are termed as acclimation, phenotypic plasticity or environmental variation (Hasegawa et al. 2000; Zhu 2002; Munns and Tester 2008; Taiz and Zeiger 2010). Acclimation process is likely an
inherent genetic trait that may also involve epigenetic responses (Borsani et al. 2005; Chinnusamy et al. 2008; Ramanjulu et al. 2007; Boyko and Kovalchuk 2011; Mirouze and Paszkowski 2011; Oh et al. 2012; Sunkar et al. 2012). Halophytes are potential genetic reservoir for “superior” alleles of known identified genes and novel loci of genes involved in Na\(^+\) homeostasis and salt tolerance (Gong et al. 2005; Flowers and Colmer 2008; Edelist et al. 2009; Oh et al. 2012). Emerging genomics and breeding technologies that include high throughput genotyping and phenotyping will facilitate whole genome approaches may reveal greater insight into salt tolerance mechanism and salt tolerance determinant identification of halophytes (Rivandi et al. 2011; Oh et al. 2012).

1.2.1 *Arabidopsis thaliana* as a glycophytic model for salinity stress responses

*Arabidopsis* is used as molecular genetic model system in abiotic stress research. By using loss- or gain-of-function experimental approaches, numerous salt adaptation and tolerance determinant were identified. These determinants were at genomics, proteomics and metabolomics levels and leads to identification of salt tolerant pathways among glycophytic plants and many useful insights to improve the tolerance mechanism in plants (Kasuga et al. 1999; Bressan et al. 2001; Apse and Blumwald 2002; Zhu 2002; 2003). Tomato is another glycophytic model that has been valuable for plant salt stress tolerance mechanisms because of its molecular genetic tractability and primary gene pool which offers substantial genetic variation for adaptive capacity (Bressan et al. 2001; Rubio et al. 2004).
1.2.2 *Thellungiella halophila* (salt cress) as a halophytic model for salinity stress responses

Salt cress (*T. halophila*) is a halophyte relative of Arabidopsis that also exhibits a high degree of freezing tolerance (*Bressan et al.* 2001; *Taji et al.* 2004). Salt cress is capable of reproduction even after exposure to 500 mM NaCl or -15°C (*Inan et al.* 2004). It is a tractable molecular genetic model because of its small plant size, prolific seed production, short life cycle, small genome (~2X Arabidopsis) and ease of transformation (*Bressan et al.* 2001). It is imperative to answer the basic questions that: do halophytes have unique salt tolerance mechanism, ‘better’ alleles that encode more effective determinants or regulatory cascades that provide these plants with greater capacity to more effective defense in response to salt imposition.

1.3 How salinity affects the plants and what is the counter mechanism

Plant responses to salinity are time dependent and depend on the onset of the salt stress. To get an insight into the physiological mechanism of salinity tolerance, it is a prerequisite to know, whether deleterious effect is due to osmotic stress of the salt in the soil or the toxic effect of the salt ions in the plant. Salinity affects plants in two phases: a rapid response to the increase in external osmotic pressure i.e. osmotic stress or hyper osmolality effect of salinity on plants and slower phase due to accumulation of Na⁺ in leaves i.e. ionic stress (*Munns and Tester* 2008). Osmotic phase starts immediately after the onset of salt stress around the roots and results in significant drop into shoot growth, reduction in the rate of leaves expansion, new leaves emerge comparatively slowly, and lateral buds development remain quiescent or slower, fewer branches or lateral shoots formation.
The second and slower response of salt on the plants is ion-specific and ionic stress. Ionic phase of stress starts when salt accumulates to toxic concentrations in the old leaves and they die because the older leaves already attained their maturity so, no longer expanding and leads to accumulation of Na\(^+\) and subsequently results into senescence of the leaves. Ionic stress causes the ionic disequilibrium at cellular level and affecting vital process \textit{viz.} photosynthesis and many enzymatic and metabolic pathways.

1.3.1 Mechanism of salinity tolerance

Plants display a diverse level of salinity tolerance. Some plants tolerate higher level of salinity and agriculturally, yield is not reduced at a given salinity as others plants do at the same salinity. This variability may be in major groups of plants (mangroves and chenopods which are dominated by salt tolerant species), between closely related species and between different varieties or even between individuals of the same varietal line. In the same way dicotyledonous and monocotyledonous plants also varies in their responses to salinity. Dicotyledonous plants could maintain higher Na\(^+\) and Na\(^+\): K\(^+\) ratios in shoots while monocotyledonous plants tolerate lesser Na\(^+\): K\(^+\) ratios and accumulate less Na\(^+\) because the former can sequester the Na\(^+\) to vacuole.

Tolerance to salinity involves a variety of processes that operate in different parts of the plants and more than one of these processes simultaneously working within plants. These processes can occur in all cells within plants or in specific cell types to confer the tolerance to salinity. This reflects that salt tolerant mechanism could be studied at two level of organization. First includes various processes which are operated at cellular level which ultimately impart salinity tolerance and second many different mechanisms working at the whole plant level enabling plant to cope up with high salinity stress.
1.3.2 Tolerance at cellular level

1.3.2.1 Intracellular compartmentation: Vacuolar sequestration

The vacuole provides a large compartment for Na⁺ storage in a mature plant cell. The plant cell adapt an effective way to reduce Na⁺ concentration in the cytosol by sequestration of Na⁺ into the vacuole which prevents the toxic effects of Na⁺ on the cytosolic cellular processes. The vacuolar membrane (the tonoplast) facilitate the Na⁺ sequestration into the vacuole through the function of tonoplasm Na⁺/H⁺ exchangers (NHX) which are driven by the proton motive force across the vacuolar membrane generated by the vacuolar H⁺-ATPase (V-ATPase) and H⁺ pyrophosphatase (V-PPase) (Apse and Blumwald 2002; Gaxiola et al. 2007).

Figure 1.1: Cytosolic sodium ion (Na⁺) homeostasis in plant cell by transporters like NHX, SOS1, HKT and NSCC. Diagram was adopted from Hasegawa (2013).
There are various types or isoforms of Na\(^+\)/H\(^+\) antiporters have been reported from Arabidopsis, rice and mammalian systems. These isoforms show differences up to certain degree in tissue specificity, regulation and expression patterns. Brett et al. (2005) classified the eukaryotic NHE (Na\(^+\)/H\(^+\) exchangers) gene family on the basis of cellular location, ion selectivity, inhibitor specificity, and protein sequence similarity, into two major clades, the intracellular (IC, endosomal/TGN, NHE8-like, and plant vacuolar) and plasma membrane (PM, recycling and resident). The IC clade can be further sub divided into two main groups: Class-I and Class-II (Pardo et al. 2006). In Arabidopsis, Class-I category (AtNHX1-4) are 56–87% similar, however Class-II (AtNHX5 and 6) are 79% similar but Class I and Class II are only 21–23% similar among either of the group (Yokoi et al. 2002). The Class-I NHX proteins, reported and characterized so far, are localized in the vacuolar membrane and form a separate clade within the IC group that is comprised of plant exchangers. However, Class-II members, present in endosomal vesicles of plants and homologous proteins with various endosomal localizations are also reported in animals and fungi (Pardo et al. 2006). The plants represent a characteristics vacuolar NHE clade which is abundantly and exclusively found in plants. The absence of ATP-powered plasma membrane sodium intracellular pumps in plants may be the reason for development of the specialized clade of vacuolar NHE in plants, which act to store high concentrations of salt and water in the vacuole.

Sequestration of Na\(^+\) into the vacuoles has been proposed as an important salt-tolerance mechanism in plant; however, the molecular nature of the vacuolar membrane transporters for Na\(^+\) sequestration was discovered only in recent years. Apse et al. (1999) found the better salt-tolerance of transgenic plants by over expressing AtNHX1 in
Arabidopsis and found that the improved salt tolerance was attributed to increased Na\(^+\) compartmentation in the vacuoles. Later on AtNHX1 was also found to confer salt tolerance in tomato and canola (Zhang and Blumwald 2001; Zhang et al. 2001). Gaxiola et al. (1999) has characterized the Arabidopsis AtNHX1 and showed that this transporter may functionally complement the yeast vacuolar membrane Na\(^+\)/H\(^+\) antiporter ScNHX1. The AtNHX1 and its role in plant salt tolerance, was studied by its overexpression in plants and established its role in salt tolerance of crop plants. NHX transporters, usually up-regulated in response to salt stress and also its regulation may be controlled by the SOS pathway (Qiu et al. 2004) however their strong constitutive expression suggested that they have functions other than vacuolar sequestration of sodium (Hanana et al. 2009). These functions may include a role in plant development (Apse et al. 2003; Hanana et al. 2007); in vesicle trafficking and protein targeting (Sottosanto et al. 2007); in the transport of monovalent cations besides Na\(^+\), such as Li\(^+\), Rb\(^+\) and in particular, K\(^+\) (Wu et al. 2005). All of which have been shown to be substrates for NHX antiport with protons; and a role in pH homeostasis. Interestingly, the NHX1 protein in morning glory (Ipomea nil) appears to be involved in the pH control of flower color; an intentional mutation in InNHX1 resulted in the partial inhibition of vacuolar alkalization, and inhibited change in floral color (Fukada-Tanaka et al. 2000). The roles of NHX in pH homeostasis, and in Na\(^+\) sequestration, are inextricably linked to the activity of proton pumps in the tonoplast; simultaneous over expression of NHX and the vacuolar pyrophosphatase AVP has led to enhanced salt tolerance in rice (Zhao et al. 2006) and A. thaliana (Brini et al. 2007). The up-regulation of NHX in response to salt stress is well documented, but vacuolar proton pumps are ambiguous. In wheat, the expression of the
pyrophosphatases showed that at least one isoform is salt-inducible (Wang et al. 2009), while earlier report showed little change in response to salt stress (Brini et al. 2005). In cucumber (Cucumis sativus), vacuolar pyrophosphatase H$^+$ electrochemical potentials across the plasma membrane, tonoplast, and endosomal membranes are largely responsible for passive and active Na$^+$ fluxes across these membranes (Figure 1.1; Niu et al. 1995; Blumwald et al. 2000; Epstein and Bloom 2005; Schumacher 2010). The plasma membrane H$^+$-ATPase, which has catalytic and regulatory domains in the cytosol, utilizes energy derived from ATP hydrolysis for vectorial H$^+$ efflux to the apoplast that generates the H$^+$ gradient across the membrane (Palmgren et al. 2001). This pump acidifies the apoplast (pH ~5.5) approximately 1.5 to 2.0 pH units relative to the cytosol (pH ~7.2) and is largely responsible for an inside negative potential of about -120 to -150 mV across the plasma membrane (Figure 1.1; Niu et al. 1995; Blumwald et al. 2000; Palmgren et al. 2001). The inside negative plasma membrane potential, and high apoplastic Na$^+$ concentrations establish a thermodynamic potential whereby Na$^+$ influx across the plasma membrane typically is passive and efflux is active (Figure 1.1; Flowers and Yeo 1992; Niu et al. 1995; Blumwald et al. 2000; Hasegawa et al. 2000; Epstein and Bloom 2005), while Cl$^-$ influx is active and efflux is passive (Teakle and Tyerman 2010).

External Ca$^{2+}$ reduces net intracellular Na$^+$ influx and help in the maintenance of K$^+$ and Na$^+$ homeostasis (Greenway and Munns 1980; Reng et al. 1992). Ca$^{2+}$ activates high-affinity K$^+$ uptake, increasing K$^+$ over Na$^+$ selective uptake (Rains and Epstein 1967; Zhu et al. 1998). Ca$^{2+}$ activation of a CBL-interacting kinase (CIPK)/calcineurin B-like (CBL) pathway is responsible for phosphorylation of AKT1 and high-affinity K$^+$ uptake,
which reduces Na\(^+\) leakage (Pardo and Rubio 2011). \([\text{Ca}^{2+}]_{\text{ext}}\) also inhibits Na\(^+\) influx that presumably occurs through NSCCs (Figure 1.1; Schachtman and Liu 1999; 2004; Tyerman et al. 2002). Physiological evidence implicates NSCCs but their molecular identity has yet to be determined. When \([\text{Ca}^{2+}]_{\text{ext}}\) levels are low (mM), however, Na\(^+\) influx through these channels may be a substantial component of intracellular uptake (Amtmann and Sanders 1998; Rus et al. 2001; 2004; Demidchik and Maathuis 2007; Plett and Møller 2010). In addition, \([\text{Ca}^{2+}]_{\text{int}}\) leads to SOS1 activation to facilitate Na\(^+\) efflux to the apoplast reducing net Na\(^+\) uptake (Figure 1.1; Shi et al. 2000; 2002; Qiu et al. 2002; Zhu et al. 2002; 2003; Pardo and Rubio 2011). There are two tonoplast H\(^+\) electrogenic pumps; a multi-subunit V-type (vacuolar type) ATPase and the AVP1 H\(^+\)-pyrophosphatase (PPase) (Figure 1.1) for which ATP or pyrophosphate (PPi), respectively, are substrates (Gaxiola et al. 2007; Paez-Valencia et al. 2011). These pumps couple energy from ATP or PPi hydrolysis to H\(^+\) transport that establishes ~1.5 to 2.0 pH unit gradients (lower in the vacuolar lumen) across the tonoplast, and contributes to a membrane potential of between 0 to - 40 mV between the cytosol and the lumen (negative) (Figure 1.1; Blumwald et al. 2000). The Na\(^+\) electrochemical potential across the membrane determine whether influx or efflux into the vacuolar lumen is active or passive (Blumwald et al. 2000). V-type ATPase activity also may facilitate Na\(^+\) sequestration into endosomes to reduce cytosolic ion levels (Schumacher K 2010).

The SOS Ca\(^{2+}\) signaling pathway plays an important role in Na\(^+\) homeostasis and facilitates Na\(^+\) efflux across the plasma membrane regulating net uptake (Figure 1.1 Zhu 2002, 2003; Pardo and Rubio 2011). However, it is not fully resolved yet, how the Na\(^+\) signal is perceived. Ca\(^{2+}\) established its role as secondary messenger in signal
transduction (Zhu, 2002, 2003). NaCl causes the induction of cytosolic Ca\textsuperscript{2+} increase; Ca\textsuperscript{2+} activated the calcineurin B and neuronal Ca\textsuperscript{2+} sensor-like protein SOS3 (CBL4), which is a myristoylated protein with Ca\textsuperscript{2+} binding sites (Liu and Zhu, 1998; Ishitani et al. 2000; Zhu, 2002, 2003; Gong et al. 2004; Pardo and Rubio 2011). Ca\textsuperscript{2+}-activated SOS3 physically binds with the auto-inhibitory domain of SOS2 (CIPK24) and this binding of auto-inhibitory domain activates kinase activity (Liu et al. 2000; Zhu 2002, 2003; Pardo and Rubio, 2011) SOS2 kinase then phosphorylates the SOS1 Na\textsuperscript{+}/H\textsuperscript{+} in the plasma membrane and activating the Na\textsuperscript{+} efflux from the cytosol to the apoplast (Qiu et al. 2002; Quintero et al. 2002; Zhu, 2002, 2003; Pardo and Rubio 2011; Quintero et al. 2011). There are evidences which support the notion that the SOS pathway is tightly conserved in plants, including crops (Oh et al. 2009; Olias et al. 2009; Feki et al. 2011).

1.3.2.2 Gene regulation under salinity stress

The high salinity response requires the induction of transcription of many genes, thus the activity of specific sets of transcription factors and their binding to specific sequences in the promoter regions of target genes are very prerequisite (Chen et al. 2002; Xiong et al. 2002). Transcription factors are integral in linking tolerance responses with the salt sensory pathways. Several sets of transcription factor family genes were found to be differentially expressed in response to elevated external salinity (Golldack, et al. 2011). Among them, the most common are the basic leucine zipper (bZIP) (Yang et al. 2009), WRKY (Jiang et al. 2009), APETALA2/ ETHYLENE RESPONSE FACTOR (AP2/ERF) (Kasuga et al. 1999), MYB (Cui et al. 2013), basic helix–loop–helix (bHLH) (Jiang et al. 2009), and NAC (Tran et al. 2004) families. These transcription factors are reported to regulate the expression levels of various genes that may ultimately influence
the level of plants salt tolerance (Figure 1.2). To counteract the water potential decrease resulting from the osmotic component of enhanced salinity, genes relevant for inorganic ion uptake and osmolyte synthesis are unregulated (Geng et al. 2013).

1.3.2.3 Synthesis of osmoprotectants or compatible solutes

Compatible solutes are water soluble, neutral or zwitterionic compounds which do not interfere the biochemical reactions in the cytoplasm. These osmoprotectants used to balance the osmotic potential of the cell as Na\(^+\) influx into the vacuole creating an imbalance of the osmotic potential. Osmoprotectants are often secondary metabolites such as quaternary ammonium compounds (e.g. glycinebetaine) polyols (e.g. mannitol, sorbitols etc) as well as core metabolites such as proline and sucrose. Over accumulation of osmolytes and compatible solutes are another mechanism, many plants adapts to escapes from the deleterious effects of the salt stress.

1.3.3 Whole plant adaptations to high salinity

Although various features of every cell within the plant that enhance cellular survival and thus increases the tolerance of the whole plant to salinity stress, plants also have a wide range of other mechanisms that involve particular activities of specific cell types. These mechanism utilizing the anatomical as well as physiological features which confers to escapes the cell from the toxic level of Na\(^+\) and thus enhancing the cell survival and in turn plant adaptation to higher salinities. These processes may be discussed in following subheadings.
1.3.3.1 Regulation of Na\textsuperscript{+} transport to the shoot

Plant adaptation to high salinity at whole plant level involve various processes which enables plant to restricts Na\textsuperscript{+} from reaching to the cytoplasm or the accumulation into the shoots. There is a general rule that crop plants are more tolerant to the salinity if there is little accumulation of NaCl in shoot and if less Na\textsuperscript{+} reaching to the cytoplasm or accumulation in the shoots plants are more tolerant to the salinity. But this is not the rule always many of the crop plants and varieties are the exception to this rule and they responded differently e.g. barley and cotton accumulating more Na\textsuperscript{+} into their shoots and they are more salt tolerant compared to wheat, accumulating less Na\textsuperscript{+} into the shoots but comparatively more salt sensitive. There are many processes have been discovered and some more hypothesized in plants tolerance to salinity and people around the globe are working for conclusive way to establish the mechanism how plants manage to protect themselves from cellular toxicity. There are three pathways for Na\textsuperscript{+} influx into cell: Two protein-mediated pathways may be distinguished by their sensitivity and insensitivity to extracellular Ca\textsuperscript{2+}, and a third pathway appears to be due to 'leakage' into the root via the apoplast.
Figure 1.2: Overview of the cellular Na\textsuperscript{+} transport mechanisms; role of different transporter under salinity tolerance in plant root cell adapted by Deinlein et al. (2014). Na\textsuperscript{+} is said to sense by an yet to unidentified sensory mechanism inside the cell. Once, the salt is sense by the cell, Ca\textsuperscript{2+}, ROS, and signaling hormones cascades are activated. CBLs, CIPKs, and CDPKs are part of the Ca\textsuperscript{2+}-signaling pathway, which can alter the global transcriptional profile of the plant. Ultimately, these early signaling pathways result in expression and activation of cellular detoxification mechanisms, including HKT, NHX, and the SOS Na\textsuperscript{+} transport mechanisms as well as osmotic protection strategies. Furthermore, the Na\textsuperscript{+} distribution in the plant is regulated in a tissue-specific manner by unloading of Na\textsuperscript{+} from the xylem. Abbreviations: NSCCs, nonselective cation channels; ROS, reactive oxygen species; CDPKs, calcium-dependent protein kinases; CBLs, calcineurin B-like proteins; CIPKs, CBL-interacting protein kinases; AP2/ERF, APETAL2/ETHYLENE RESPONSE FACTOR; bZIP, basic leucine zipper; NHX, Na\textsuperscript{+}/H\textsuperscript{+} exchanger; SOS, SALT OVERLY SENSITIVE.

1.3.3.2 The Ca\textsuperscript{2+}, a player in mitigating the Na\textsuperscript{+} toxicity

The Ca\textsuperscript{2+} role in mitigating the toxic effect of Na\textsuperscript{+} has been reviewed thoroughly and its role is well established in cellular Na\textsuperscript{+} and K\textsuperscript{+} homeostasis. Ca\textsuperscript{2+} effects are very
complex but play important role in the protection of Na$^+$ toxicity by reduced inhibition of the Na$^+$ accumulation into roots and shoots of plants while stimulating the accumulation of K$^+$. The Ca$^{2+}$ mediated reduction in accumulation of Na$^+$ is partly due to a partial inhibition of the unidirectional influx of Na$^+$ into the roots and also minimizing the Na$^+$ induced efflux of K$^+$ (Cramer et al. 1985). It is confirmed now that most likely pathway for the important Ca$^{2+}$- sensitive Na$^+$ influx is non-selective cation channels (NSCCs) (White 1999; Davenport and Tester 2000; Demidchik et al. 2002). There are many candidate genes which encode these non-selective cation channels (NSCCs). The two major candidate genes for non-selective cation channels which functional validation have been established are; cyclic nucleotide gated channels (the CNGCs) and the putative glutamate activated channels (the GLRs) (Leng et al. 2002; Lacombe et al. 2001). In Arabidopsis, out of the 20 predicted cyclic nucleotide-gated channels, AtCNGC1, 3, 4 and 10, have been shown to catalyze Na$^+$ uptake while AtCNGC2 was shown to exclude Na$^+$ and be permeable to K$^+$, indicating that there are majority of the family members are involved into Na$^+$ uptake compared to only certain family members might be responsible for influx of Na$^+$ (Balague et al. 2003; Hua et al. 2003; Gobert et al. 2006; Guo et al. 2008; Leng et al. 2002). In rice, OsCNGC1 expression was down regulated in a salt-tolerant rice cultivar upon salt stress compared with a more salt-sensitive rice cultivar; this down-regulation might have observed because the salt-tolerant cultivar allowed the restricted Na$^+$ influx (Senadheera et al. 2009).

The study of 20 glutamate receptors family (GLRs) of ion channels in Arabidopsis indicates that there might be three classes of receptors, each activated by different group of amino acids. Transgenic plants overexpressing AtGLR3;2 were hypersensitive to ionic
stress of Na\(^+\) and K\(^+\) (Kim et al. 2001). Many different studies revealed that the different types of GLRs are involved in the Na\(^+\) transport.

1.3.3.3 HKT: a Ca\(^{2+}\) independent Na\(^+\) influx

The Na\(^+\) influx by Ca\(^{2+}\) is not completely inhibited so Na\(^+\) influx probably occurs to some extent through non-selective cation channels independent of Ca\(^{2+}\) (Davenport & Tester 2000). Several other transporters have been reported potentially to mediate this influx, including those encoded by the HKT gene family (Platten et al. 2006). HKT gene family may be categorized into two: class I and II on the basis of sequence and transport analyses. Class I HKT transporters; in most cases mediate more Na\(^+\)-selective transport (Uozumi et al. 2000; M a`ser et al. 2002) whereas Na\(^+\)–K\(^+\) co-transport was attributed to Class II HKT transporters (Rubio et al. 1995). Disruption mutations in AtHKT1;1 the sole HKT gene in Arabidopsis, encoding a class I transporter, cause Na\(^+\) hypersensitivity of leaves coupled with Na\(^+\) overaccumulation in leaves with a concomitant reduction in root Na\(^+\), upon salinity stress (Ma`ser et al. 2002, Berthomieu et al. 2003, Horie et al. 2006). Detailed studies of AtHKT1;1 and its rice ortholog OsHKT1;5 have further demonstrated a major role in the removal of Na\(^+\) from the xylem sap into the xylem parenchyma cells and thereby minimizing the leaves cellular Na\(^+\) toxicity (Ren et al. 2005, Sunarpi et al. 2005, Horie et al. 2006, Davenport et al. 2007) (Figure 1.2). Tissue specific over-expression of AtHKT1;1 in the stele enhances salt tolerance (Moller et al. 2009). In vivo analyses by patch clamping on root stellar cells from Athkt1;1 mutant and wild type plants have provided evidence that AtHKT1;1 mediates passive Na\(^+\) channel-like transport (Xue et al. 2011). AtHKT1;1-mediated Na\(^+\) removal from the xylem has been suggested to stimulate indirect K\(^+\) loading into xylem vessels via outward-rectifying
K⁺ channels, resulting in a high K⁺/Na⁺ ratio in leaves which is a prerequisite for the cellular survival and salinity tolerance (Ren et al. 2005, Sunarpi et al. 2005). TaHKT1;4 gene which encodes a class I HKT transporter, was found to protect leaf blades from Na⁺ overaccumulation by Na⁺ removal from xylem in the leaf sheath (Huang et al. 2006). There are certain regulators of AtHKT1;1 have been identified and to engineer more salt-tolerant plants, further insights is required about synergistic effects of certain traits combinations for the tolerance. The plant hormone cytokinin is negative regulator of the expression of AtHKT1;1 in Arabidopsis roots via the type B response regulators ARR1 and ARR12, therefore, in response to salinity stress cytokinin level decreases and AtHKT1;1 expression increases (Mason et al. 2010). Recently, Shkolnik-Inbar et al. (2013) reported a negative regulation of root AtHKT1;1 expression through ABA-INSENSITIVE 4 (ABI4), a transcription factor. Loss-of-function mutations analysis revealed that ABI4 gene rendered soil-grown plants more salt tolerant, with lower Na⁺ levels in shoots thus correlating with increased AtHKT1;1 expression. By analogy, ABI4 overexpressing lines were found hypersensitive due to lower AtHKT1;1 expression. (Shkolnik-Inbar et al. 2013).

1.4 Epigenetics and salt tolerance

Information that is passed from one generation to next; to daughter cells at cell division or from parent to offspring- but is not coded in DNA sequences is referred as epigenetics. Chromatin modifications or epigenetic modifications have been proposed to contribute to the adaptation potential of plants under different environmental stresses (Baek et al. 2011; Zhu 2008). Several studies have claimed that chromatin modification is the result of resistance responses of plants to salt stress in the same generation as the
stress occurs. In this context, Arabidopsis was reported for hyperosmotic priming that is plants have been treated with mild salt stress in the seedling stage, followed by cultivation under control conditions. Under the control condition, no visible differences between pretreated plants and control plants were observed. However, after another salt stress application, the pretreated groups were found to accumulate less Na\(^+\) and thus were more salt tolerant. This observed phenotype was attributable to epigenetic histone modifications that mainly affected expression of transcription factors (Sani et al. 2013). The study of Arabidopsis phenotypes also revealed that induction of HKT1 expression was lower in controls compared to pretreated plants, which might be the reason for better salt tolerance of pretreated plants (Sani et al. 2013). Epigenetics involve methylation of bases in DNA and RNA which is responsible for epigenetic attributes and many recent studies confirmed that the DNA methylation of many promoters and coding regions of four transcription factors in soybean (Glycine max) were affected with the salinity. This is an insight to the hypothesis that chromatin modification of certain genes could enhance salinity tolerance of plant (Song et al. 2012).

### 1.5 Groundnut

Groundnut (Arachishypogaea L.) is one of the major oilseed leguminous crop plant grown in subtropical and tropical regions of the world. Groundnut is the rich source of oil (40-60%), protein (10-20%) and carbohydrates (Pandey et al. 2012). It is widely used as edible oil throughout the world and ranked third among all the edible oils (Kumari et al. 2012). India is the second largest producer of groundnut after China while USA ranked third in the terms of production whereas India has the highest area under cultivation (USDA 2014). Groundnut oil serves as a rich source of vitamins, minerals,
antioxidants, monounsaturated fatty acids and also a source of medicinally important compounds. The major vitamins found in groundnut oil are Thiamin, Riboflavin, Niacin, Pantothenic acid, vitamin B₆, Folate, vitamin C and vitamin E. Its oil contains almost all macronutrients such as calcium, magnesium, potassium and important micronutrient like zinc, iron and phosphorus. The antioxidant and medicinally important compounds viz. $p$-coumaric acid and resveratrol, polyphenols, flavonoids and isoflavones are also reported to present in the groundnut oil (Janial et al. 2013). It has been found a good source of energy, 100g kernels of groundnut provide around 564 Kcal energy after combustion (Jambunathan 1991). Due to ease of availability and laden with nutritious value groundnut become one of the essential commodities to implement in the routine food. The therapeutic food plumpy nut was based on groundnut and it was used extensively to fight the malnutrition poor children in Niger and have been saved the thousands of life.

In India groundnut oil is used extensively as cooking medium. Around 60% of the total global production is used for the oil extraction for edible and industrial purposes and remaining 40% is used for the storage and seed for the next generation crops (Singh and Diwakar 1993; Janial et al. 2013). The waste cake by-product of oil extraction, used preferably for the feeding of animals and it was reported to contain more nutritious value compared to cereal feed. The oil cake is also used by fertiliser industry to make fertilisers. All these values of groundnut make the groundnut a commercial cash crop and so efforts are being made to increase its production and area under cultivation.

1.5.1 Taxonomy and biology
The cultivated groundnut (*Arachishypogaea* L.) is an annual herb belonging to the family fabaceae (leguminosae), grouped into two subspecies, subsp. *fastigiata* Waldron and subsp. *hypogaea* Krap. et. Rig. The subsp. *fastigiata* contains four botanical varieties, var. *vulgaris*, var. *fastigiata*, var. *peruviana*, and var. *aequatoriana*. The subsp. *hypogaea* contains two varieties, var. *hypogaea* and var. *hirsuta*. Plant, pod, and seed characteristics of each botanical type are different (Krapovickas and Gregory 1994). Groundnut is an allotetraploid (2n = 2x = 40) with “AA” and “BB” genomes. All species, except the cultivated species (*A. hypogaea* and *A. monticola*) in Section *Arachis*, and certain species in Section *Rhizomatosae*, are diploid (2n = 2x = 20). The “AA” and “BB” genomes of *Arachis hypogaea* contributed by diploid progenitors *A. duranensis* and *A. ipaensis*, respectively (Kochert et al. 1996). Groundnut belongs to NEW WORLD crops and its center of origin is supposed to be Southern Bolivia and Northern Argentina (Kochert et al. 1996).

Groundnut is an annual herb. Its rooting system is very strong tap root while stem length or plant height grows up to 50 cm with stretched or erect stems. The leaves may be compound, pinnate, antiparallel with long stalk containing four leaflets. The flower is tubular calyx cleistogamous with self-pollination. After fertilization, stalk of the ovary elongates geotropically which is termed as peg or gynophore. Peg penetrates into the soil up to depth of approximately 7 cm for the pods development (ICRISAT, India). The flowering generally starts within the 17-35 days after sowing depending on the cultivar and environmental conditions. The complete life cycle of groundnut is 90-130 days, depending on the cultivars or genotype used.
Based on the growing and branching habit groundnut is categorised in four groups (Rajgopal et al. 2002). These groups are Spanish (having synchronous maturity and shorter duration), Virginia Runner, and Valencia and Virginia bunch. The bunch cultivars have the erect growth of the shoot above the ground whereas runners grow parallel to the earth surface. There are several cultivars have been raised and used for the cultivation from all the groups around the globe. The cultivars from the different groups varied in terms of oil yielding potential, application, flavours and also productivity.

1.5.2 Status of groundnut production in India

Groundnut is an important source of edible oil, food, and feed legume crop grown in over 100 countries. It covered 24 million ha area worldwide with a total production of 38 million tons in 2010 (FAO 2008). Statistics by the Food and Agriculture Organization (FAO 2009) of the United Nations show that peanut is the world’s 13th most important food crop, the 4th most important source of edible oil and the 3rd most important source of vegetable protein. India has the largest area (around 5.0-5.4 mha) under groundnut cultivation whereas it stands second in total annual production after China. Among groundnut producing states in India, Gujarat is the largest producer (2.64 million metric tons) contributing 38.14% of total production. Tamil Nadu ranked second in terms of production while its productivity is maximum. Table 1.1 represents the state wise groundnut production, area under cultivation and productivity from the major groundnut producing states of India. According to DAGS (2011), Saurastra region contributes almost 90% of the groundnut production in Gujarat. The seven districts Amreli, Bhavnagar, Jamnagar, Junagadh, Kutch, Porbandar and Rajkot are the groundnut producing districts of Gujarat while Jamnagar district is the largest among all the districts.
Table 1.1: Groundnut production, area under cultivation and productivity from the major groundnut producing states of India

<table>
<thead>
<tr>
<th>State</th>
<th>Year: 2011-2012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Production (million metric tons)</td>
</tr>
<tr>
<td>Gujarat</td>
<td>2.64</td>
</tr>
<tr>
<td>Tamilnadu</td>
<td>1.07</td>
</tr>
<tr>
<td>Andhra Pradesh</td>
<td>0.85</td>
</tr>
<tr>
<td>Rajasthan</td>
<td>0.80</td>
</tr>
<tr>
<td>Karnataka</td>
<td>0.50</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>0.36</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>0.34</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>0.09</td>
</tr>
<tr>
<td>Odissa</td>
<td>0.07</td>
</tr>
</tbody>
</table>


Globally groundnut is an important source of protein and cattle feed and originated in the South America. Then groundnut were distributed globally and many of the countries are the principally growing it as oilseed plants. It is also called crop of the poor
According to USDA (2014), China shares the highest production to global groundnut production however USA is the highest in terms of yield although it ranked third global production. India is the second largest producers in the world though yield per hectare is much lower compared to USA and China which demands the need to improve the production to become the world leader in global groundnut production (Table 1.2).

**Table 1.2:** Description of area under cultivation, yield and annual production of groundnut in top 10 producing countries of the world in the year 2012/13 and projected data for the year 2013/14

<table>
<thead>
<tr>
<th>Country</th>
<th>Area (million ha)</th>
<th>Yield (metric tons per ha)</th>
<th>Production (million metric ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>4.70</td>
<td>4.75</td>
<td>3.55</td>
</tr>
<tr>
<td>India</td>
<td>5.00</td>
<td>5.40</td>
<td>1.00</td>
</tr>
<tr>
<td>USA</td>
<td>0.65</td>
<td>0.42</td>
<td>4.73</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2.50</td>
<td>2.50</td>
<td>1.20</td>
</tr>
<tr>
<td>Indonesia</td>
<td>0.68</td>
<td>0.67</td>
<td>1.68</td>
</tr>
</tbody>
</table>

1.5.3 Factors affecting groundnut production

Groundnut is the major leguminous oilseed crop plants and grown into subtropical region of the world. According to USDA (2014), there is lot of variation among the yield and productivity across different region of the world. Top five countries producing groundnut, India has the minimum yield (1.0-1.02 metric tons per hectare) while USA has the average production of 4.24- 4.74 metric tons per hectare. In India, causes of least
productivity are the various biotic and abiotic cues. Major biotic factors responsible for the yield loss in groundnut are the viruses, bacteria, fungi, aphids and thrips. The pathogens causing significant loss of productivity include *Peanut mottle virus* (mottle virus), *Cercospora arachidicola* (early leaf spot), *Cercosporidium personatum* (late leaf spot), *Groundnut rosette virus* (rosette virus), *Puccinia arachidis* (rust), *Aphis craccivora* (aphids), *Frankliniella* spp. (thrips) and, *Amrasca devastans* (jassids) worldwide (Singsit et al. 1997; Swathi-Anuradha et al. 2008; Culbreath and Srinivasan 2011; Culbreath et al. 2013, Pandey et al. 2012), certain insect pests *viz. Apro aeremamodicella, Spodoptera litura, Amsacta* spp., *Heliothis* spp., Lepidopteran and termites cause major yield losses (Prasad and Gowda 2006; Tiwari et al. 2008). Major abiotic factors which affect the growth, causes yield penalty and significantly reduces the productivity are drought, salinity, high temperature and excessive or low light intensity.

Although several efforts have been made to enhance crop productivity through conventional breeding, the results are not satisfactory. However, complex genetic behavior of the majority of desired traits; conventional breeding is in adequate to address the increasing demand for peanut higher productivity. In terms of genetic improvement, the narrow genetic base, the tetraploid and complex nature of the genome of cultivated peanut, are some of the serious bottlenecks. Only limited genetic diversification has been achieved in the past through interspecific hybridization between cultivated peanut and other species of section *Arachis* due to differences in ploidy levels and the linkage drag. Eliminating the linkage drag involves back crossing, a lengthy process that also results in dilution of the level of resistance present in wild relatives of *Arachis*. In India, where most of the groundnut production dependent over rainfall, there are only two ways to enhance
the production to meet the increasing demand, either to increase the yield per hectares or to expand the growing area under cultivation which is largely affected with many environmental factors like salinity, sodicity and alkalinity. As conventional breeding and hybridization proven to inefficient to combat the increasing demand of groundnut production, there is a need to look into modern tools of genetic engineering and plant tissue culture to find better solution to fulfill the increasing demand and production.

1.5.4 Genetic transformation of groundnut

Conventional plant breeding has put many milestones for the improvement of crop yields and in development of many resistance varieties to enhance the production of crop plants. But legumes and especially groundnut which is produced by a single hybridization events and have very narrow germplasm, doomed the plant breeders. Genetic engineering proved to be useful tool to introduce the desired traits and characters into such plants. Many important agronomic traits have to improve to develop new cultivar or variety to mimic the deleterious effect of many abiotic and biotic cues for better production. There are three important aspects for a successful transformation experiment to conduct. The first key step is to develop a well regeneration system and second to increase the number of transformation events and third selection of the transformed event. Therefore, a successful tissue culture or regeneration protocol must be established for the efficient transformation protocol or to develop the transgenic groundnut for improved traits.

Plant regeneration or tissue culture system may be accomplished either by somatic embryogenesis or through direct organogenesis. In groundnut, several reports of plant regeneration systems based on organogenesis and somatic embryogenesis have been published. The explants used for regeneration through organogenesis were zygotic
embryo (Joshi et al. 2008; Livingstone and Birch 1999), axillary meristem (Singh and Hazra 2009), epicotyl (Little et al. 2000) and immature leaflets (Joshi et al. 2003). Bibliographic searches revealed a plethora of regeneration protocol of groundnut through direct organogenesis or somatic embryogenesis. Recently, plantlet regeneration procedure through direct organogenesis from different explants viz. cotyledon (Radhakrishnan et al. 2000; Venkatachalan et al. 2000; Venkatchalam et al. 1998), de-embryonated cotyledon (Asif et al. 2011; Qin et al. 2011; Tiwari et al. 2011; Tiwari and Tulli, 2008; Sharma and Anjaiah 2000), zygotic embryo (Khandelwal et al. 2003), cotyledonary node (Iqbal et al. 2012; Verma et al. 2009; Swathi-Anuradha et al. 2006; Swathi-Anuradha et al. 2008; Venkatchalam et al. 1998), hypocotyl (Dodo et al. 2008; Matand and Prakash 2007; Swathi-Anuradha et al. 2006; Venkatchalam et al. 1998), epicotyl and immature leaflets (Shan et al. 2009) has been established. Most of the recent reports on regeneration and transformation of groundnut used vertical halves of de-embryonated cotyledon explant. Regeneration from the cotyledon explant used different concentration of BAP in combination with different concentration of 2, 4-D in most of the regeneration protocol. The MS-salts (Murashige and Skoog 1962) and Gamborg’s B5 medium (Gamborg et al. 1968) supplemented with 3% sucrose, 20 μM BAP and 10 μM 2,4-D at pH 5.8, induced multiple adventitious shoot buds in 95.5% of explants after 15 days of culture (Sharma and Anjaiah 2000). Similarly, Qin et al. (2011) has utilized 10 mg/l BAP and 1 mg/l 2,4-D phytohormone as efficient inducer of shoot buds from the transformed de-embryonated cotyledons. The best combination for efficient regeneration of transformed explants is 4 mg/l BAP and 0.1 mg/l NAA (Asif et al. 2011). In contradictory to these combination of phytohormones, Radhakrishnan et al. (2000), Tiwari and Tulli (2008; 2012), and Tiwari et
al. (2008; 2011) have used BAP with the modified MS to final concentration of 15, 20, 20, 20 and 20 mg/l, respectively for efficient regeneration of shoot buds. Since, different genotype/cultivars used in the above studies with genetic variation responds to phytohormones differently and this might be the reason for great variability in use of hormones or concentration. For, elongation of regenerated explants, cytokinin (BAP) and auxin (2,4-D) are used at lower concentration than as used during regeneration. Sharma and Anjaiah (2000) have used reduced concentration of (BAP upto 2 µM) for shoot elongation while Tiwari and Tulli (2008; 2012) have optimized 3 mg/l BAP for elongation. Whereas rooting media was also utilised by different authors were different. Media optimized for the rooting was MS basal (Radhakrishnan et al. 2000), MS basal supplemented with 5 µM NAA (Sharma and Anjaiah 2000), 1 mg/l NAA (Tiwari and Tulli 2008; 2012; Tiwari et al. 2008; 2011) and 0.3 mg/l IBA (Khandelwal et al. 2003).

1.5.4.1 Particle gun-mediated transformation

Microprojectile or particle bombardment or gene gun mediated genetic transformation is a major physical process of transforming a different range of plants including many recalcitrant, but agronomically important crops which are otherwise difficult to be transformed via Agrobacterium (Christou 1992). Particle bombardment facilitates a wide range of transformation strategies, high molecular weight DNA delivery into plant cells and simultaneous multiple gene transformation with no biological constraints and host limitations (Altpeter et al. 2005).

There are several reports of generating fertile transgenic peanut plants using particle bombardment method. An approach to vaccine production against virus in plants Bluetongue VP2 gene (BTVP2) coding for capsid protein is bombarded upon the zygotic
embryos. Successful development of transgenic plants from these embryos through somatic embryogenesis provided an efficient protocol for transgenic groundnut development and opened an avenue for vaccination of cattle for viral diseases (Athmaram et al. 2006). Somatic embryos induced by using mature zygotic embryos with Piclorams, developed for several groundnut cultivars were transformed through particle bombardment and successful regeneration of transgenic plants was achieved (Livingstone and Birch 1999; Dodo et al. 2005; Chu et al. 2008). Chu et al. (2013) have optimised the protocol for particle delivery where they used very low concentration of plasmid gold particle (50 µg/shot), DNA (70 ng/shot) and increased the transformation efficiency and number of transgene copy number per transformation. Since, particle bombardment method has advantage that it has no host or genotype dependency (Livingstone and Birch 1999). But, the biolistic transformation generally produces transgenic plants with high copy number (Travella et al. 2005; Dai et al. 2001), transgene instability and vector backbone integration along with the T-DNA (Khanna and Raina 2002; Hu et al. 2003; Kohli et al. 2010). Economically it is little costly as it requires tungsten or gold particle for the bombardment.

1.5.4.2 Agrobacterium-mediated transformation

Agrobacterium is known as natural genetic engineer. The Agrobacterium-mediated transformation of plants utilizes pathogenicity of a gram-negative soil bacterium of Rhizobaceae family, Agrobacterium tumefaciens and A. rhizogene. It is generally considered superior to biolistic method due to the advantages of low copy transgene events in transgenic plants and better stability of transgene expression (Harwood 2012). There are several factors like endogenous phytohormones (Liu and Nester 2006; Nonaka
et al. 2008; Yuan et al. 2008) secondary metabolites and phenolic compounds (Sahi et al. 1990; Zhang et al. 2000) and host defence mechanisms (Yuan et al. 2007; Anand et al. 2008) which negatively regulate the transformation efficiency in plants.

There are several factors which affect the Agrobacterium mediated transformation efficiency. Generally it’s the growth of Agrobacterium cultures in the late log phase (OD$_{600}$ 1.0-1.6) were reported to give higher transformation efficiency in groundnut (Akcay et al. 2009; Qin et al. 2011; Tiwari and Tulli 2012). Acetosyringone, a phenolic compound, activates the Agrobacterium for T-DNA transfer and several reports have claimed increased transformation efficiency in several plant species after its addition in co-cultivation media (Tiwari and Tulli 2008; 2012; Patel et al. 2013). The oxidative burst, at the time of co-cultivation of Agrobacterium with the explant, is one of the earliest observable aspects of defence (Wojtaszek 1997; Qiusheng et al. 2005). It affects transformation efficiencies by generating ROS which may kill the Agrobacterium or inhibit their growth (Wu et al. 1995) or the hypersensitive response to Agrobacterium causes rapid death of transformed cells (Greenberg et al. 1994) and strengthening of cell wall to avoid transformation (Bradley et al. 1992). Qiusheng et al. (2005) has used different antioxidants to obtain a higher transformation efficiency and regeneration. Antioxidants protect cells from oxidative stress by scavenging free radicals when used in the co-cultivation media (Tiwari and Tulli 2012; Dutt et al. 2011). The L-cysteine, an antioxidant enhances the transformation efficiency in several crop plants while used in the co-cultivation media at time of transformation (Tiwari and Tulli 2012; Frame et al. 2002; Olhoft et al. 2003; Kumar et al. 2011). Use of 2,4-D in co-cultivation media have been reported to enhance transformation efficiency in several plants (Mannan et al. 2009; Pena
The phenolic compounds which are generally released from the explants at wound site may get oxidized after exposure to air and the explants turns brown and died (Egnin et al. 1998). Washing of explants with ½ MS salts solution containing myo-inositol or PVP protect explants from oxidation and results in the survival of explants (Tiwari and Tulli 2012; Egnin et al. 1998). Reducing monosaccharide viz D-glucose, assist transformation by enhancing VirA-VirG sensitivity to phenol and also by elevating the saturating concentration of phenol (Lacroix et al. 2011; Cangelosi et al. 1990; Cangelosi et al. 1990) which is proven worth full in groundnut transformation (Tiwari and Tulli 2008; 2012). Lower pH (5.6-5.7) of the co-cultivation media also reported to increase the transformation efficiency by activating the VirG expression (Charles and Nester 1993). Temperature play an important role in transformation, Uranbey et al. (2005) has described the temperature dependency of the vir genes expression and T-DNA delivery in plant tissue. The optimum temperature for groundnut transformation was reported by Tiwari and Tulli (2012) for co-cultivation of explants and agrobacterium at 18-21 °C for highest efficiency. Cotyledons, because of their higher regeneration efficiency compared to leaf, are preferred explants for transformation in most of the annual plants (Schmidt and Willmitzer 1988; Sujatha et al. 2012; Dai et al. 2014, Sharma and Anjaiah 2000; Guo et al. 2005; Dai et al. 2007).

There are several successful transformation events in groundnut using Agrobacterium-mediated transformation. For example, transgenic groundnut plants with a rice chitinase gene showed enhanced resistance to Aspergillus flavus infection in root and LLS and rust disease in the foliar tissues (Iqbal et al. 2012; Prasad et al. 2013). The two Indian cultivars of groundnut Kadiri 6 (K6) and Kadiri 134 (K134) have been transformed...
with TSV coat protein (CP) gene, the defense mechanism of transgenic plants against stem necrosis disease caused by TSV is enhanced significantly (Mehta et al. 2013). Transgenic lines (cultivar JL24) overexpressing Cry1EC (δ-endotoxin) gene under control of PR-1a inducible promoter showed 100% mortality of the Spodoptera litura at all the developmental stages (Tiwari et al. 2011). Increased tolerance towards the leaf spot disease causing fungal pathogens like Pheaoisariopsis personata and Cercospora arachidicola was exhibited by the transgenic groundnut over expressing a mustard defensin gene (Anuradha et al. 2008).

AtAVP1 overexpressing groundnut, showed improved biomass production, photosynthetic rates and tolerance towards drought and salinity stress (Qin et al. 2013). Ectopic expression of AtNHX1 gene into groundnut plants showed more chlorophyll content, higher photosynthetic rates and biomass productivity as compared to wild type groundnut plants at 150mM NaCl stress (Banjara et al. 2012). The transgenic groundnut plants over expressing AtNAC2 showed improved ability to tolerate drought and salinity tolerance compared to wild-type (Patil et al. 2014). Groundnut plants over expressing PDH45, a pea helicase gene revealed better chlorophyll stability, water use efficiency and improved biomass production in stressed or non-stressed conditions (Manjulatha et al. 2014). Asif et al. (2011) has reported that transgenic groundnut over expressing AtNHX1 whose expression is driven by 35S CaMV promoter showed improved tolerance property towards the salinity and drought stress condition. Qin et al. (2011) has reported that over expression of IPT gene improves the drought tolerance and 51 to 65% higher yield in the field condition without affecting the oil quality.
1.6 Salt tolerance and SbNHX-1 gene

The vacuole of a mature plant cell, functions as a large compartment for Na⁺ storage. A vacuolar Na⁺/H⁺ antiporter pumps Na⁺ into the vacuole by making use of the H⁺-ATPase and H⁺-PPiase coupled with the vacuolar H⁺-translocating enzymes, the H⁺-ATPase and the H⁺-PPiase, which produce electrochemical H⁺ gradients (Blumwald 1987). The activity of Na⁺/H⁺ antiporter was shown to be present in tonoplast vesicles from red beet storage tissue (Blumwald et al. 1985). The first plant Na⁺/H⁺ antiporter, AtNHX-1, was isolated from Arabidopsis by functional genetic complementation of a yeast mutant defective for endosomal Na⁺/H⁺ activity and was found similar to the mammalian NHE transporter (Apse et al.1999; Gaxiola et al.1999; Quintero et al. 2002). Several crop plants have been reported to transform with NHX-1 gene and shown to enhance their performance under high NaCl environment. Most of the studies reported in the Table 1.3 and their improved attributes under NaCl condition. Table 1.3 was adapted from Zhang et al. (2013).
Salicornia brachiata Roxb. (Amaranthaceae) is an extreme halophyte, grows in saline ecosystem along the coastal areas of Gujarat. The halophytes have a unique genetic makeup allowing them to grow and thrive well under salt stress conditions (Agarwal et al. 2010). The experimental model for study of salt tolerance in our laboratory concentrated on an extreme halophyte, Salicornia brachiata. Salicornia can grow in a wide range of salt concentrations (0.1–2.0 M) and can accumulate quantities of salt as high as 40% of its dry weight (Agarwal et al. 2010). This unique characteristic provides an advantage for the study of salt tolerance mechanisms. Salicornia accumulates salt in the pith region, which reflects the fact that antiporter genes are necessary to maintain homeostasis in extreme salinity. This plant may serve as a model plant to study the salt responsive genes. Several stress responsive known genes like SbASR-1 (Jha et al. 2009, 2012), SbGSTU (Jha et al. 2011b), SbpAPX (Singh et al. 2014), SbDREB2A (Gupta et al. 2010), SbMT2A (Chaturvedi et al. 2012) and SbSOS1 (Yadav et al. 2012) from S. brachiata have been cloned and characterized. A salt-tolerant SbNHX1 gene has been cloned from S. brachiata (NCBI accession number: EU448383) and its functional validation was carried out in transgenic tobacco (Jha et al. 2011) and Jatropha (Jha et al. 2013). Transgenic tobacco over-expressed with SbNHX1 gene showed better growth, increased biomass and chlorophyll content as compared to wild type tobacco plants under NaCl concentrations up to 300 mM. The SbNHX-1 gene, further transformed to Jatropha which has displayed better salt tolerant characteristics at green house Therefore, SbNHX1 gene cloned from S. brachiata may be utilized to develop salt tolerant crop plants for better performance under saline conditions. Groundnut, a principal crop plant in Gujarat, which production is compromised by high salinity, a matter of concern for the farmers, have chosen in present
study to develop its salt tolerance which may be better utilized in salinity affected areas, leads to enhance production. To validate this hypothesis, an effort was made to develop the transgenic groundnut with enhanced salt tolerance by using a local cultivar, following objective have been decided in the present work

a) Genetic transformation of groundnut (A. hypogaea) with salt tolerant gene SbNHX-1 cloned from Salicornia brachiata

b) Raising the transgenic lines

c) Molecular and physiological analysis of transgenic plants for salt tolerance at different stages of growth