1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) also known as peanut, is allotetraploid (4x=40) and an important cash crop for farmers particularly in the semi-arid tropical and sub-tropical region between 40° N and 40° S (Shoba *et al.*, 2012). The genus *Arachis* belongs to the *Fabaceae* family and included in subfamily *Papilionoidae*, tribe *Aeschynomeneae* and subtribe *Stylosanthinae* (Holbrook and Stalker, 2003). The species, *hypogea*, is an annual herb and grouped into two subspecies *viz.* subspecies *hypogaea* which has two botanical varieties (*hypogaea* and *hirsuta*), while subspecies *fastigiata* has four botanical varieties (*fastigiata*, *vulgaris*, *peruviana*, and *aequatoriana*) (Krapovickas and Gregory, 1994).

*A. hypogaea* has six centres of diversity but the greatest amount of variations are found in Brazil where species of *Arachis* originally evolved (Gregory *et al*., 1980) and it is also thought to be the centre from where the distribution of *Arachis* had been occurred (Krapovickas, 1973).

RFLP analysis and cytological studies has proved that two set of genomes "A" and "B" in *Arachis hypogaea*. Each genome has identical pair and was contributed by *A. duranensis* and *A. ipaensis* respectively as a result of a single hybridization event, thus indicating its single origin. Further chloroplast analysis indicated that *A. duranensis* was the female progenitor of the cross (Kochert *et al*., 1996). The chromosomes of *Arachis* species are small and ranging from 1.4 to 3.9 µm in length (Holbrook and Stalker, 2003) and the size of the peanut genome is 2,813 Mb/IC (Arumuganathan and Earle, 1991). Recently, the population explosion has shown an alarming concern across the globe and it is expected that the world will be outnumbered with nine billion by the end of year 2050. While, crop production is decreasing due to the various effects of biotic and abiotic stresses, and thereby minimizing the economic losses is a concern for all nations to cope with the increasing demand of food by supplying sufficient nutrition to the mankind.

There are various stresses affecting development and production of crop plants; however loss due to abiotic stresses is more prominent as compared to the loss that occur due to biotic stress like insect/pests, weeds and diseases. Among abiotic stresses, salinity and drought are the most pervasive yield limiting factors. Salinity
affects 7 % of land across the globe accounting for 930 million ha of land (Szabolcs, 1994). It is estimated that increased salinization of arable land will have profound deleterious effects across the globe which could result in 30 % land loss within next 25 years, and it may go up to 50 % by the year 2050. Irrigation systems are also prone to salinization. It is estimated that about half of the existing irrigation systems of the world are under the influence of salinization, alkalization or water logging (Szabolcs, 1994). Irrigation scheme covers only 15 % of the cultivated land of the world (227 million ha in 1987). Rest of the rainfed area also suffers from drought due to inadequate and erratic rainfall. In India, more than 60 % of the agricultural land is rainfed and subject to varying levels of abiotic stresses particularly water deficit stress.

Groundnut is the world's 4th most important source of edible oil and the 3rd most important source of vegetable protein. It was grown in about 21.77 million ha of land, yielding 38.61 million tons of pods with an average yield of 1773.68 kg/ha across the globe. India is the second largest producer of groundnut in the world and this crop was cultivated at about 5.26 million ha of land, yielding 6.96 million tons of pods with an average yield of 1322.96 kg/ha; while in Gujarat, it is grown in 1.68 million ha of land producing 2.71 million tons of pods with a productivity of 1611.50 kg/ha (FAO, 2012).

Groundnut is extensively grown in the semi-arid tropics by resource-poor farmers where many abiotic and biotic factors limit its productivity and quality. Several biotic and abiotic stresses limit groundnut production all together causing annual yield losses of over $ 3.2 billion (Dwivedi et al., 2003). Biotic and abiotic stresses happen to be the major constraints in groundnut production. The major abiotic factors affecting groundnut production include drought, low availability of phosphorus especially under acidic soil conditions, non-availability of iron in calcareous soils (Dwivedi et al., 2003), and salinity stress (Srivastava et al., 2007). However, in some countries particularly in India and Vietnam, tolerance to cold temperature is required as the low temperature prevailing during the sowing results in delayed germination and a reduced growth rate thus delaying the harvest (Dwivedi et al., 2003). Even though groundnut crop is fairly drought-tolerant, production fluctuates considerably as a result of rainfall variability (Reddy et al., 2003). There are reports that photosynthesis and vegetative growth of groundnut is...
well adapted to high temperature, while reproductive growth is extremely sensitive to high temperature (Craufard et al., 2003).

As water is a scarce resource, there is little hope in improvement of production scenario by bringing more areas of land under irrigation programme; in such a situation it is better to concentrate on development of crops with increased tolerance capability to various abiotic stresses.

So, one of the important ways to enhance the productivity in stressful environment is to breed crops that are more tolerant to stress. Classical plant breeding methods involving inter-specific or inter-generic hybridization, induced mutation and in vitro induced variation have been applied to improve the abiotic stress tolerance of various crop plants but without much success.

These techniques have limitations because tolerance to stress is controlled by many genes, and their simultaneous selection is difficult (Richards, 1996; Yeo, 1998) and tremendous effort is required to eliminate undesirable genes that are also co-transferred during breeding programme (Richards, 1996).

The success in transferring stress resistance traits from wild Arachis species to cultivated groundnut through conventional breeding has been limited mainly because of cross compatibility barriers, the linkage of resistance trait with many undesirable pod characteristics, and the long period of time required for developing stable tetraploid interspecific derivatives (Dwivedi et al., 2003). Further, lack of efficient selection procedures particularly under field conditions (Ribaut et al., 1997) also makes the effort more tedious.

The earlier molecular technologies showed low level of genetic polymorphism in A hypogaea, but recent advanced molecular markers such as SSR detected more frequent genetic polymorphism with their co-dominant nature resulted in the construction of moderate density genetic linkage maps (Holbrook et al., 2011). However, till date there is no successful report on development of abiotic stress tolerant groundnut exploring molecular breeding strategy due to identification of only minor QTLs for improved water use efficiency (Bhatnagar-Mathur et al., 2014) and their subsequent utilization in marker assisted selection, lacking of appropriate breeding strategy to be integrated with marker assisted selection (e.g. foreground selection, background selection) and unavailability of high-throughput
economical assays for identification of large number of markers (Hoolbrook et al., 2011).

Genetic transformation offers an alternative approach to address the problems of breeding strategies by making the transfer of genes from alien sources across the higher taxonomic units. This strategy is feasible for generating transgenic plants possessing tolerance to biotic and abiotic stresses. Genetic transformation in plants is based on the principles of cellular totipotency and bacterial natural genetic transformation. However, this strategy has some issues such as environmental safety concern, economic constraints and chances of horizontal transfer of transgene.

Genetic engineering has been considered to be worthy and versatile technique in developing and rendering several crops tolerance to various abiotic stresses. Genetic engineering offers a unique approach for developing tolerant crop in a faster and more precise way to achieve improved tolerance to stress (Cushman and Bohnert, 2000), because it avoids the transfer of unwanted chromosomal regions. However, through genetic engineering, multiple genes can be assembled and simultaneously introduced to the crop of interest. Already single action heterologus genes for biotic and abiotic stress tolerance have been introduced in groundnut genome. But very few reports are available as to abiotic stress tolerant groundnut till date. In order to restore cellular function and to make plant more tolerant to multiple abiotic stresses, transferring single action gene may not suffice the required level of tolerance (Bhatnager-Mathur et al., 2008). There are many functional target areas in abiotic stress signal transduction and metabolic pathways of plant for engineering tolerance to various abiotic stresses.

Abiotic stress tolerance is a polygenic character involving a large number of genes by their up and/or down regulation mechanisms in response to a particular stress. So, enhancing tolerance towards multiple stresses simultaneously and at a higher level required to transfer a single gene that encodes for a transcription factor which regulates expression of stress inducible multiple genes upon exposed to stresses. Transcription factors have been shown to produce multiple phenotypic alterations in transgenics as compared to wild type by their expression at molecular level, many of which are involved in stress responses. The dehydration-responsive element binding proteins (DREBs) are important transcription factors
comprise of conserved ERF/AP2 DNA-binding domain and regulate expression of a set of abiotic stress inducible, downstream genes by binding to their cis acting element region (such as DRE/CRT) of promoter and impart stress tolerance to plants. The DREB transcription factors could be grouped into DREB1/CFB (CRT binding factor) and DREB2, which are involved in two separate signal transduction pathways under low temperature; salt and dehydration respectively (Agrarwal et al., 2006). DREB genes have been isolated from various plant species and genetically engineered plants overexpressing DREB genes for abiotic stress tolerance have been reported (reviewd by Agarwal et al., 2006). \textit{AtDREB1A/ CBF3} gene(s) have been introduced in \textit{Arabidopsis thaliana} to play a crucial role in promoting the expression of stress tolerance genes and to confer stress tolerance in transgenic plants; and these transgenics showed tolerance to various abiotic stresses (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Kasuga et al., 1999). Heterologus expression of \textit{AtDREB1A} gene in various transgenic crops such as tobacco, rice and wheat conferred enhanced tolerance to low temperature, salinity and drought stresses respectively (Kasuga et al., 2004; Datta et al., 2012; Pellegrineschi et al., 2004).

So genetic engineering of groundnut by overexpression of heterologus stress tolerance gene could be a viable option to the constraint of breeding programme. Transgenic groundnut var. JL24, a Spanish type variety presently phased out of cultivation in India, overexpressing \textit{AtDREB1A} gene under the control of \textit{rd29A} promoter showed tolerance to water deficit condition with improved transpiration efficiency (TE) (Bhatnagar-Mathur et al., 2007). Overexpression of \textit{AtDREB1A} gene in transgenic groundnut triggered increased biosythesis of antioxidant enzymes and proline, reduced the level of lipid peroxidation, but these antioxidative machinery did not play causative role in improved TE (Bhatnagar-Mathur et al., 2009a). However, \textit{AtDREB1A} transgenic groundnut showed improved growth-related traits like Harvest index, root:shoot ratio, root length (Jagana et al., 2012; Vadez et al., 2013) and 20-30 % lower reduction in pod yield (Bhatnagar-Mathur et al., 2014) than wild type under progressive soil moisture deficit stress. However, till date no report is available on \textit{AtDREB1A} transgenic groundnut which shows tolerance to salinity stress.
Keeping the views in mind, the present study has been carried out to develop transgenic groundnut by incorporating the transgene AtDREB1A in cv. GG20; premium, a high yielding, bold seeded, Virginia bunch type cultivar; and to express AtDREB1A gene under the control of stress inducible rd29A promoter in those transgenics for improved tolerance to various stresses like drought, salinity, and soil moisture deficit stresses at vegetative (pre-flowering) and reproductive (flowering and peg formation) phases; and their subsequent molecular, physio-biochemical characterization; analysis of growth-related parameters of those transgenics after maturity; establishment of correlation between transgene expression, physio-biochemical and growth-related parameters; and to identify better transgenic events than presently available for further utilization in crop improvement programme as pre-breeding resources. The present research work was carried out with the following objectives:

1. To develop transgenic groundnut resistant to abiotic stress by expressing AtDREB1A gene.

2. To characterize the putative transformants for integration, expression and inheritance of introduced gene.

3. To carry out evaluation of transgenic plants for resistance to abiotic stress under glasshouse condition.