1. **INTRODUCTION**

In the current scenario of burgeoning human population (FAO, 2009) and alarming global climate change (Thuiller et al., 2005) there is an overall need to sustain and enhance agricultural productivity. This is especially true in the context of abiotic stresses in general and drought in particular, that constitute a major constrain for agricultural productivity worldwide (Boyer, 1982; Araus et al., 2002; Dita et al., 2006). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is an international research centre committed to agricultural research in the dry land tropics, covering over 55 countries in Asia and sub-Saharan Africa that is abode to the worlds poorest of the poor. The semi-arid tropics (SAT) are characterized by the unpredictable weather, limited and erratic rainfall, nutrient-poor soils, and occurrence of several pests and diseases being the major constrains limiting crop productivity (Serraj et al., 2005; www.icrisat.org).

Drought is the number one factor limiting crop productivity in the rain-fed production systems of the SAT. In addition to its direct effects on yield, drought reduces both, carbon assimilation through photosynthesis due to limited gas exchange as well as symbiotic nitrogen fixation (SNF), in legume crops (Serraj et al., 1999). Besides, drought also leads to aflatoxin contaminations that result in significant reductions in crop yield, soil fertility and crop value. Climatologists believe that the ensuing global climate changes might produce even more severe and widespread dry conditions in these
regions with potentially serious consequences for global agriculture, crop productivity and food availability in the near future (Fischer et al., 2001; Wenzel and Wayne, 2008). Food security would be indefensible in the absence of significant increase in the crop yields in these marginal rain-fed agricultural lands of SAT that contribute significantly to the world food production (Bhatnagar-Mathur et al., 2010).

Peanut (*Arachis hypogaea* L.) is an annual legume also known as groundnut, earthnut, monkey-nut or goobers, is a valuable cash crop for millions of small-scale farmers living mostly in the SAT. It is world’s fourth most important source of edible oil, third most important source of vegetable protein and thirteenth most important food crop of the world (http://www.cgiar.org). Peanut is one of the three mandatory legume crops of ICRISAT, which is a designated world repository for peanut germplasm with over 14,000 accessions of cultivated and 450 accessions of wild *Arachis* species currently held in public trust. About 70% of the world’s peanut is produced in the SAT, the exclusive agro-ecosystem focus of ICRISAT (Nigam and Lenné, 1996). Although, peanut originated in South America it is mainly produced in Asia and Africa; India, China and USA are its largest producers. The crop is grown in 25.2 million ha throughout the world in over 100 countries between the latitudes 40° N and 40° S with a total global production of 36.5 m t (FAO, 2008).
While different parts of the peanut plant including kernel (seeds), root, haulms and shell are used, the seeds are a rich source of high-quality, edible oil (45–50%), easily digestible protein (23–25%), minerals, and vitamins (Savage and Keenam, 1994; Wrenshall, 1949). About two-third of world’s peanut production is used for oil, the remaining one third is consumed as food. It’s cake is used as feed or for making other food products, while the haulms provide quality fodder (Nigam and Lenne, 1990). The cultivation of peanut also helps to improve soil fertility as it leaves behind a substantial amount of nitrogen in the soil. Peanut kernels are widely acknowledged as a rich and cheap source of vegetable protein, and if included in the daily diet, can help decrease the dietary protein deficiencies (Nigam et al., 2003; Nigam and Aruna, 2008).

India has the largest peanut growing area with 6.7 million ha (27.3%) and stands second in the production at 6.5 million t (18.2%). Acreage, production and productivity of peanut in India has shown large amount of fluctuations since 1993-94 to 2006-07. Rainfall pattern during the pre-sowing months and availability of substitute high-value oilseed crops like soybean and sunflower of short durations requiring less water had significant negative impact on acreage allocation decisions of the farmers (Patil et al., 2009). The average yield per unit area in India, 970 kg ha\(^{-1}\), is deplorably lower than that of Israel where the average yields of 5,401 kg per hectare have been reported (FAO, 2005; Patil et al., 2009). The low productivity of peanut in India is mainly due to the fact that, 80% of the crop is grown under
rainfed conditions by the resource poor farmers (Kaushik, 1993). Moreover, a big gap exists between the realized yield and potential yield of peanut at both subsistence and commercial systems of production in Asia and Africa due to both abiotic and biotic factors. The major abiotic factors affecting peanut production include drought, high temperature, low soil fertility, low soil pH and iron deficiency. Among the biotic factors, diseases caused by pathogenic fungi, virus and bacteria, nematodes, foliar insect pests and contamination with mycotoxins caused by *Aspergillus flavus* are important (Sharma and Ortiz, 2000; Dwivedi, 2003). In the rainfed subsistence agriculture, drought is the major cause for low and erratic pod yield in peanut that contributes to over 6.7 m t loss in annual world peanut production (Subbarao et al., 1995), that results in estimated monetarily losses of over US $ 520 million, annually (Sharma and Lavanya, 2002). Yield losses in peanut due to water deficits vary depending on timing, intensity and duration of the deficit, coupled with other location-specific environmental stress factors such as high irradiance and temperature (Nigam et al., 2001). Genetic improvement and improved management practices can help bridge this gap. Drought management strategies, whether agronomic or genetic, in the water scarce SAT regions, needs to focus on maximizing extraction of available soil moisture and the efficiency of its use in crop establishment (Serraj et al., 2005).

Both, conventional and trait-based approaches are being used for improving drought tolerance in crop plants (Saxena, 2002;
Bänziger M and Araus, 2007; Ashraf, 2010; Cázares, 2011). Conventional plant breeding approaches for drought tolerance are based on selection for yield and its components in a given drought environment. While such approaches have been partly successful, they require large investments in land, labor and capital to screen a large number of progenies (Araus et al., 2002). The progress in the direct screening for grain yield has been hampered by the low heritability, polygenic control, epistasis, significant genotype-by-environment (G X E) interaction and quantitative trait loci (QTIs)-by-environment (QTL X E) interaction (Piepho, 2000a; Cattivelli et al., 2008). While plant breeding efforts in the past relied heavily on empirical approaches for drought tolerance in crop plants, these were hindered mainly by the quantitative genetic base and poor understanding of the physiological basis of yield under water-limiting conditions (Blum, 1998; Passioura, 2002), evidently leading to marginal returns. Thus, currently there is a broad consensus on adapting strategic approaches based on sound physiological and genetic understanding of yield for achieving further yield gains (Jackson et al., 1996; Miflin 2000; Slafer 2003; Snape et al., 2001; Serraj et al., 2005; Bhatnagar-Mathur et al., 2009).

Recent breakthroughs in the biotechnology research have encouraged targeted breeding of crops for drought tolerance and use of new genomic tools to enhance crop water productivity (Jones et al., 1997; Prioul et al., 1997; Ribaut and Poland, 2000; Varshney et al., 2005; 2009a, 2010). Accordingly, at ICRISAT a holistic approach of
crop improvement is being pursued where the traditional breeding practices are complemented with modern tools of biotechnology, i.e., genomics for carrying our marker-assisted plant breeding, and genetic engineering for developing transgenic crops with biotic and abiotic stress tolerance (Sharma and Ortiz, 2000; Sharma and Bhatnagar-Mathur, 2006a).

Marker-assisted breeding is revolutionizing the improvement of temperate field crops (Toenniessen et al., 2003) and will have similar impacts on breeding of tropical crops (Dwivedi et al., 2003). Compared to conventional approaches, genomics offers unprecedented opportunities for dissecting quantitative traits into their single genetic determinants, the so-called quantitative trait loci (QTLs), thus paving the way to marker-assisted selection (MAS) (Morgante and Salamini, 2003; Varshney et al., 2005, 2006; Ribout et al., 2007) and, eventually, cloning of QTLs (Salvi and Tuberosa, 2005) and their direct manipulation via genetic engineering (Bhatnagar-Mathur et al., 2008; Varshney et al., 2009). The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops indicates a growing interest in this approach. Furthermore, next-generation sequencing technologies, new genomics platforms and continuous evolution of bioinformatics have all added new dimensions for deciphering and manipulating the genetic basis of drought tolerance (Tuberosa et al., 2002, 2005; Varshney et al., 2005, 2009, 2010, 2011a). However, despite all the recent technological breakthroughs, the overall contribution of genomics-assisted breeding
to the release of drought-resilient cultivars has so far been marginal mainly due to the major challenge of the implementation of accurate, high through-put phenotyping for drought tolerance traits which again is due to the rudimentary understanding of the physiology of yield under drought and difficulty in predicting the phenotypic value of a new assembly of alleles, as well as very high cost of applying genomics strategies and tools in breeding program (Varshney et al., 2005; Tuberosa and Salvi, 2006; Bhatnagar-Mathur et al., 2008; Varshney and Dubey, 2009b).

Genetic engineering appears to be an attractive option where genes of interest originate either from cross barrier species, distant relatives or from non-plant sources that cannot be introgressed through normal breeding. Genetic engineering has been successfully used to complement traditional breeding methods in crop improvement (Fernandez-Cornejo J et al., 2002; James, 2009). This technology continues the trend of improving crops with more precise methods, permitting the transfer of a single gene with a known function into existing crop varieties, in contrast to the cross breeding techniques which transfer thousands of genes of unknown functions into crops (Sharma et al., 2005). Transfer of genes from heterologous species provides the means of selectively introducing new traits into crop plants and expanding the gene pool beyond what has been available to traditional breeding systems (Rajaram and Borlaug, 1997). Further, it is possible to control the timing, tissue-specificity,
and expression level of the transferred genes for their optimal function (Sharma and Ortiz, 2000).

A major limitation in the successful utilization of the transgenic technology is the requirement of a highly efficient tissue culture based regeneration and genetic transformation system, specifically for the desired crop as well as ways to minimize the possible unexpected and unwanted side-effects as the function of the introduced homologous or heterologous genes in plant species (Takeda et al., 2008). At ICRISAT, a highly efficient tissue culture and transformation system for peanut has been developed, which is genotype independent and works efficiently for different cultivars belonging to both Spanish and Virginia types (Sharma and Anjaiah, 2000; Sharma and Bhatnagar-Mathur, 2006). This *Agrobacterium tumefaciens* based transformation protocol has been optimized using cotyledon explants for the development of genetically engineered peanut cultivars (at high transformation frequencies of 55 to 70%) for routine use in transformation as part of basic studies in gene expression (Sharma and Anjaiah, 2000). Using this method, it is routinely possible to generate a large number of independently transformed transgenic events of peanut that do not show any morphological abnormalities and set viable seeds in a period of 6 to 8 months from the start of tissue cultures.

Transgenic plants of peanut for better drought tolerance were thus developed at the Genetic Transformation Laboratory of ICRISAT.
through genetic transformation of peanut cultivar JL 24 by introducing the DREB1A gene, a transcription factor under the control of stress inducible promoter from the rd29A gene, both being derived from Arabidopsis thaliana (Bhatnagar-Mathur et al., 2004, 2007). Molecular characterization for the presence, integration and expression of the transgene (rd29A:DREB1A) in the transgenic events of peanut was carried out through PCR, inverse PCR, RT-PCR, Southern blot analysis and DDRT-PCR, as well as by cloning the genes specifically expressed under imposed water limiting conditions (Reddy, 2008). Simultaneously, 14/50 transgenic events of peanut at T2 and T3 generation were screened for varying transpiration efficiency (TE) in the two pot-based drydown experiments under contained greenhouse conditions (Bhatnagar-Mathur et al., 2007). The adaptive response of the transgenic plants was compared with the untransformed control parent of peanut variety JL 24 under the imposed water stress by recording the changes in leaf area development, dry matter partitioning, transpiration and TE (Bhatnagar-Mathur et al., 2007; Devi, 2008).

The present study was an extension of the work done so far at ICRISAT, taking further closer to the realization of the production of peanut transgenic plants for drought tolerance. Based on the above mentioned studies (Bhatnagar-Mathur et al., 2007; Devi, 2008; Reddy, 2008) six transgenic events of peanut cultivar JL 24, namely, RD2, RD11, RD12, RD19, RD20 and RD33, having a single copy of the transgene insert and varied TE, were selected for further evaluation of
their drought response at genetic and physiological level. Main objectives of the present investigation were:

1. Development of new $rd29A$:DREB1A transgenic events of peanut through $A$. tumefaciens-mediated genetic transformation of the peanut genotype ICGV86031, a high yielding germplasm line with multiple resistances against Spodoptera, leaf minor, jassids and thrips.

2. Physiological evaluation for induced drought tolerance in the six transgenic events of peanut to identify the best events in terms of better yield performance under imposed drought conditions in the contained greenhouse and field conditions.

3. Biochemical studies: ABA estimations in the six selected transgenic events of peanut under drought stress and control conditions to confirm the ABA independent expression of $rd29A$:DREB1A transgene.

4. Introgression of the $rd29A$:DREB1A gene from the selected transgenic event into the peanut variety ICGV 86031 through back-crossings.