5. DISCUSSION

Peanut, cultivated worldwide as a food and cash crop contributes significantly to food security in the drought-prone and resource-poor regions of the semi-arid tropics (Wrenshall, 1949; Savage and Keenam, 1994; Nigam et al., 2003; Nigam and Aruna, 2008). Being grown mostly in the rainfed subsistence agricultural lands, drought remains the major constrain affecting crop productivity and yield (Kaushik, 1993; Sharma and Ortiz, 2000; Sharma and Lavanya, 2002; Dwivedi et al., 2003). Drought tolerance in plants is a multigenic complex trait being regulated by diverse drought adaptive mechanisms (Blum, 2005; Reynolds and Tuberosa, 2008; Pinto et al., 2010). Recent advances in the basic understanding of gene expression, transcriptional regulation and signal transduction in plant responses to drought (Zhu et al., 2010) associated with simultaneous progress in the acquaintance of molecular and genomic tools influencing gene discovery (Abe et al., 2003; Tran et al., 2004; Seki et al., 2001) has enabled genetic engineering of transcription factors, regulating multiple pathways for inducing biotic and abiotic stress tolerance in plants (Agarwal et al., 2006; Hussain et al., 2011). Dehydration-responsive element-binding (DREB) transcription factors have been well recognized and reported to improve drought resistance in transgenic plants including tomato, peanuts, rice, barley, and wheat (Kasuga et al., 1999; Pellegrineschi et al., 2004; Oh et al., 2005; Bhatnagar-Mathuret al., 2007; Wang et al., 2008; Xiao et al., 2009; Morranet al., 2010).
At ICRISAT, transgenic events of peanut overexpressing \textit{DREB1A} gene from \textit{Arabidopsis thaliana} along with the \textit{rd29A} stress inducible promoter, also from \textit{A. thaliana}, were produced through \textit{Agrobacterium tumefaciens}-mediated genetic transformation (Bhatnagar-Mathur et al., 2007). After successfully establishing the transgenic status of 50 transgenic events of peanut, six transgenic events were screened mainly for varied transpiration efficiency (TE) under greenhouse conditions (Bhatnagar-Mathur et al., 2007; Devi, 2008; Reddy, 2008). This was based on Passioura’s (1977) concept of yield evaluation under drought stress (DS), through the preliminary pot based drydown experiments (Sinclair and Ludlow, 1986). Present study takes these efforts further by attempting to comprehensively test these six events of transgenic peanut under greenhouse and field conditions for drought tolerance and finally screen the most promising transgenic event/s for eventual development of drought tolerant transgenic peanut varieties.

During this study, the transgenic status of each of the transgenic event, from T3 to T7 generation was confirmed through PCR and RT-PCR analysis using gene specific primers, while Southern analysis at T5 generation ascertained the stable insertion of the transgene at higher generations of the transformants. With increased stringency and obligatory need of comprehensive evaluation of the transgenic material before being allowed to their next level of assessments in open field, there is an essential need of realistic greenhouse experimental protocols and robust systems in place for
successful screening and identification of high performance drought tolerant transgenic events. Quite a lot of studies, reportedly meant for assessing transgenic crops, conducted mainly under artificial stress conditions in small pots at an early seedling stage, for a very short duration covering a transient phase, has mostly lead to deceptive conclusions from both agronomic and physiological perspective (Bhatnagar-Mathur et al., 2008; Yang et al., 2011). Therefore, in the present study, for all the drydown experiments (Sinclair and Ludlow, 1983) were carried out initially in the contained greenhouse and later in the confined field conditions, long PVC tubes or the lysimeters (Vadez et al., 2008; Ratnakumar et al., 2009). The lysimeters helped hold far more water (~8 l) after saturation of soil profile, compared to pots, resulting into extended experimental period, viz., 7-8 wk of gradual drying in greenhouse conditions without any external addition of water, thereby covering almost the entire flowering and pod filling phase. Again, the use of polythene beads as the top layer on the soil, instead of polythene covers, helped completely reduce the soil-surface evaporation, as well as allowed no hindrance to pegging and pod development and hence, facilitating yield evaluation under drought stress (DS). Thus, a robust and reliable lysimetric system based drydown procedure was adapted and followed for the comprehensive evaluation of drought tolerance in the six transgenic events of peanut. The lysimetric system allowed studying simultaneously, the effect of drought on relative differences in the water uptake (Transpiration), biomass accumulation per volume of
water captured (TE) and pod formation (Yield), as well as facilitated the study of roots and shoot growth patterns across the genotypes with more precision, helping to record their natural adaptive responses under stress in a consistent manner during the successive experiments.

Reproductive stage being the most sensitive to water deficit (O’Toole, 1982) has been widely acknowledged as the most prevalent problem in rainfed drought prone agriculture, mainly because in most rainfed ecosystem the crop season’s rains diminish towards flowering and harvest time. Therefore, irrespective of biomass production up to flowering, the adaptive ability of a genotype, either due to reduced leaf area or inherent stomatal regulation reducing the transpirational loss, along with increased root growth to access water from deeper soil thus leading to effectively use of the available soil moisture into the reproductive growth stage, becomes the key for reproductive success (Merah, 2001; Kato et al., 2008). The drought stress was always imposed at flowering stage and maintained throughout the reproductive phase of the peanut crop while screening for transgenic events having best chance of reproductive success under DS.

In the present study, total water captured or cumulative transpiration (T) across the genotypes under the drought stress treatment was recorded as low as 30-35% in the contained greenhouse while it was 50% in outdoor conditions, when compared to the total water captured under the well-watered conditions (WW),
thereby suggestive of significantly different water regimes, under DS and WW treatments. For example, high yielding genotypes of cotton, wheat and rice have greater stomatal conductance and transpiration under DS (Blum et al., 1982; Sanguineti et al., 1999; Araus et al., 2002; Izanloo et al., 2008) or WW (Shimshi and Ephrat, 1975; Lu et al., 1994; Lu and Zeiger, 1994; Reynolds et al., 1994; Fischer et al., 1998; Horie et al., 2006). Recently, lysimetric system-based study with peanut breeding lines indicated that the pattern of water extraction was critical in explaining yield differences under intermittent drought (Ratnakumar et al., 2009). In this study, although there were no significant genotypic differences in the pattern of water uptake under WW, the transgenic events in general showed higher rates of transpiration under DS relative to the untransformed wild type controls (WT), in particular until half-way (0.5 NTR) after which all the genotypes showed similar rates of transpiration, probably due to lowered stomatal conductance under stress, as an adaptation, in the transgenic events, as has been reported earlier (Bhatnagar-Mathur, et al., 2007).

Cumulative transpiration in the transgenic events RD20 and RD33, in general, under WW across the experiments was significantly higher (P<0.05) than the WT. Under terminal DS where no water was added after the plants reached end point of 0.1 NTR, the transgenic events RD2, RD11, RD19, RD20 and RD33, in general showed significantly higher cumulative transpiration than WT, mainly due to the induced root response in deep soil layers (below 40
consequently enhancing water uptake up to 20-30% in these
transgenic events. Thus, in this study a positive and significant
(P<0.001) correlation was found between root dry weight and
evapotranspiration in DREB1A transgenics of peanut (Vadez et al.
2007). Very few studies done on roots in peanut have reported rooting
differences under various water regimes (Robertson et al., 1980, Boote
et al., 1982; Ketring et al., 1982; Pandey et al., 1984).

During the present study, further efforts were made to ascertain
whether root length density and water extraction are closely related. It
is still a matter of debate, though there are supportive arguments
tendered for the existence of a positive correlation between root length
density and water uptake (Passioura, 1983; Monteith, 1986, Lafolie et
al., 1991). While there were other studies which showed poor
relationships between water uptake and RLD across several cereals
and legumes (Hamblin and Tennant, 1987; Dardanelli et al., 1997;
Katayama et al., 2000, Amato and Ritchie, 2002; Zaman-Allah et al.,
2011). More precise and comprehensive data related to root length
density involving root volume and root surface area at varying soil
depths under both DS and WW regimes has clearly confirmed the
positive relationship between the rooting response and water
extraction from deeper soils. Here, it was observed that DS promoted
root growth in the transgenic events more than in the wild type, which
was possibly in part related to the promotion of root growth in deep
layers. This led to a higher water extraction in three transgenic events
than in the WT under DS, which was probably accountable for water
extraction differences in the three weeks that followed stress imposition. Finally, the water extraction was well related to both the root length density and the root dry weight at depth, but not with the average root length density.

The stimulation of root dry weight under DS was reported in earlier studies on peanut (Allen et al. 1976), although it has also been shown that the root growth decreased upon water deficit (Meisner and Karnok, 1992), although not as much in the deeper layer. Root depth was also reported to increase upon exposure to water stress in other studies (Lenka and Misra, 1973; Narasimham et al., 1977; Ketring and Reid, 1993). In the present study, it was found that several transgenic events had higher root growth, especially in the deep soil layers. This study is one of the first to report when a genetic transformation has led to an increase in the root biomass, except for the transformation with vacuolar H\textsubscript{\textminus}-pyrophosphatase (H\textsubscript{\textminus}-PPase) AVP1 which increased root growth in tomato (Park et al., 2005). Modeling studies have shown the benefit of improving crop rooting depth (Sinclair and Muchow, 2001; Hammer et al., 2009). The capacity for deep rooting and water extraction in these transgenics opens-up a scope for using this characteristic towards the development of lines that are better adapted to water limitation, for environments with deep soil and availability of water in the deep layers.
Despite the remaining controversy about the relationship between root length density and water extraction, the results from the present study showed a clear relationship between either root length density or root dry weight in the deep layer (below 60 cm) and water extraction. These results are somewhat different to others in peanut, using genetically diverse breeding materials, where poor relationship between the root length density, or root dry weight, and water extraction were found (Vadez et al., 2008; Ratnakumar and Vadez, 2011). The reasons for these differences remain unclear. According to Dardanelli et al. (2004), crop species could be characterized by a common uptake coefficient $K$ that is lower in peanut than in other crops, provide a maximum rate of water absorption once the root length density is above a minimum threshold. The close relationship between the root length density in the 60-90 cm layer and water extraction, but not between the average RLD and water extraction agrees with Dardanelli’s statement and would indicate that 0.50-0.70 cm cm$^{-3}$ is below the minimum RLD for maximum water extraction in peanut. This would agree with the report of peanut variety JL 24 having a relatively poor maximum root depth and among the highest root length density; although lower than 0.50 cm cm$^{-3}$ (Ratnakumar and Vadez, 2011), and here with the relatively heterogeneous distribution of roots in the different soil layers. For instance, the root dry weight of peanut variety JL 24 below 60 cm was about half that above 60 cm, whereas the root weight was about the same across the layers in the peanut transgenic events RD11 or RD33. Similar results
were observed for root length density. Therefore, it seems that the
effect of genetic transformation on roots under DS conditions was to
alter the distribution of the root system to make it more uniform
across the soil profile, thereby leading to increase in the RLD at each
level closer to a value allowing maximum water extraction rate.

Breeding efforts to improve the adaptation of peanut to drought
have been undertaken mostly focusing on trying to improve its water
use efficiency (Hubbick et al., 1986; Wright et al., 1994;
Krishnamurthy et al., 2007). For most effective use of the water
resource, three principal factors are involved: maximizing water
uptake; efficient conversion of water extracted into biomass
(transpiration efficiency), and efficient conversion of biomass into a
harvestable product such as grain (HI). These factors can be
expressed symbolically as, \( Y = T \times TE \times HI \) (Passioura, 1977). Pod yield,
according to the equation is a function of Transpiration\((T)\), water lost
through the plant canopy; Transpiration efficiency\((TE)\), biomass
produced per unit of water transpired; and the Harvest Index\((HI)\), the
ratio of grain yield to standing biomass.

Peanut being a crop grown under rainfed conditions, it has been
a strongly followed belief that improving water use efficiency \((TE)\)
would be the best strategy to cope with periodic intermittent drought.
Hence, a lot of research programmes intended to enhance the drought
tolerance of peanut has actually using transpiration efficiency \((TE)\) as
a selection criterion (Condon et al., 2002; Rebetzke et al., 2002). TE is
considered as an important component trait of WUE, as well as a major source of yield variation (NageswaraRao and Wright, 1994; Wright et al., 1994; Krishnamurthy et al., 2007) under DS. However, there have been contradictions in accepting TE as an independent variable affecting yield, since results have been inconsistent, ranging from no relationship to negative or positive relationships, between TE and yield in various crops under changed experimental conditions (Hall et al., 1994; Matus et al., 1996; Monneveux et al., 2007; Morgan et al., 1993; Munoz et al., 1998; Ngugi et al., 1994,1996; Read et al., 1991; Saranga et al., 2004; Sayre et al., 1995; Specht et al., 2001).

Biomass of a genotype is a function of crop transpiration. Effective use of water (EUW) implies maximal soilmoisture capture for transpiration which being tightly linked with biomass production becomes the most important target for yield improvement under drought stress (Blum, 2009). Enhancement of biomass production under drought stress can be achieved primarily by maximizing soilwater capture, while diverting the largest part of the available soil-moisture towards stomatal transpiration. Thus, it is the EUW and not transpiration efficiency (TE) that is the major engine for agronomic or genetic enhancement of crop production under a limited water regime (Blum, 2009). In the present study, a close relationship between the volume of water captured and accumulated biomass was recorded, while the genotypic differences in TE remained inconsistent across the experiments.
Deep or dense root system that would promote soil moisture capture and WU is correlated across genotypes with low WUE or TE. Pinheiro et al. (2005) and Kobata et al. (1996) concluded that “the high dry matter production of those rice cultivars has been known to be drought resistant under field conditions is caused not by high WUE, but by high ability to maintain transpiration, which is supported by deep root systems.” Thus, it is not surprising that favorable genotypic plant water status under drought stress as reflected in measurements of relative water content or canopy temperature is correlated with low WUE across genotypes (Araus et al., 1993; Frank et al., 1997; Read et al., 1991; Zong et al., 2008). Genotypic variation in WUE under limited water regimes is affected more by variation in the water uptake (Transpiration) rather than by variation in the accumulated biomass (Blum, 2005). This has also been determined for TE and stomatal conductance at the single leaf level (Juenger et al., 2005; Monclus et al., 2006; Monneveux et al., 2006). Hence, the selection for high WUE under limited water supply tends to result in a genetic shift towards plant traits that limit crop water use, such as early flowering and smaller leaf area (Martin et al., 1999; Menendez and Hall, 1995; Ngugi et al., 1994; Sayre et al., 1995). The successful and widely cited case for dryland wheat grain yield improvement with selection for high WUE through low carbon isotope discrimination in NSW Australia (Condon et al., 2002) can be explained by the fact that wheat is grown there mainly on stored soil moisture.
The *DREB1A* transgenic events of peanut, RD20 and RD33 under WW showed significantly higher (P<0.05) biomass compared to the WT, while RD11 and RD19 had lower shoot biomass. In both cases, it was the volume of water captured that made the difference as the former events had significantly higher water uptake (Transpiration), though their TE inconsistently was at par or higher with the WT, while the events RD11 and RD19 with significantly lower (P<0.5) cumulative transpiration (water captured) also had significantly lower TE under WW. Under terminal drought stress in greenhouse, significant positive root response increased the water capturing capacity in almost all the transgenic events except RD12 which allowed them to keep a higher rate of transpiration in the initial three weeks of stress, thus resulting into significantly higher water uptake that eventually lead to higher biomass as compared to the WT. However, under terminal DS, the WT and RD12 had significantly higher TE than other transgenic events which contradicts earlier findings (Bhatnagar-Mathur et al., 2007) where most of these transgenic events had significantly higher TE in pot-based drydown experiments, thereby indicating the ambiguity of TE as a trait to be used for screening genotypes for drought tolerance (Blum, 2009).

Interestingly, under intermittent DS, addition of water after the plants reached 0.1 NTR released the stress as well as allowed time to the WT to capture the available water in the cylinders, hence, nullifying the initial advantage the transgenic events had with higher rate of transpiration. Thus, after the stress release, the volume of
water added at intervals being lowest and similar, meant for survival across the genotypes lead to loss of the genotypic differences in transpiration by the end of the experiment. Hence, under intermittent DS, the total biomass accumulation was similar across the genotypes, also resulting into loss of the genotypic differences in TE. Interestingly, the genotypes though having similar amount of biomass with similar volume of captured water under the intermittent DS, differed in partitioning pattern towards shoot, root and pod. The average biomass partitioning observed during all the experiments under the water limiting conditions showed the WT to have significantly higher shoot biomass (~60-75%) and less of the pod (~23-32%) as compared to that found in transgenic events RD2, RD11 RD19 and RD33 which had significantly higher pod formation (~40-50%) with lower shoot biomass partitioning. Thus, it was not the TE, biomass accumulated per volume of water, but the enhancement of biomass accumulation with increase in volume of water captured (EUW) along with the partitioning towards productive yield and better root:shoot ratio for better water capture that made the difference in the better performing transgenic events under DS.

Cumulative transpiration (T) in general did not show any correlation with the accumulated pod dry weight and HI. Instead, a significantly positive correlation was seen between the transpiration occurring during 37 to 79 day, flowering and pod filling stage, of the greenhouse trial with pod dry weight/yield ($r = 0.443$) as well as with the HI ($r = 0.321$). However in the confined field trial, it was seed dry
weight which showed a positive correlation (P< 0.05) with the total water uptake viz., cumulative transpiration (r = 0.296). Transpiration efficiency (TE) showed no correlation with either pod weight or HI during both the trials. Root: shoot ratio in the DS plants from confined outdoor trial showed significant positive correlation with dry weights of pod and seed, as well as with cumulative transpiration, while had negative correlation with shoot dry weight and TE.

Under DS, the reduction in biomass and pod yield is certain in peanut as was seen in the present study that has also been reported previously (Pimratch et al., 2008; Jongrungklang et al., 2008; Ratnakumar and Vadez, 2011). Delta pod weight, indicative of the reproductive success under DS, the reduction of pod yield under DS in WT along with RD20 was significantly higher (P<0.05) than that in the events RD11 and RD19. As a result, under DS the genotypes susceptible or sensitive to drought stress i.e., RD20 and WT had higher leaf dry weight, shoot dry weight, aerial biomass and lower HI and yield. In contrast, the drought tolerant transgenic events, RD11 and RD19, had significantly lower shoot, leaf dry weight but had significantly higher HI with higher seed number and pod yield. Interestingly, the transgenic events RD33 and RD2 were significant in having relatively higher shoot biomass as well as high HI, thereby showing efficient utilization of the limited water available under intermittent DS by partitioning optimally to both shoot biomass and pod/seed accumulation, and could be termed as high yielding events performing well under both WW and DS conditions. Water deficits during pod fill generally reduces pod and kernel weight (Pallas et al., 1977, 1979; Pandey et al., 1984a; Lenka and Misra, 1973; Nageswara Rao et al., 1985; Wright et al., 1991). The transgenic
events RD2, RD11 and RD33 had significantly (P<0.05) superior pod fill as compared to that in the WT under intermittent drought stress conditions. In general, under irrigated conditions the HI of all the genotypes were similar with very few deviations in one or more trials. Under DS in field trials, the HI decreased on an average by ~18% in the WT, thus, indicating that the stress affected the reproductive processes. In contrast, under drought stress treatments, the transgenic events RD11, RD2 and RD33 had consistently higher HI that changed by mere 1-8% in the contained field under DS when compared to the WW.

Abscisic acid (ABA) integrates environmental constraints that are linked to changes in water availability with the metabolic and developmental programs of plants. Induced ABA synthesis acts as a signal for common additional sets of pathways for collective adaptive response in plants. Plants that are challenged by drought and salt stress use ABA as an endogenous signal to initiate adaptive responses (Zhu, 2002). Competitive ELISA was standardized for the estimation of free ABA using the polyclonal antibodies and standards developed previously at ICRISAT that could detect up to 4 ng ml\(^{-1}\) free ABA. In the present study, the immunoassay of ABA showed its significant increase across the transgenic events as well as the control WT under DS. However, there were no significant differences in the estimated ABA content under DS across the genotypes. Reports suggesting that the \textit{DREB1A} transcription factor is ABA-independent and is self-regulating while inducing plants adaptive response under water deficit stress has been based on the response of exogenous application of ABA rather than actual estimations of the levels of ABA induced under
stress (Shinozaki et al., 1997, 2000). This is the first ever study showing the independent nature of the ABA induction pathway through estimation of free ABA in DREB1A transgenic plants.

Extensive evaluation for drought tolerance in the six transgenic events under both WW and DS in greenhouse and field has repeatedly shown them to be significantly different when compared to the WT. Consistent pattern in genotypic variation of component traits confirms the heritability of the genotypic response under stress as well as under control conditions. Genetic transformation thus resulted in the induction of permanent and heritable changes in these transgenic events. Several factors have been attributed for the observed variations in transgene expression that include transgene locus in the chromosome, copy number and transgene fidelity (Matzke and Matzke, 1998; Kooter et al., 1999). In the present study, the variation under stress could be explained and related to the corresponding stress-induced overexpression of the DREB1A transgene that is supposed to be tightly regulated by the stress inducible promoter rd29A, as confirmed by the RT-PCR analysis of these transgenic events. However, the fact that few transgenic events had shown significant variation even under WW conditions, without any corresponding over-expression of the transgene, as it was observed, and could not be explained in the previous study (Bhatnagar-Mathur, et al., 2007) as well as in present study.
Only the event RD2 seems to be similar to the WT JL 24, under WW and performs better under DS, which can be explained through the corresponding overexpression of the DREB1A transgene. But the events RD19 and RD11 under WW conditions were significantly different than the WT. They show drought tolerant features viz., significantly smaller leaf area, short plant height, lower transpiration and transpiration efficiency even under WW conditions. They also differ in having significantly lower 100 seed weight than WT with half the size of the WT seeds. On the other hand, the events RD20 and RD33 seem to be like high yielding varieties produce significantly higher biomass and pod yield under WW as compared to the WT. Only explanation to this completely different genotypes under control conditions seem to be the epigenetic changes which might have occurred due to the insertion of foreign DNA through genetic transformation, as being seen in animals where it has lead to unorthodox genetic and epigenetic changes (reviewed in Matzke et al., 1999; Rieseberg, 2001; Paterson et al., 2003; Adams and Wendel, 2004; Levy and Feldman, 2004; Madlung and Comai, 2004; Soltis et al. 2004). Introgression of alien DNA into plant genomes through sexual crossing and genetic engineering commonly used in plant breeding, which conceivably, apart from transfer of the target gene(s) and insertional disruption, may cause structural changes involving extensive alterations in DNA methylation and transcription of both cellular genes and Transposon elements related DNA, consequently, transcription can be altered. This may explain, in part, the frequent
observation of novel traits in wide hybridization-derived plants, which often exhibit transgressive phenotypes (Rieseberg et al., 2003; Liu et al., 2004). To explain and prove the reason of variation seen in transgenic peanuts, RD11 and RD19 particularly, further study from the perspective of epigenetics is required. Experiments to test changes in the methylation pattern if any in the transposon-like elements and adjacent candidate genes corresponding to the changed traits in these events could further our understanding about the genetic changes the introgression of an alien DNA could cause.

Thus, the preliminary optimistic results from the above-mentioned studies encouraged us to further introduce the transgene rd29A:DREB1A in the elite high-yielding variety of peanut, ICGV86031 via A.tumefaciens-mediated genetic transformation. In addition, best performing transgenic event of peanut variety JL 24 (WT), RD2 was used as a source of the transgene for introgression into peanut variety ICGV 86031 through backcrossing for investigating in future the influence if any, of the gene transfer method on the produced transgenics.

Prerequisite for the successful production of transgenic plants through genetic engineering is the availability of an efficient tissue culture system for plant regeneration duly supported by an effective genetic transformation system (Vasil, 1987; Brasileiro and Dusi, 1999; Pérez-Molphe-Balch and Ochoa-Alejo, 1998). In the case of peanut, a highly efficient regeneration and transformation system as reported by
Sharma and Anjaiah (2000) was used for the development of new transgenic events with the \textit{rd29A:DREB1A} transgene. The regeneration of direct adventitious shoot buds is considered more effective than the regeneration of shoot buds through an intervening callus as it avoids possible somaclonal variations and leads to higher regeneration frequency (Fontanna et al., 1993). Furthermore, the regeneration potential is also affected by the age of the explants (Pierik, 1987; Welander, 1988; Sharma et al., 1990). As suggested by Sharma and Anjaiah, (2000), in the present study, the use of juvenile de-embryonated cotyledonary explants for direct shoot regeneration without any callus formation proved very effective that resulted in a regeneration efficiency of \(~90\%\). \textit{A.tumefaciens}-mediated gene transfer has been the preferred mode of genetic transformation in peanut as it results in the production of a large number of stable transgenic plants (Lacorte et al., 1991; Mansur et al., 1993; Eapen and George, 1994; Mckentley et al., 1995; Cheng et al., 1997; Venkatachalam et al., 1998, 2000; Li et al., 1997; Rohini and Rao, 2000; Sharma and Anjaiah, 2000; Daniel, 2002; Khandelwal et al., 2003; Anuradha et al., 2006). Accordingly, \textit{A.tumefaciens} strain C58 was used in the present investigation. The binary vector used in this study had kanamycin as the selectable marker along with \textit{rd29A:DREB1A} as the candidate gene. During the course of this study, 24 new putatively transformed shoots were developed that were successfully transplanted to the greenhouse that produced the T1 generation seeds. Transgenic status of the T1, T2 and T3 generation plants was confirmed through PCR
using gene specific primers, while southern analysis validated the presence of transgene at T3 generation.

Similarly, preliminary introgressions were initiated for studying the response *rd29A:DREB1A* in a different genomic background of peanut. For this, the high TE and high yielding transgenic event RD2 was selected as the donor parent, while a popular breeding line of peanut, ICGV 86031 having multiple resistance to various abiotic stress with good yield but long duration was selected as the recipient or the recurrent parent providing the enriched background. Initial cross between RD2 parent (pollen) and ICGV 86031 (female) resulted in introgressed lines having the transgene as confirmed through PCR. Thereafter, the F1 progenies were again backcrossed with the pollens from ICGV 86031 parents for BC2 and BC3 crosses. Progenies from each of the crosses were screened for the transgene and only the PCR positive plants were further used for crossings. Each of the crosses from the very initial hybridization gradually caused the change in the genomic background closer to that of ICGV 86031, with the transgene acting as a marker for selection of the hybrids. First cross resulted in the progeny having genomic background with 50% share from JL24 and 50% from ICGV 86031; which changed with increased share of ICGV 86031 (75%) in BC1F0 population, again to 87.5% in BC2F0 population and finally to 93.75% of ICGV 86031 genomic share in BC3F0 population.
The present BC3F0 population having the transgene \textit{rd29A:DREB1A} had distinct ICGV 86031 features viz., pigmentation in stem, dark green and waxy leaves. Thus, the genomic background has been almost converted to that of ICGV 86031 through the three successive backcrosses, and following the BC4 cross, the process of producing isogenic populations through successive selfing followed by physiological evaluation could be carried out.