4. Results

4.1 Genetic transformation studies

Experiments on *Agrobacterium tumefaciens*-mediated genetic transformation of peanut cv. ICGV 86031 resulted in the successful production of transgenic plants carrying *rd29A:DREB1A* transgene intended for inducing enhanced drought tolerance in them.

4.1.1 Regeneration and transformation

**Regeneration**- Reasonably high frequency of multiple shoot buds was consistently produced at the nodal region of each of the responding explant. On the shoot induction medium (SIM), the explants responded, initially by turning green followed by their enlargement within 3 d of culture initiation. Overall, 70% of the responding explants initiated multiple shoot buds within 14 d at the proximal cut end. However, no further elongation of these shoot buds occurred even after 4 wk on SIM. Hence, the explants bearing shoot buds were cut at the nodal region into two to four pieces each, and transferred to the shoot elongation medium (SEM) for at least three passages of 3 wk each while the elongated shoots were rescued at the end of each passage. Frequently, three to five shoots were recovered from each explant, although over ten shoots could be recovered if the explants were sub-cultured on SEM for 2-3 extra subcultures. The elongated shoots were rooted on MS medium containing 5 μM NAA, where the adventitious roots appeared within 2 wk in all the shoots, thus
showed 100% rooting efficiency. These roots developed further in 4 wk when the rooted shoots were ready for transplantation. Upon transplantation, all the plants survived and appeared phenotypically normal producing flowers and pods with viable seeds.

**Restriction analysis of plasmid carrying the binary vector pBI29ApNot:** Total size of the plasmid \( rd29A:DREB1A \) isolated from cells of the transformed \( A.\ tumefaciens \), strain C58 was 13.49 kb. Restriction digestion of the plasmid (Fig. 4.1) with \( \text{HindIII} \) restriction enzyme having two restriction sites resulted in the release of a 947 bp fragment representing the \( rd29A \) promoter. Similarly, digestion with \( \text{BamHI} \) released 654 bp fragment of \( DREB1A \). The restriction digestion of the plasmid with either \( \text{EcoRI} \) or \( \text{XbaI} \), both having single restriction sites produced a single band of 13.49 kb, while with \( \text{NcoI} \) gave four bands were observed (between 1.65 kb and 5.0 kb) while \( \text{PstI} \) produced three bands between 3.0 kb and 6.0 kb as expected.

**A. tumefaciens mediated genetic transformation of peanut var. ICGV 86031:** In four transformation experiments that were carried out during the present study, over 40% of the co-cultivated explants (Fig. 4.2A) when cultured on SIM containing 100 mg L\(^{-1}\) kanamycin for two weeks showed shoot bud regeneration (Fig. 4.2B, C), while rest of the explants enlarged in size, induced callus and turned yellow on the margins with no shoot induction. Elongation of the induced shoots on SEM supplemented with 75 mg L\(^{-1}\) kanamycin (Fig. 4.2D) for further 4 wk resulted in screening of the putative transformants with only 22%
remaining green, while rest turning yellow, showing bleaching that is typical symptom of kanamycin effect. Finally, overall 24 putatively transformed shoots were obtained which successfully rooted in the root induction medium (RIM) containing 50 mg L\(^{-1}\) kanamycin. These \(rd29A:DREB1A\) transformants of peanut variety ICGV 86031 were transplanted in earthen pots, hardened and finally transferred to the greenhouse (Fig. 4.2E-G) where the transgenic status was further confirmed through PCR analysis.

4.2 Molecular analysis

4.2.1 PCR screening of ICGV 86031 DREB1A transgenics

PCR analysis for both \(npt\)II and \(DREB1A\) transgenes was carried out using the genomic DNA isolated with the modified CTAB method (Dellaporta et al., 1983; Porebski et al., 1997), and from leaf tissue of untransformed and \(rd29A:DREB1A\) transformed plants of peanut cultivar ICGV 86031. Spectrophotometric optical density of the isolated genomic DNA, (mini and maxi preparations) was found to be between 1.8 and 2.0 at 260 nm to 280 nm wavelength. 200 ng of this DNA were used as a template for PCR based analysis.

All the 24 T0 putative transformants of peanut cv. ICGV 86031, which rooted in the presence of 50 mg l\(^{-1}\) kanamycin showed the expected amplicon of 700 bp coding region of the \(npt\)II gene, and 300 bp amplicon representing the junction fragment of the \(rd29A\) promoter and \(DREB1A\) transgene, respectively. No amplification was observed when genomic DNA from the untransformed control ICGV 86031
plants were used as template during the PCR analysis. Similarly, PCR based screening of the T1, T2 and T3 progeny from the PCR positive T0 transgenic parent showed presence or absence of the expected amplicon of 700 bp and 300 bp (Fig. 4.3 A, B) size corresponding to the coding region of \textit{npt} II gene and \textit{rd29A}:\textit{DREB1A} specific junction primers, respectively, mostly in agreement with the Mendelian ratio of 3:1, as detailed in Table 4.1. No amplification was observed in the genomic DNA of untransformed ICGV 86031 peanut plants with both \textit{npt} II and the junction primers.

**4.2.2 Transgene segregation studies in six DREB1A transgenic events of peanut var. JL 24**

All the selected PCR positive plants from the six transgenic events viz., RD2, RD11, RD12, RD19, RD20 and RD33 in their T3 generation were advanced to T7 generation and PCR based screening. Miniprep genomic DNA from leaves of plants representing the segregating population of T3, T4, T5, T6 and T7 generations respectively, used for PCR with \textit{npt} II gene-specific primers showed presence or absence of the expected 700 bp coding region of the \textit{npt} II gene (Fig. 4.4A). No amplification was observed when the genomic DNA from untransformed control plants was used as template. PCR with the primers designed using regions from \textit{rd29A} promoter and \textit{DREB1A} junction amplified the expected 760 bp fragment in all the \textit{npt} II positive transgenic plants (Fig. 4.4B). While the PCR negative plants were screened out from each generation, a minimum number of
seeds as required by each of the drydown experiments from PCR positives plants were used for physiological evaluation.

Details of the number of plants analyzed through PCR for the presence of transgene \((npt\text{II} \text{ and } rd29A:DREB1A)\) and the results confirming inheritance of the transgene in the PCR positive plants while segregating out the negative plants in each of the transgenic event at each generation are shown in Tables 4.2 to 4.7. During this study, homozygosity was reached in three transgenic events including, RD2, RD11 and RD19, as all the seeds from a single plant from each of these events were tested and used up during various drydown experiments, all of which were PCR positive.

### 4.2.3 Southern blot analysis

\(Hind\text{III}\) restriction digestion of the genomic DNA extracted from leaves of PCR confirmed transgenic plants of both peanut cultivars, ICGV 86031 and JL 24, resulted in the release of the \(rd29A\) gene, a 947 bp fragment, seen as an independent band at similar position in their respective lanes, while no band was seen in the control untransformed lanes of parents ICGV 86031 and JL 24, respectively (Fig. 4.5A, B).

### 4.2.4 RT-PCR studies-

Quality and integrity of the total RNA extracted by using TRIzol\textregistered reagent was confirmed by the presence of two clear bands of 28S and 18S rRNA species on 1% TBE agarose gel. Expression of the \(npt\text{II}\) and \(DREB1A\) genes in the transgenic events from both cultivars, ICGV 86031 and JL 24, respectively, was
determined in individual plants by RT-PCR. The \textit{nptII} gene was constitutively expressed in all the plants, both WW and DS, while expression of the \textit{DREB1A} gene was observed only in the DS plants (Fig. 4.6). All the tested transgenic plants under DS showed an amplification of the expected 499 bp \textit{DREB1A} fragment (Fig. 4.7A, B).

### 4.3 Physiological evaluation under greenhouse conditions

In quest for identifying the best performing event/s among the initially screened six DREB transgenic events of peanut var. JL 24 under drought stress, a series of drydown experiments were planned and carried out. The physiological evaluation of the induced drought tolerance in DREB transgenics primarily involved studying variations in genotypic responses to the changes in water uptake viz., transpiration, biomass accumulation, comprehensive root traits and finally the pod and seed yield under imposed DS relative to the WW conditions in contained greenhouse as well as in outdoor field conditions. Experiment-wise results are presented in the following section.

#### 4.3.1 Experiment 1

Main objective of the experiment conducted during Sept. 4 to Nov. 17, 2006 was to study the pattern of transpiration and root growth response under the imposed terminal DS in the selected five transgenic events of peanut cv. JL 24 viz., RD2, RD11, RD12, RD19 and RD20 in comparison with their untransformed parent JL 24 control (WT).
**Pre-harvest biomass** - The initial, pre-harvest biomass (leaf, stem and roots dry weight) recorded at the beginning of the experiment was found to be similar across all the genotypes, except for the leaf area in the transgenic event RD19 (812.376 cm$^{2}$ plant$^{-1}$) and root dry weight in RD11 (1.06 g plant$^{-1}$), which were significantly higher (P<0.05) than that in JL 24 (Table 4.8).

**Transpiration pattern** - Transpiration (or the water uptake) under DS reduced by about 50–60% relative to that under WW treatment following 12 d after the imposition of DS in all the tested genotypes. Thereafter, the transpiration rate during the drying phase, under DS remained above that of the WT in most of the transgenic events. The daily transpiration rate in the transgenic events was higher than the untransformed WT for most of the time from 7 d after withdrawing irrigation, more particularly in the events RD11, RD2, RD19 and RD20. By contrast, the profile of transpiration under WW was essentially the same in all the genotypes (Fig. 4.8). Under DS, across the genotypes, average cumulative transpiration was 3143 ml plant$^{-1}$ (grand mean) i.e., a drop of 55% compared to 7007 ml plant$^{-1}$ that was recorded under WW conditions (Table 4.9). Within the same treatment, under DS, the water uptake was similar across the transgenic events as each of them showed significantly higher (P<0.05) transpiration ranging from 21 to 42%, than that was observed in WT. In contrast, the total water uptake under WW was within a close range viz., 0.3 –19%, that was still significantly higher (P<0.05) in the transgenic events than in WT (Fig. 4.9A).
Post harvest gross biomass- At end of the experiment, gross biomass recorded across the genotypes under DS (15.5 g plant⁻¹) reduced by 45% when compared to that under the WW (27.98 g plant⁻¹) (Table 4.10). Within the DS treatment, each of the transgenic events accumulated biomass significantly higher (P<0.05), ranging from 19% to 50% more than that in the WT [12.18 g plant⁻¹] (Fig. 4.9B). Within the transgenic events, the event RD20 had significantly higher biomass (18.32 g plant⁻¹) than the rest, while the event RD2 had the lowest gross biomass (14.48 g plant⁻¹). Under WW conditions, the biomass accumulation in the transgenic events RD20 (32.55 g plant⁻¹) and RD2 (30.05 g plant⁻¹) was significantly higher (P<0.05) than that in WT (25.86 g plant⁻¹), while others did not show any significant difference.

Gross leaf area across the genotypes under DS (1290 cm² plant⁻¹) was reduced by 55% as compared to that under WW conditions (2895 cm² plant⁻¹) (Table 4.10). Within the DS treatment, RD20 (1440 cm² plant⁻¹) had the highest and RD2 (1120 cm² plant⁻¹) had the lowest leaf area, but along with other transgenic events, both were in parity with that of the control JL 24 (1265 cm² plant⁻¹). However under WW, the event RD20 (3330 cm² plant⁻¹) had significantly higher (P<0.05) leaf area than that in WT (2577 cm² plant⁻¹) while RD2 (3212 cm² plant⁻¹) and RD12 (2958 cm² plant⁻¹) were at par with both RD20 and WT (Fig. 4.10A).
Total leaf dry weight under DS conditions (5.66 g plant$^{-1}$) was reduced by 40% when compared to that in WW plants (9.36 g plant$^{-1}$) (Table. 4.10). Within DS, all the transgenic events were at par with each other with similar leaf dry weights. However, compared to JL 24 (5.078 g plant$^{-1}$), the leaf dry weight in RD20 (6.464 g plant$^{-1}$) was significantly higher (P<0.05). Similarly, under the WW treatment, the pattern was similar with no significant differences in leaf dry weights among the transgenic events, but when compared to JL 24 (8.51 g plant$^{-1}$), the leaf dry weight in transgenic event RD20 (10.73 g plant$^{-1}$) was significantly higher (P<0.05).

A marginal (17%) decrease in the total stem dry weight (Table 4.10) was found across the genotypes under DS (7.23 g plant$^{-1}$) as compared to that in WW (8.72 g plant$^{-1}$). Within the DS treatment, the accumulated total stem dry weights in events RD20 (8.69 g plant$^{-1}$), RD11 (7.73 g plant$^{-1}$), RD19 (7.46 g plant$^{-1}$) and RD12 (7.38 g plant$^{-1}$) were significantly higher (P<0.05) than that in JL 24 (5.67 g plant$^{-1}$), while RD2 (6.45 g plant$^{-1}$) was at par with JL 24. Under WW, only the event RD20 had significantly higher (P<0.05) stem dry weight (10.18 g plant$^{-1}$) when compared to the WT (8.21 g plant$^{-1}$), while other transgenic events were at par with the later (Fig. 4.11A).

Roots dry weight across the genotypes under DS showed a significant increase of 43% when compared to that under WW (Table 4.10). Under DS, the transgenic events showed a significant increase ranging between 43-75% in the accumulated root dry weight while the
untransformed WT showed marginal increase (8%). The transgenic events RD11 (2.47 g plant\(^{-1}\)), RD12 (2.31 g plant\(^{-1}\)) and RD19 (2.65 g plant\(^{-1}\)) had significantly higher (P<0.05) root dry weight under DS relative to that of JL 24 (1.73 g plant\(^{-1}\)) (Fig. 4.11B). However, under WW, all the six genotypes had roots dry weights within a narrow range of 1.52–1.65 g plant\(^{-1}\), which was similar to that recorded in the WT (1.61 g plant\(^{-1}\)).

Under DS, all the transgenics had more profuse rooting in deep soil layers (Fig. 4.12A) as shown by the roots dry weights per plant at 40–120 cms. This was significantly higher (P<0.05) in the transgenic events RD11 (1.218 g plant\(^{-1}\)), RD19 (1.504 g plant\(^{-1}\)), RD2 (1.29 g plant\(^{-1}\)) and RD20 (1.33 g plant\(^{-1}\)) than that in WT (0.795 g plant\(^{-1}\)). In contrast, under WW conditions, there was no difference in the pattern of root distribution over different soil depths (Fig. 4.12B). Similarly, it was observed that while the DS WT had no pods, all the transgenic plants did have few pods (Table 4.10). In contrast, all the genotypes yielded under WW in terms of pod number and pod weight, except for RD2 (10.02 g plant\(^{-1}\)) that had significantly higher (P<0.05) pod weight than the WT (7.3 g plant\(^{-1}\)).

**Net change in biomass:** In general, across all the genotypes, the net change in the biomass under DS reduced by 44% as compared to that in WW conditions. Within the DS treatment, all the transgenic events had significantly higher net biomass per plant ranging from 35 to 72% as compared to WT (7.185 g plant\(^{-1}\)). Transgenic event RD20 had
significantly higher net biomass (12.33 g plant\(^{-1}\)) than that accumulated individually in other transgenic events, which in turn were in parity with each other (Table 4.11).

Under DS, there was a significant decrease of 74% in leaf area, (Fig. 4.13A) and of 52% in leaf dry weight (Fig. 4.13B) across the genotypes as compared to that under WW. However, while only RD20 (2585 cm\(^2\) plant\(^{-1}\)) had a significantly higher (P<0.05) net leaf area under WW conditions, there were no significant differences in the net leaf area, under DS treatment, between individual transgenic events and control WT. Similarly, the net leaf dry weight of the transgenic event RD20 was significantly higher (P<0.05) than that in WT under DS as well as WW conditions (Table 4.11).

In general, under DS there was a marginal reduction in the net stem dry weight (22%) per plant across the genotypes as compared to that under WW. The transgenic event RD2 (4.81 g plant\(^{-1}\)) and WT (3.74 g plant\(^{-1}\)) had a significant decrease of 30% in the stem growth as compared to that under WW, while there was only a marginal decrease of 2-17% in most of the transgenic events (Fig. 4.14A). As a result, except for the event RD2, all the transgenic events had significantly higher (P<0.05) stem dry weights under DS when compared to the WT. Under WW conditions, only the event RD20 had significantly higher (P<0.05) net stem dry weight than that in JL 24 (6.27 g plant\(^{-1}\)), while rest of the transgenic events had net stem dry weights individually in parity with the later.
**Net root dry weight** accumulation across the genotypes under DS, in general was significantly higher (81%) than that under WW (Fig. 4.14B). Although, the net root dry weights in all the transgenic events was higher in general, in the events RD19 (1.632 g plant⁻¹) and RD2 (1.502 g plant⁻¹), it was significantly higher (P<0.05) than that in WT (0.939 g plant⁻¹). Under WW however, there was no significant difference in the pattern of root growth across the genotypes as the net root growth was within a narrow range of 0.5 - 0.85 g plant⁻¹.

**Transpiration efficiency (TE)** – In general, the gross transpiration efficiency (TE) under DS across the genotypes was 22% higher than that under WW conditions. Within the WW treatment, no significant difference was seen across the genotypes with respect to gross as well as net TE. However, under DS all the genotypes had similar gross TE, the net TE recorded in the transgenic event RD20 (3.59 g kg⁻¹ water transpired per plant) was significantly higher (P<0.05) than that in the WT (3.022 g kg⁻¹ water transpired per plant) (Table 4.12). Among the transgenic events, RD11 (3.53 g kg⁻¹ water transpired per plant) showed lowest TE under WW, but had a higher TE (5.04 g kg⁻¹ water transpired per plant) under DS, almost equivalent to RD20 (5.2 g kg⁻¹ water transpired per plant). RD20 had the highest, gross and net, TE recorded under both WW and DS conditions.

**Correlations between the component traits of yield:** Under WW, the cumulative transpiration showed positive and significantly higher (P<0.001) correlation with gross as well as net growth in leaf area, leaf
and stem dry weight (Fig. 4.15A), but had no correlation with the accumulated gross or net roots dry weights. Likewise, TE had no correlation with water uptake under WW. In contrast, the cumulative transpiration showed a significant positive correlation (P<0.001) with the root dry weight, both gross as well as net root dry weights under the DS treatment (Fig. 4.15B). Correlation was even better ($r^2=0.41$) with the root dry weight within the 40–120 cm soil depth (Fig. 4.16). Under DS although, the gross TE had no correlation with water uptake, the net TE showed a positive correlation (P<0.05, $r^2=0.41$) with cumulative transpiration. Pod weight also showed a positive (P<0.05) relationship with water uptake under DS.

**4.3.2 Experiment 2**

The main objective of this experiment conducted during July-October, 2007, was to evaluate pod yield in the selected six transgenic events, RD2, RD11, RD12, RD19, RD20 and RD33 along with their untransformed parent JL 24 as a control (WT) under the terminal DS that was gradually imposed through drydown procedure at the peak flowering stage. A periodic measurement of the water uptake (transpiration) across the genotype under WW and DS was also recorded during the course of this experiment.

**Transpiration**- Water uptake in all the genotypes under DS declined gradually and within 18 d after the last irrigation, it reached almost half (55%) the rate of transpiration seen under WW conditions. The end point (0.1 NTR) in DS plants was reached by 56 d after the last
irrigation when they showed only 10% of the transpiration seen in WW plants. Under DS, during the initial three weeks when NTR was 0.5, most of the transgenic events, with RD33 and RD2 in particular, maintained a significantly higher rate of transpiration than WT, but thereafter, there was no significant difference in water uptake across the genotypes (Fig. 4.17). Average cumulative transpiration across the genotypes under DS was 3876 ml plant\(^{-1}\) that was about 38% of that under WW (10155 ml plant\(^{-1}\)). At the end of the experiment, under DS the cumulative transpiration was similar in all the transgenic events (Table 4.13), with only RD11 (4230 ml plant\(^{-1}\)) showing significantly higher (P<0.05) water uptake than the WT (3510 ml plant\(^{-1}\)). The transgenic event RD12 had the lowest cumulative transpiration of 3408 ml plant\(^{-1}\) amongst the transgenic events under DS. Under WW conditions, only RD33 showed significantly higher (P<0.05) rate of transpiration than the WT throughout the experimental period. This resulted in it having a cumulative transpiration of 15758 ml plant\(^{-1}\), which was significantly higher (P<0.05) than that showed by other transgenic events as well as the WT (10130 ml plant\(^{-1}\)).

**Biomass and Yield:** In general, under the terminal DS across the genotypes, average leaf dry weight (8.16 g plant\(^{-1}\)) and stem dry weight (9.73 g plant\(^{-1}\)) was reduced significantly by 41% & 47%, respectively, as compared to that under WW viz., leaf (13.75 g plant\(^{-1}\)) and stem (18.34 g plant\(^{-1}\)) (Table 4.14). Within the DS treatment, the transgenic event RD33 had significantly higher (P<0.05) leaf (11.85 g \(\text{plant}^{-1}\)) and stem (14.44 g plant\(^{-1}\)) dry weight that resulted in significantly higher
(P<0.05) shoot growth (26.86 g plant⁻¹) than rest of the genotypes that were at par with WT for leaf and stem growth and the ensuing shoot growth under DS. The pattern was same under WW where the transgenic event RD33 again had significantly higher (P<0.05) shoot growth (63.36 g plant⁻¹) than the WT (25.428 g plant⁻¹), while other transgenic events had shoot growth at par with the later (Fig. 4.18A).

Yield in terms of pod number, pod weight, seed number and seed weight showed significant variation across the genotypes under DS. In general, the average pod number (9.4 pods plant⁻¹) and pod weight (8.79 g plant⁻¹) was reduced by 48% and 38%, respectively, under DS across the genotypes, as compared to the pod number (18.21 pods plant⁻¹) and pod weight (14.27 g plant⁻¹) under WW (Table 4.14). Under the terminal DS, the transgenic event RD19 (11.6 pods plant⁻¹) had significantly higher (P<0.05) average pod number followed by RD11 (10.5 pods plant⁻¹) and RD2 (10.5 pods plant⁻¹) than in the events RD12 (7.8 pods plant⁻¹) and RD33 (7.5 pods plant⁻¹). WT with an average pod number (9.5 pods plant⁻¹) was statistically at par with all the transgenic events. Similarly, under WW, no significant difference was seen in pod number between the transgenic events and WT (Table 4.14).

Under terminal DS, the transgenic events RD2 (10.11 g plant⁻¹) and RD11 (9.63 g plant⁻¹) along with the control WT (11.42 g plant⁻¹) had significantly higher (P<0.05) pod weight than RD33 (6.64 g plant⁻¹) and RD12 (7.24 g plant⁻¹). However, under WW, the event RD33 had
the highest pod weight (21.36 g plant$^{-1}$), though there were no significant differences across all the genotypes (Fig. 4.18).

Seed number (15.12 seeds plant$^{-1}$) and seed weight (6.81 g plant$^{-1}$) under terminal DS across the genotypes was reduced by 43% and 33% respectively, than that under the WW conditions, viz. 26.48 seeds plant$^{-1}$ and 10.16 g plant$^{-1}$, respectively (Table 4.14). Under terminal DS, the seed numbers in WT (18.4 seeds plant$^{-1}$), RD2 (18.17 seeds plant$^{-1}$), RD19 (18.0 seeds plant$^{-1}$) and RD11 (16.17 seeds plant$^{-1}$) showed significantly higher (P<0.05) seed filling than RD33 (9.33 seeds plant$^{-1}$) and RD12 (12.0 seeds plant$^{-1}$). However, under WW conditions RD33 had the highest number of seeds (35.2 seeds plant$^{-1}$) while RD12 had the lowest (16.6 seeds plant$^{-1}$). Similarly, while under terminal DS, the seed weight in the WT (8.75 g plant$^{-1}$), RD2 (8.71 g plant$^{-1}$) and RD11 (7.44 g plant$^{-1}$) was significantly higher (P<0.05) than that in RD33 (4.58 g plant$^{-1}$) and RD19 (5.08 g plant$^{-1}$), no significant difference in seed weight across the genotypes under WW conditions was detected.

In general, the gross biomass across the genotypes under the terminal DS reduced by 44% when compared to that under WW conditions (Table 4.14). The transgenic event RD33 was conspicuous in having the highest biomass under both DS and WW. Under terminal DS, the event RD33 (31.64 g plant$^{-1}$) had significantly higher (P<0.05) biomass accumulated than the WT (25.89 g plant$^{-1}$), while other transgenic events had biomass ranging between 29.4 g plant$^{-1}$ in
RD20 to 21.84 g plant$^{-1}$ in RD11, but were statistically at par showing no significant difference with WT. Under WW conditions, the transgenic event RD33 (84.72 g plant$^{-1}$) had significantly higher (P<0.05) biomass than all the other transgenic events as well as the WT. Other transgenic events had biomass ranging between 51.89 g plant$^{-1}$ in RD20 to 26.38 g plant$^{-1}$ in RD12 that were statistically at par with that of WT (39.82 g plant$^{-1}$).

**Transpiration Efficiency (TE):** TE or the efficiency of biomass accumulation measured across the genotypes under the terminal DS (6.03 g kg$^{-1}$ water transpired) showed a marginal 20% increase than that under WW (5.02 g kg$^{-1}$ of water transpired) (Table 4.15). Within a treatment under terminal DS, the transgenic event RD12 (7.25 g kg$^{-1}$ water transpired) and the WT (6.29 g kg$^{-1}$ water transpired) had significantly higher (P<0.05) TE than the rest. The events RD11 (5.054 g kg$^{-1}$ water transpired), RD2 (5.68 g kg$^{-1}$ water transpired) and RD19 (5.69 g kg$^{-1}$ water transpired) had the lowest TE recorded under terminal DS (Fig. 4.19A).

While under WW conditions, the TE recorded for the event RD33 (5.81 g kg$^{-1}$ water transpired) was at par with that of RD12 (5.46 g kg$^{-1}$ water transpired), RD2 (5.34 g kg$^{-1}$ water transpired), RD11 (4.87 g kg$^{-1}$ water transpired) and RD20 (4.75 g kg$^{-1}$ water transpired), it was significantly higher (P<0.05) than RD19 (4.43 g kg$^{-1}$ water transpired) and WT (4.47 g kg$^{-1}$ water transpired).
**Harvest Index (HI):** Proportion of the pods formed to the biomass accumulated (HI) across the genotypes under terminal DS was at an average of 0.342, that was almost similar to that found under WW conditions (0.314). Under terminal DS, HI of the transgenic event RD11 (0.415) as well as the WT (0.425) was found to be significantly higher (P<0.05) than the events RD33 (0.2), RD12 (0.29) and RD20 (0.32), that were statistically at par with the HI recorded in RD2 (0.38) and RD19 (0.37) (Fig. 4.19B). Under WW, all the transgenic events and the WT had HI within a close range (0.248 to 0.346) with no significant differences.

**Correlations - cumulative transpiration with shoot and pod dry weight:** Under WW, the cumulative transpiration showed a high positive correlation (P<0.001) with pod yield and shoot biomass as also with leaf and stem individually (Fig. 4.20A). In contrast, under terminal DS, although the water uptake correlated positively and significantly (P<0.001) with the stem dry weight ($r^2 = 0.178$), it had no correlation with both leaf dry weight ($r^2 = 0.040$) and pod yield ($r^2 = 0.078$) (Fig. 4.20B). The TE had no correlation with water uptake under both DS as well as WW conditions. Pod yield ($r^2 = 0.287$) was positively correlated (P<0.001) with TE only under WW conditions (Fig 4.21), while husk (pod shells) and shoot dry weight correlated well (p>.001) with TE under terminal DS.
4.3.3 Experiment 3

This experiment was conducted during April- August, 2008 in the greenhouse (GH) to evaluate the pod yield in the selected six transgenic events, including RD2, RD11, RD12, RD19, RD20 and RD33 along with the untransformed wild type JL 24 as the control (WT) under intermittent DS imposed during flowering and pod filling stages. Water uptake was recorded periodically every 7-10 days during the course of the experiment. The observations on various parameters studied were as follows:

**Transpiration:** Average water uptake under DS reduced to half (NTR 0.48) of that under WW within 16 d after last irrigation (Fig. 4.22). Cumulative transpiration across the genotypes under DS (10264 ml plant\(^{-1}\)) was significantly reduced (65%) compared to that shown under WW (30375 ml plant\(^{-1}\)) (Table 4.16). Under DS, no significant differences were found in the cumulative transpiration between the transgenic events and WT, while total water uptake in events RD12 (26602 ml plant\(^{-1}\)) and RD19 (26864 ml plant\(^{-1}\)) under WW was significantly lower (P<0.05) than WT (3067 ml plant\(^{-1}\)).

**Biomass and Yield:** Average shoot biomass under the intermittent DS (19.13 g plant\(^{-1}\)) in the GH across the genotypes was reduced by 56% as compared to that under WW (43.54 g plant\(^{-1}\)) (Table 4.17). Within the WW, the shoot dry weight of the events RD33 (58.19 g plant\(^{-1}\)), RD20 (57.38 g plant\(^{-1}\)) and WT (49.15 g plant\(^{-1}\)) was significantly higher (P<0.05) than that found in RD11 (34.47 g plant\(^{-1}\))
and RD19 (24.55 g plant\(^{-1}\)). Again, under the intermittent DS, shoot dry weights in RD11 (13.81 g plant\(^{-1}\)) and RD19 (14.62 g plant\(^{-1}\)) were significantly lower (P<0.05) than that in RD12 (24.55 g plant\(^{-1}\)), WT (21.65 g plant\(^{-1}\)) and RD20 (20.89 g plant\(^{-1}\)) (Fig. 4.24A).

Average yield in terms of pod weight (12.81 g plant\(^{-1}\)) and seed weight (8.69 g plant\(^{-1}\)) across the genotypes under the intermittent DS showed a reduction of 70% and 73%, respectively, as compared to that under WW conditions, viz., average pod weight (43.027 g plant\(^{-1}\)) and seeds weight (32.30 g plant\(^{-1}\)) (Table 4.17). Within the intermittent DS treatment, pod weight (Fig. 4.23) in the transgenic events RD2 (15.68 g plant\(^{-1}\)), RD19 (15.23 g plant\(^{-1}\)) and RD11 (14.81 g plant\(^{-1}\)) was significantly higher (P<0.05) than the WT (8.014 g plant\(^{-1}\)). In contrast, under WW, the average pod weight of all the transgenic events, except RD19 (28.39 g plant\(^{-1}\)) were at par, with no significant differences with the WT (50.66 g plant\(^{-1}\)).

Similarly, under the intermittent DS, the seed weight in the transgenic events RD2 (12.18 g plant\(^{-1}\)), RD11 (10.77 g plant\(^{-1}\)) and RD19 (10.69 g plant\(^{-1}\)) were significantly higher (P<0.05) than that in the WT (4.47 g plant\(^{-1}\)). Under WW, the seed weight in all the transgenic events and WT was similar (Table 4.17), except for significantly lower seed weight in the event RD19 (21.71 g plant\(^{-1}\)).

The average biomass, including the shoot weight and pod dry weight under intermittent DS in across the genotypes (31.55 g plant\(^{-1}\)) was reduced by 63%, than that under WW (85.79 g plant\(^{-1}\)). Within
the intermittent DS, the transgenic event RD12 had the maximum biomass accumulation (36.24 g plant\(^{-1}\)) than rest of the transgenic events that was significantly higher (P<0.05) than that in WT (31.37 g plant\(^{-1}\)). While under the WW conditions, the average biomass dry weight in RD19 (52.94 g plant\(^{-1}\)), RD11 (68.57 g plant\(^{-1}\)) and RD2 (82.22 g plant\(^{-1}\)) were significantly lower (P<0.05) when compared to that in WT (104.82 g plant\(^{-1}\)).

**Transpiration efficiency (TE):** Average TE in the DS plants across the genotypes (3.23 g kg\(^{-1}\) water transpired) was marginally higher (12%) than that under WW (2.87 g kg\(^{-1}\) water transpired) (Table 4.17). Within the WW conditions, TE in the WT (3.252 g kg\(^{-1}\) water transpired) along with the transgenic events RD12 (3.246 g kg\(^{-1}\) water transpired), RD20 (3.139 g kg\(^{-1}\) water transpired) and RD33 (3.136 g kg\(^{-1}\) water transpired) were similar, although significantly higher (P<0.05) than that found in the transgenic events RD19 (2.051 g kg\(^{-1}\) water transpired), RD2 (2.748 g kg\(^{-1}\) water transpired) and RD11 (2.543 g kg\(^{-1}\) water transpired) (Fig. 4.24A). In contrast, under the intermittent DS, all the transgenic events had TE similar to WT. The TE in RD12 (3.464 g kg\(^{-1}\) water transpired) was significantly higher (P<0.05) than RD11 (2.94 g kg\(^{-1}\) water transpired) under intermittent DS.

**Harvest Index (HI):** The pod to biomass ratio (HI) under DS (0.41) across the genotypes was 20% lower than that recorded under WW (0.51). Within the DS, average HI in the three transgenic events RD11
(0.522), RD19 (0.513) and RD2 (0.48) was significantly higher (P<0.05) than that in the WT (0.311), RD33 (0.314) and RD12 (0.322). In contrast, under the WW, except for the event RD33 that had the lowest HI (0.407), all the transgenic events had HI similar to the WT (0.311) (Fig. 4.24B).

**Correlation between the component traits of yield:** Cumulative transpiration under WW correlated well (P<0.001) with the shoot dry weight, pod weight, biomass and TE. However, under the intermittent DS, it correlated positively and significantly (P<0.05) only with shoot dry weight. The volume of water transpired during the period between 37 and 79 d from the day of sowing correlated well with the pod and seed weight, under both WW (P<0.05) as well as intermittent DS conditions (p>0.005) (Fig. 4.25). Under WW, TE was significantly (P<0.001) correlated with pod and seed weight, but not with yield under DS. TE correlated well with shoot dry weight under both WW and intermittent DS.

**4.3.4 Experiment 4**

This experiment was conducted during November 25, 2008 to February 9, 2009, in the greenhouse to comprehensively study the root traits in the selected six transgenic events, including RD2, RD11, RD12, RD19, RD20 and RD33 along with the WT under the terminal DS imposed during flowering and pod filling stages. Water uptake was recorded periodically every 7-10 d during the course of the
experiment. The observations on various parameters studied were as follows:

**Pre-harvest (PH) biomass:** The pre-harvest dry weights measured at the start of the DS in the transgenic events viz., RD11, RD2, RD20 and RD33 were at par with the WT at with statistically similar standing biomass, except for the lower biomass recorded in RD12 and RD19, at PH stage. (Table 4.18).

The pre-harvest leaf dry weights in the transgenic events RD20 (3.98 g plant\(^{-1}\)) and RD2 (3.60 g plant\(^{-1}\)) were at par with that of WT (4.22 g plant\(^{-1}\)), while in RD11 (3.54 g plant\(^{-1}\)), RD33 (3.178 g plant\(^{-1}\)), RD12 (3.07 g plant\(^{-1}\)) and RD19 (2.32 g plant\(^{-1}\)), it was significantly lower (P<0.05) compared to that in WT. Except for the event RD19 that had the lowest initial leaf area (405 cm\(^2\) plant\(^{-1}\)), all the transgenic events and WT had statistically similar leaf area at the pre-harvest stage which ranged from 532 cm\(^2\) plant\(^{-1}\) in RD12 to 654 cm\(^2\) plant\(^{-1}\) in RD11. Similarly, the initial stem dry weight varied significantly in all the tested genotypes where only RD20 (3.16 g plant\(^{-1}\)) was at par with that of the WT, while rest had initial stem dry weight that was significantly lower (P<0.05).

The initial dry weights of the roots in the events RD20 (0.72 g plant\(^{-1}\)) and RD33 (0.725 g plant\(^{-1}\)) were similar to the WT, while rest of the transgenic events had significantly lower (P<0.05) roots dry weight at the pre-harvest stage. Similarly, except for the event RD19 (41.8 cms plant\(^{-1}\)), all the transgenic events had statistically similar
initial root length that ranged from 49.6 cm in RD12 to 55.4 cm in RD20 and 54.33 cm in the WT.

**Transpiration:** Within about 3 wk from the date of last irrigation (saturation) the water uptake decreased by 60% in DS plants compared with that of the WW plants, and in 6 wk it reduced by 80% (Fig. 4.26). Accordingly, during the experimental period, the cumulative transpiration recorded under DS (6272 ml plant⁻¹) was 51% lower than that under WW treatment (12700 ml plant⁻¹). Although, there were no significant differences in cumulative transpiration across the genotypes under DS, under WW the transgenic event RD20 (15230 ml plant⁻¹) had significantly higher (P<0.05) water uptake than RD11 (10860 ml plant⁻¹) and RD12 (10720 ml plant⁻¹). The WT under both DS (6245 ml plant⁻¹) as well as WW (12805 ml plant⁻¹) had cumulative transpiration at par with all the transgenic events (Table 4.19).

**Post harvest gross biomass and TE:** At the end of the experiment, the total leaf dry weight (grand mean) across the genotypes under DS (11.01 g plant⁻¹) remained unchanged as compared to that under WW conditions (10.9 g plant⁻¹) (Table 4.20). Within the WW conditions, the WT (12.378 g plant⁻¹) had leaf dry weight at par with all the transgenic events where it ranged from 9.5 g plant⁻¹ in RD12 to 12.614 g plant⁻¹ in RD33, except for RD11 (8.583 g plant⁻¹) that had the lowest leaf dry weight. However, under DS, when compared to WT (10.876 g plant⁻¹), the leaf dry weight in RD33 (12.616 g plant⁻¹) was significantly higher
(P<0.05) while that in RD19 (9.5 g plant\(^{-1}\)) it was significantly lower (P<0.05). Gross leaf area of under DS (1556 cm\(^2\) plant\(^{-1}\)) was significantly reduced by 45% across the genotypes when compared to that in the plants under WW conditions (2819 cm\(^2\) plant\(^{-1}\)). Within WW, the leaf area in WT (3222 cm\(^2\) plant\(^{-1}\)) was in parity with RD2 (3451 cm\(^2\)/plant), RD20 (3171 cm\(^2\) plant\(^{-1}\)) and RD33 (3460 cm\(^2\) plant\(^{-1}\)), whereas it was significantly lower (P<0.05) in RD11 (1939 cm\(^2\) plant\(^{-1}\)), RD12 (2120 cm\(^2\) plant\(^{-1}\)) and RD19 (2372 cm\(^2\) plant\(^{-1}\)). While, only RD12 had significantly higher (P<0.05) gross leaf area of 1740 cm\(^2\) plant\(^{-1}\) than that in JL 24 (1436 cm\(^2\) plant\(^{-1}\)) under DS, with others being at par with the later.

There was absolutely no change in the average gross stem dry weight in general, across the genotypes under DS when compared to that under WW (Table 4.20). Within the WW, highest gross stem weight was recorded in the WT (21.11 g plant\(^{-1}\)) and the transgenic events RD2 (18.8 g plant\(^{-1}\)) and RD33 (20.47 g plant\(^{-1}\)). Compared to the WT under WW conditions, the gross stem dry weight was significantly lower (P<0.05) in RD11 (12.7 g plant\(^{-1}\)), RD12 (14.4 g plant\(^{-1}\)), RD19 (14.42 g plant\(^{-1}\)) and RD20 (17.53 g plant\(^{-1}\)). In contrast, under the DS treatment, all the tested genotypes including the WT had similar total stem dry weights.

There was no significant difference in the average gross shoot growth in plants across the genotypes under DS in comparison to that under WW (Table 4.20). Within WW, four transgenic events RD20
(29.35 g plant\(^{-1}\)), RD19 (23.74 g plant\(^{-1}\)), RD12 (23.15 g plant\(^{-1}\)) and RD11 (21.28 g plant\(^{-1}\)) had significantly lower (P<0.05) shoot biomass than that in WT (35.04 g plant\(^{-1}\)) and RD33 (35.27 g plant\(^{-1}\)). While under DS, only RD19 (23.81 g plant\(^{-1}\)) had shoot biomass significantly lower than that in the WT (28.634 g plant\(^{-1}\)). RD20 (30.732 g plant\(^{-1}\)) and RD33 (30.188 g plant\(^{-1}\)) had the highest shoot biomass accumulated under the DS conditions.

Average gross roots dry weight across the genotypes increased by 100% under DS (8.2 g plant\(^{-1}\)) compared to that under WW conditions (4.09 g plant\(^{-1}\)). Under WW, the gross roots dry weight in the transgenic event RD11 (3.38 g plant\(^{-1}\)) was significantly lower (P<0.05) than that in the WT (4.56 g plant\(^{-1}\)), while in rest of the transgenic events it was at par with that in WT. In contrast, under the DS treatment, RD20 had the highest root dry weight (10.268 g plant\(^{-1}\)) that was significantly higher (P<0.05) than that in WT (7.497 g plant\(^{-1}\)). No significant differences were observed in rest of the transgenic events when compared to WT (Table 4.20).

On an average, about 30% reduction was observed in the pod and seed yield across the genotypes under DS as compared to that in WW (Table 4.20). Within the treatment under WW, the transgenic event RD2 had pod dry weight (29.184 g plant\(^{-1}\)) and seed weight (20.302 g plant\(^{-1}\)) that was significantly higher (P<0.05) by 19% and 28%, respectively, than the pod weight (24.54 g plant\(^{-1}\)) and seed weight (15.86 g plant\(^{-1}\)) in the WT. While under DS in RD2, the pod
dry weight was 11.414 g plant⁻¹ and seed dry weight was 8.008 g plant⁻¹, which was significantly higher (P<0.05) viz., 81% and 85%, respectively than that in WT (6.289 g plant⁻¹ pod wt; 4.32 g plant⁻¹ seed wt). Under WW, the transgenic events RD20, RD19 and RD12, while under DS, the event RD33 had significantly lower (P<0.05) pod and seed yield when compared to that in WT. The event RD11 was at par with JL 24 under both the test conditions (Fig. 4.27).

Overall, the total biomass across the genotypes under DS was 21% lower than that in WW conditions. Within the WW treatment, biomass in the events RD2 (63.92 g plant⁻¹), RD33 (62.84 g plant⁻¹) and WT (65.686 g plant⁻¹) was significantly higher (P<0.05) than that in RD20 (54.54 g plant⁻¹), RD19 (48.88 g plant⁻¹), RD12 (44.04 g plant⁻¹) and RD11 (41.87 g plant⁻¹). However, under DS, only RD2 (49.485 g plant⁻¹) had significantly higher (P<0.05) gross biomass compared to that in the WT (41.386 g plant⁻¹) and other transgenic events, except for RD20 (45.697 g plant⁻¹) which was at par with RD2.

The average gross TE across the genotypes under DS (6.46 g kg⁻¹ water transpired) compared to that under WW conditions (3.85 g kg⁻¹ water transpired) was 68% higher (Fig. 4.28). Within the WW conditions, TE in the WT (4.315 g kg⁻¹ water transpired) was at par with that of RD20 (3.965 g kg⁻¹ water transpired) and RD12 (3.995 g kg⁻¹ water transpired) but was significantly higher (P<0.05) than that found in RD11 (3.55 g kg⁻¹ water transpired), RD19 (3.53 g kg⁻¹ water transpired), RD2 (3.846 g kg⁻¹ water transpired) and RD33 (3.713 g kg⁻¹ water transpired).
water transpired). While under DS, only RD33 (5.824 g kg\(^{-1}\) water transpired) had TE significantly lower (P<0.05) than that found in the WT (6.515 g kg\(^{-1}\) water transpired), with rest of the transgenic events being at par with the later.

**Post harvest net biomass and TE:** In general, the net growth in the leaf area across the genotypes under DS (935 cm\(^2\) plant\(^{-1}\)) was 58% lower to that under WW (2220 cm\(^2\) plant\(^{-1}\)) (Tables 4.21). Within the DS treatment the transgenic events RD12 (1057 cm\(^2\) plant\(^{-1}\)), RD19 (955 cm\(^2\) plant\(^{-1}\)), RD2 (937 cm\(^2\) plant\(^{-1}\)) and RD33 (988 cm\(^2\) plant\(^{-1}\)) had net leaf area that was significantly higher (P<0.05) than that in WT (784 cm\(^2\) plant\(^{-1}\)). The net leaf area in RD11 (904 cm\(^2\) plant\(^{-1}\)) and RD20 (920 cm\(^2\) plant\(^{-1}\)) were in parity with the WT as well as other transgenic events under DS. While under WW conditions, WT (2569 cm\(^2\) plant\(^{-1}\)) along with RD2 (2815 cm\(^2\) plant\(^{-1}\)), RD20 (2495 cm\(^2\) plant\(^{-1}\)) and RD33 (2818 cm\(^2\) plant\(^{-1}\)) had net leaf area significantly higher (P<0.05) than that in RD11 (1285 cm\(^2\) plant\(^{-1}\)), RD12 (1588 cm\(^2\) plant\(^{-1}\)), and RD19 (1967 cm\(^2\) plant\(^{-1}\)).

Overall, there were no differences in the net leaf and stem dry weights in the net shoot dry weights between the plants under WW and DS conditions recorded across the genotypes (Table 4.21). Within the WW conditions, the net change in leaf dry weight, was significantly low in RD11 (5.043 g plant\(^{-1}\)) in comparison to the WT (8.16 g plant\(^{-1}\)), while other transgenic events were at par with the former. However, under DS, there was parity in net leaf dry weight between the WT and
all the transgenic events, except for the RD33 (9.32 g plant$^{-1}$) which had significantly higher (P<0.05) net leaf dry weight compared to WT (6.66 g plant$^{-1}$) as well as to the transgenic events RD20 (7.737 g plant$^{-1}$), RD19 (7.175 g plant$^{-1}$), RD11 (7.352 g plant$^{-1}$) and RD12 (6.653 g plant$^{-1}$).

Net stem dry weight recorded under DS in all the transgenic events and UT were mutually at par with no significance difference between each other. However, the growth of stem under WW showed significant differences among the genotypes. Net stem dry weight under WW in WT (17.34 g plant$^{-1}$) was significantly higher (P<0.05) than that in RD11 (9.92 g plant$^{-1}$), RD12 (11.92 g plant$^{-1}$), RD19 (12.54 g plant$^{-1}$) and RD20 (14.22 g plant$^{-1}$) but was in parity with that recorded in events RD2 (15.67 g plant$^{-1}$) and RD33 (17.29 g plant$^{-1}$). Therefore, under DS (22.058 g plant$^{-1}$) there was no reduction in the average net shoot dry weight as compared to that under WW (22.259 g plant$^{-1}$) across the genotypes. Within the DS treated plants, no significant difference was seen in the net shoot growth in all the genotypes, although under WW, the transgenic events RD11 (14.959 g plant$^{-1}$), RD12 (18.018 g plant$^{-1}$) and RD19 (19.54 g plant$^{-1}$) had significantly lower net shoot growth than shown by RD33 (55.674 g plant$^{-1}$) and WT (57.526 g plant$^{-1}$).

The response of roots under DS in terms of growth in length and root biomass was highly significant (P<0.001) in comparison to that under WW. Thus, across the genotypes, average net increase in root
length under DS (73.26 cms plant\(^{-1}\)) was 71% higher than that under the WW (42.92 cms plant\(^{-1}\)), while average roots net dry weight was 117% higher under DS (7.43 g plant\(^{-1}\)) than that under WW conditions (3.424 g plant\(^{-1}\)) (Fig. 4.29).

Within the treatment under DS, there was no significant difference in the net root response across the genotypes for net growth in the root length as well as net roots biomass (Table 4.21). But under the WW conditions, the net root length in the WT (42.34 cms plant\(^{-1}\)) was significantly lower (P<0.05) than that in the transgenic events RD33 (60.2 cms plant\(^{-1}\)) and RD19 (56.4 cms plant\(^{-1}\)), while the net root dry weights in the transgenic event RD11 (2.75 g plant\(^{-1}\)) was significantly lower (P<0.05) than that in WT (3.76 g plant\(^{-1}\)) and RD33 (4.09 g plant\(^{-1}\)).

Also, the net biomass in general under DS (36.523 g plant\(^{-1}\)) was reduced by 25% as compared to that under WW (47.996 g plant\(^{-1}\)). Within the same treatment, under DS only RD2 (42.14 g plant\(^{-1}\)) had significantly higher (P<0.05) net biomass than the WT (33.226 g plant\(^{-1}\)), while under WW JL 24 (57.526 g plant\(^{-1}\)) along with the transgenic events RD2 (56.578 g plant\(^{-1}\)) and RD33 (55.674 g plant\(^{-1}\)) had significantly higher (P<0.05) net biomass than RD20 (47.746 g plant\(^{-1}\)), RD19 (41.404 g plant\(^{-1}\)), RD12 (38.389 g plant\(^{-1}\)) and RD11 (34.916 g plant\(^{-1}\)).

Net TE under DS (5.494 g kg\(^{-1}\) water transpired) across the genotypes increased 62%, than that under WW (3.39 g kg\(^{-1}\) water
transpired). Within DS, only RD2 (5.978 g kg\(^{-1}\) water transpired) had significantly higher (P<0.05) net TE than that in the WT (5.27 g kg\(^{-1}\) water transpired), while others were in parity with the later. In contrast, under WW, only RD12 (3.515 g kg\(^{-1}\) water transpired) had net TE at par with WT (3.876 g kg\(^{-1}\) water transpired), as the later had significantly higher (P<0.05) net TE than the rest of the transgenic events (Fig. 4.28).

Overall, under DS, the average gross root length (128 cms plant\(^{-1}\)) per plant across the genotypes was about 35% more than that under WW (94 cms plant\(^{-1}\)). Within the WW treatment, root length per plant in the transgenic event RD33 (111.8 cms plant\(^{-1}\)) was significantly higher (P<0.05) than that in the control JL 24 (98 cms plant\(^{-1}\)) and rest of the transgenic events. However, under DS conditions all the genotypes were in absolute parity with respect to total root length per plant.

**Root traits:** Roots scan studies yielded detailed root traits analysis with respect to segment-wise root length density (RLD), root surface area, root diameter, root volume per plant under both WW and DS across the genotypes, which are briefed below.

**Root length density:** RLD of the roots per plant across the genotypes under DS increased by 45% when compared to that under WW conditions. Genotypic variation in RLD per plant under DS was significant only at deeper layers of soil, mainly 30-120 cms depth,
while upper 0-30 cms had similar RLDs under both the treatments across the genotypes (Table 4.22).

At 30-60 cms depth, the events RD2 (0.883 plant$^{-1}$) and RD12 (0.825 plant$^{-1}$) had significantly higher (P<0.05) RLD per plant than that in WT (0.705 plant$^{-1}$), while at 60-90 cms depth RLD in RD2 (0.749 plant$^{-1}$) and RD33 (0.687 plant$^{-1}$) were significantly higher (P<0.05) than that in the WT (0.494/plant). Only RD2 (0.669 plant$^{-1}$) had RLD per plant significantly higher (P<0.05) than that in WT (0.509 plant$^{-1}$) at 90-120 cms depth.

The total RLD per plant in RD2 (3.465 plant$^{-1}$) under DS was in parity with that of RD20 (3.245 plant$^{-1}$) and RD33 (2.931 plant$^{-1}$), but was significantly higher (P<0.05) than that in WT (2.713 plant$^{-1}$) as well as to that in the transgenic events viz., RD11 (2.82 plant$^{-1}$), RD12 (2.80 plant$^{-1}$) and RD19 (2.66 plant$^{-1}$). In contrast, under WW, only the event RD33 (0.585 plant$^{-1}$) had significantly higher (P<0.05) RLD than that of WT (0.398 plant$^{-1}$) while there were no significant differences found in total RLD per plant between the WT and all the transgenic events, except RD11, which had significantly lower (P<0.05) total RLD per plant at 60-90 cm depth.

Overall, the root surface area per plant across the genotypes under DS enhanced by 61% when compared to that under WW conditions (Table 4.23). While there were no significant differences at 0-60 cms depth, but beyond it, mainly at 60-90 cm depth, significantly higher (45%) increase in roots surface area per plant was
observed when compared to WW under DS conditions. Within DS treatment, significant differences (P<0.05) in root surface area per plant between WT and transgenic events was found beneath 30 cm depth.

At 30-60 cm depth, the transgenic events RD2 (1481 cm² plant⁻¹) and RD33 (1340 cm² plant⁻¹) showed significantly higher (P<0.05) roots surface area per plant compared to that of WT (1134 cm² plant⁻¹). Similarly, at 60-90 cm depth, all the transgenic event in general had higher root surface area per plant than the WT (964 cm² plant⁻¹), with significantly higher (P<0.05) in RD11 (1668 cm² plant⁻¹), RD2 (1576 cm² plant⁻¹), RD33 (1446 cm² plant⁻¹), RD19 (1358 cm² plant⁻¹) and RD20 (1257 cm² plant⁻¹), (Fig. 4.31). Only RD33 (6399 cm²/plant) had total root surface area per plant significantly higher (P<0.05) than WT (4949 cm² plant⁻¹), while rest of the transgenic events had total surface area per plant in parity with that of the later. Under WW conditions, gross root surface area per plant in WT and in almost all the transgenic events, except RD33, separately at all depths as well as together in general, were in parity with each other and had no significant differences. Only RD33 had roots surface area at 60-90 cms depth (1156 cm² plant⁻¹) and the total roots surface area (4132 cm² plant⁻¹) significantly higher than that in WT, which had 907 cm² plant⁻¹ roots surface area at 60-90 cms depth and 3574 cm² plant⁻¹ total roots surface area, under WW conditions.
The total volume of roots per plant across the genotypes under DS was found to be significantly higher (78%) than that under WW conditions (Table 4.24). Within the treatment under DS, while there was parity across the genotypes in the volume of roots per plant at the upper 0-30 cm soil depth, it varied at deeper layers. The volume of roots per plant at 30-60 cm depth in RD2 (20.187 cm³ plant⁻¹) and RD33 (19.554 cm³ plant⁻¹) as well as at 60-90 cm depth in RD11 (31.412 cm³ plant⁻¹), RD2 (28.141 cm³ plant⁻¹) and RD33 (29.024 cm³ plant⁻¹) under DS were significantly higher (P<0.05) than that in WT, which had a lower root volume per plant at 30-60 cm (15.43 cm³ plant⁻¹) and at 60-90 cms (15.95 cm³ plant⁻¹) soil depth.

The total volume of roots per plant in the event RD2 (94.178 cm³ plant⁻¹) was found to be significantly higher (P<0.05) than that in WT (65.897 cm³ plant⁻¹), but rest of the transgenic events were in parity with the later. In contrast, under WW conditions, all the transgenic events and WT had total volume of roots as well as root volumes at varying depths per plant with no significant differences except for in RD33 which had root volume at 60-90 cms depth (17.421 cm³ plant⁻¹) as well as total roots volume (58.59 cm³ plant⁻¹) significantly higher (P<0.05) than that found in the WT (Fig. 4.32).

The average diameter along the whole length of the roots per plant across the genotypes under DS was around 67% higher than that under WW conditions. There was no significant difference in the average diameter of roots per plant across the genotypes under DS, while in WW conditions, the WT (1.717 mm) had average diameter of
roots per plant significantly lower (P<0.05) than that in RD33 (2.104 mm plant<sup>-1</sup>) while was in parity with all other transgenic events for the same.

**Correlations between the component root traits and cumulative transpiration:** Cumulative transpiration under WW across the genotypes showed positive and significantly high correlations with leaf area (P<0.001), leaf dry weight (P<0.001), stem dry weight (P<0.001), shoot dry weight (P<0.001), root length (P<0.001), roots dry weight (P<0.001), pod and seeds dry weight (P<0.001). Under DS, total water uptake had positive and significantly high correlations with leaf area (P<0.001), stem dry weight (P<0.01), shoot dry weight (P<0.05), root length (P<0.001), root dry weight (P<0.005), and pod and seeds dry weight (P<0.05). However, water uptake had no correlation with leaf dry weight, TE and HI. Notably, the root:shoot ratio correlated positively and significantly with HI (P<0.05), as well as with pods /seed yield (P<0.001) under DS (Fig. 4.33).

TE under WW conditions correlated well with shoot dry weight (P<0.001) and the pod /seed yield (P<0.05) but had a negative correlation with HI (P<0.05) and no correlation with root:shoot ratio. While under the DS conditions, though TE correlated well with shoot dry weights (P<0.005), it had no correlation with pod/seed yield, HI as well as with the root: shoot ratio.
4.4 Physiological evaluation under field conditions

This was the first ever lysimetric system based drydown experiment conducted for the screening of transgenic events under natural field conditions during March 4th to May 15th, 2009. Main emphasis was on the evaluation of yield in the six transgenic events relative to the untransformed control variety JL 24 (WT) under WW and intermittent DS imposed in the contained field conditions. During the experiment, periodic uptake of water was recorded which was correlated with the accumulated biomass including shoot, root and pod weight under both WW and DS conditions. Salient findings from the confined outdoor trial in lysimeters are given below.

Transpiration - After the last irrigation given at 46 d from the date of sowing, water uptake (transpiration) recorded in plants across the genotypes under DS declined to about 60% of that recorded in WW plants in around 15 d period and further to 30% in next 3 d (Fig. 4.34). Overall, under intermittent DS, the cumulative transpiration in plants across the genotypes was 17296 ml plant\(^{-1}\) that was about 50% of that under WW conditions (34202 ml plant\(^{-1}\)).

Within the DS treated plants, there were no significant differences in total water uptake (cumulative transpiration) between the WT and the transgenic events. However, the second check, breeding line ICGV 86031 (17735 ml plant\(^{-1}\)) showed significantly higher (P<0.05) cumulative transpiration than RD2 (16736 ml plant\(^{-1}\)), and RD12 (16929 ml plant\(^{-1}\)) under DS. In contrast, under WW, the
WT (29342 ml plant\(^{-1}\)), RD11 (29972 ml plant\(^{-1}\)) and RD12 (25643 ml plant\(^{-1}\)) showed significantly lower (P<0.05) cumulative transpiration than rest of the transgenic events, viz., RD19 (36642 ml plant\(^{-1}\)), RD2 (36242 ml plant\(^{-1}\)), RD20 (37690 ml plant\(^{-1}\)), RD33 (38383 ml plant\(^{-1}\)) and the breeding line ICGV 86031 (39706 ml plant\(^{-1}\)) (Fig. 4.35).

**Biomass - Shoot, Root and Pod dry weights** - Accumulated leaf dry weight across the genotypes under DS was reduced by about 30% to that found in the WW plants (Table: 4.29). Within the DS treatment, the transgenic events RD11 (8.07 g plant\(^{-1}\)), RD19 (9.23 g plant\(^{-1}\)) and RD2 (9.24 g plant\(^{-1}\)) had leaf dry weights significantly lower (P<0.05) than that in the WT (10.98 g plant\(^{-1}\)), while rest of the transgenic events had leaf dry weights statistically at par with both WT and ICGV 86031. Under WW, the transgenic events RD20 (19.11 g plant\(^{-1}\)) and RD33 (17.91 g plant\(^{-1}\)) had significantly higher (P<0.05) leaf dry weight than the WT (12.81 g plant\(^{-1}\)), while rest of the transgenic events as well as ICGV 86031 had leaf dry weight at par with it.

The stem dry weight under DS, in general, showed a reduction of about 37% across the genotypes, as compared to that found in WW treated plants. Under the DS treatment, the breeding line ICGV 86031 (10.18 g plant\(^{-1}\)) and the transgenic events RD11 (8.28 g plant\(^{-1}\)), RD19 (9.99 g plant\(^{-1}\)), RD2 (10.5 g plant\(^{-1}\)) and RD33 (10.10 g plant\(^{-1}\)) had significantly lower (P<0.05) stem dry weight than that in WT (12.08 g plant\(^{-1}\)). Under the WW conditions, no significant differences were seen across the genotypes for the accumulated stem dry weight.
Thus, an overall reduction of 33% was recorded under DS in the accumulated shoot dry weight across the genotypes as compared to that accumulated in WW conditions. Within the DS treatment, the transgenic events RD11 (16.41 g plant\(^{-1}\)), RD19 (19.21 g plant\(^{-1}\)), RD2 (19.74 g plant\(^{-1}\)), and RD33 (20.68 g plant\(^{-1}\)) recorded a significantly lower (P<0.05) shoot dry weight compared to that accumulated in WT (23.0 g plant\(^{-1}\)). While under WW conditions, the transgenic event RD33 (37.57 g plant\(^{-1}\)) had significantly higher (P<0.05) shoot dry weight than rest of the transgenic events, WT JL 24 (28.87 g plant\(^{-1}\)) and ICGV 86031 (33.84 g plant\(^{-1}\)) (Table 4.25).

The root dry weight in general under DS (5.25 g plant\(^{-1}\)), across the genotypes showed an insignificant increase of only 4% as compared to that found in WW treated plants (5.05 g plant\(^{-1}\)). Within the DS treatment, the transgenic events RD12 (4.72 g plant\(^{-1}\)), RD19 (4.61 g plant\(^{-1}\)) and RD2 (3.73 g plant\(^{-1}\)) had significantly lower (P<0.05) roots dry weight than that found in the WT (5.96 g plant\(^{-1}\)), while other transgenic events were at par with the WT as well as with ICGV 86031. In contrast, under WW, the events RD19 (6.66 g plant\(^{-1}\)) and RD33 (6.69 g plant\(^{-1}\)) had roots dry weight significantly higher than that in WT (4.65 g plant\(^{-1}\)).

Yield was recorded as pod number, pod weight, seed number and seed weight per plant under both intermittent DS (Fig. 4.36) and WW conditions (Fig. 4.37). Pod number under DS (21.15 pods plant\(^{-1}\)) reduced by 37% across the genotypes when compared to that under
WW conditions (33.7 pods plant$^{-1}$) (Table 4.25). Under DS, the transgenic event RD11 (25.75 pods plant$^{-1}$) had significantly higher (P<0.05) pod numbers than that in WT (21.0 pods plant$^{-1}$), while other transgenic events as well as ICGV 86031 had pod numbers in parity with the later. However, under WW conditions, along with the elite breeding line ICGV 86031 (37 pods plant$^{-1}$), the pod number in the transgenic events RD11 (35.14 pods plant$^{-1}$), RD19 (37.33 pods plant$^{-1}$), RD2 (39 pods plant$^{-1}$) and RD20 (36.2 pods plant$^{-1}$) were significantly higher (P<0.05) than that in the WT (27 pods plant$^{-1}$).

There was a higher reduction of pod dry weight in plants across the genotypes under the intermittent DS (7.16 g plant$^{-1}$) in the outdoor field conditions as it reduced by 66% in comparison to that found under WW conditions (20.89 g plant$^{-1}$). Under the intermittent DS, though insignificant, the transgenic events RD11 (9.04 g plant$^{-1}$) and RD33 (8.59 g plant$^{-1}$) had relatively higher pod weights than WT (7.42 g plant$^{-1}$) and were in parity with other transgenic events except RD12 (5.25 g plant$^{-1}$), which had significantly lower pod weight than that of WT. In contrast, under WW conditions, pod weights in the breeding line ICGV 86031 (26.49 g plant$^{-1}$) and transgenic events RD2 (22.96 g plant$^{-1}$), RD20 (26.28 g plant$^{-1}$) and RD33 (24.62 g plant$^{-1}$) were significantly higher (P<0.05) than that in WT (16.59 g plant$^{-1}$), with remaining transgenic events being in parity with the later.

Similarly, the number of seeds per plant across the genotypes under DS (21.45 seeds plant$^{-1}$) reduced by 56% when compared to
that under WW (48.72 seeds plant$^{-1}$). Thus, under DS, the number of seeds per plant in transgenic events RD11 (32.71 seeds plant$^{-1}$), RD19 (24.0 seeds plant$^{-1}$), RD2 (23.57 seeds plant$^{-1}$) and RD33 (24.33 seeds plant$^{-1}$) were significantly higher (P<0.05) than that in the WT (17.57 seeds plant$^{-1}$), while others were in parity with the later. Also under WW conditions, the transgenic event RD2 (58.83 seeds plant$^{-1}$) had significantly higher (P<0.05) seed numbers than that in WT (42.5 seeds plant$^{-1}$), while rest of the transgenic events and ICGV 86031 had seed numbers at par with the later.

Seed weight under the DS (4.21 g plant$^{-1}$) reduced drastically (71%) across the genotypes as compared to that found under WW (14.47 g plant$^{-1}$) treatment in the field. Within DS, the seed weight in the transgenic events RD11 (6.37 g plant$^{-1}$) and RD33 (5.85 g plant$^{-1}$) was significantly higher (P<0.05) than that in WT (4.18 g plant$^{-1}$), while other genotypes were in parity with the later. Also under WW conditions, the transgenic events RD2 (16.84 g plant$^{-1}$), RD20 (18.98 g plant$^{-1}$), and RD33 (18.74 g plant$^{-1}$), along with the control breeding line ICGV 86031 (18.15 g plant$^{-1}$), had significantly higher (P<0.05) seeds weight (10.88 g plant$^{-1}$), while the seed weights in the remaining transgenic events were not different from the WT. Thus, overall biomass across the genotypes under DS (33.53 g plant$^{-1}$) was reduced by 42% when compared to that accumulated under WW conditions (57.379 g plant$^{-1}$) (Table 4.25). Within the DS treatment, the events RD11 (31.16 g plant$^{-1}$), RD12 (32.65 g plant$^{-1}$), RD19 (31.3 g plant$^{-1}$) and RD2 (30.38 g plant$^{-1}$) had significantly lower (P<0.05) biomass
than that in the WT (36.22 g plant⁻¹). While under WW, the events RD20 (70.01 g plant⁻¹), RD33 (69.09 g plant⁻¹) and the breeding line CGV86031 (64.63 g plant⁻¹) had significantly higher (P<0.05) biomass than that in WT (50.12 g plant⁻¹). Biomass per plant accumulation under WW conditions was similar to the WT in rest of the transgenic events.

Under the intermittent DS conditions, in general, the HI reduced by 38% across the genotypes when compared to that under WW conditions (Table 4.26). The transgenic event RD11 (0.287 plant⁻¹) had significantly higher (P<0.05) HI than that in WT (0.206 plant⁻¹) as well as the elite breeding line ICGV 86031 (0.219 plant⁻¹). However, RD2 (0.396 plant⁻¹) and ICGV 86031 (0.403 plant⁻¹) had significantly higher (P<0.05) HI than WT (0.313 plant⁻¹) under the WW conditions (Fig. 4.38A).

**Transpiration Efficiency** (TE) in plants across the genotypes under intermittent DS (1.94 g kg⁻¹ water transpired) was marginally higher by 17% than that under WW (1.65 g kg⁻¹ water transpired) (Table 4.30). Only the transgenic event RD11 (1.44 g kg⁻¹ water transpired) had significantly lower (P<0.05) TE than that in WT (1.67 g kg⁻¹ water transpired) under WW, while the TE in others were in parity with the later. In contrast, under DS treatment, the breeding line ICGV 86031 (1.87 g kg⁻¹ water transpired) and the transgenic events RD11 (1.77 g kg⁻¹ water transpired), RD12 (1.94 g kg⁻¹ water transpired), RD19 (1.79 g kg⁻¹ water transpired) and RD2 (1.81 g kg⁻¹ water transpired)
had significantly lower TE (P<0.05) than that in the WT (1.87 g kg\(^{-1}\) water transpired) (Fig. 4.38B).

**Root: shoot ratio** - Comparative root: shoot ratio in plants across the genotypes under DS (0.25 plant\(^{-1}\)) enhanced by 64% than that under WW (0.15 plant\(^{-1}\)) (Table 4.26). Within the treatment under DS, the transgenic event RD11 (0.32 plant\(^{-1}\)) had significantly higher (P<0.05) root: shoot ratio than that in WT (0.255 plant\(^{-1}\)), while that in the events RD12 (0.21 plant\(^{-1}\)) and RD2 (0.195 plant\(^{-1}\)), it was significantly lower (P<0.05). Under WW conditions, the root: shoot ratio was found to be similar in all the genotypes.

**Correlations:** Under intermittent DS during the confined outdoor trial, yield (Y) was found to be significantly (P<0.001) and positively correlated with the cumulative transpiration (R\(^2\) = 0.372), HI (R\(^2\) = 0.787) and root: shoot ratio (R\(^2\)=0.760). The root: shoot ratio also correlated significantly (P<0.001) and positively with HI (R\(^2\) =0.443), (Fig. 4.39). However, the TE showed no correlation with either pod weight or harvest index in the field trial. In the field trial, the cumulative transpiration (T), TE and yield efficiency showed significant positive correlation (P<0.001) with respect to each other under WW, while HI had no correlation with TE but showed negative correlation (P<0.05) with T. In the confined outdoor trial, HI, TE and yield showed a very high degree of correlation (P<0.001) with respect to each other as well as with the aerial biomass. HI, unlike TE and yield showed no correlation with cumulative transpiration, although it
had significant positive correlation (P<0.05) with the transpiration during 46-82 d of the trial.

4.5 ABA estimations during outdoor field trial

The ABA content in leaves under DS (69.44 ng gm⁻¹ leaf fresh wt.), in general, was significantly higher than that under the WW (27.10 ng gm⁻¹ leaf fresh Wt.) across all the genotypes (Table 4.27). The ABA content generally showed a significant increase ranging from 142% to 189% across the transgenic events while it raised to 200% in WT and 87% in ICGV 86031 under DS when compared to WW. However, statistically there were no significant differences across the genotypes in the measured ABA content neither under DS nor under WW conditions (Fig. 4.40).

4.6 Introgression studies

F1 hybrids - The initial cross between the elite germplasm line ICGV 86031 (♀) and the transgenic event RD2 (♂) resulted in the production of the F1 progeny. Thirty one F1 plants were produced from the crosses between seven individual plants of ICGV 86031 with RD2-8-3-1-2 (batch I), while 50 F1s resulted from the crosses between 6 different plants of ICGV 86031 and RD2-8-3-1-5 (batch II). Out of the 81 F1 seeds sown and screened for presence of the transgene, 61 F1 plants were positive for both nptII and rd29A:DREB1A transgenes, while the rest of the 20 F1s were found to be negative (Table 4.28). PCR analysis of the 16 plants (T4 progeny) of the transgenic event
RD2 that was used for introgression showed two PCR negative plants while 14 plants were positive.

**BC1** - A total of 86 BC1F0 seeds were produced from the backcross (BC1) between the recurrent parent ICGV 86031 and 26 PCR positive F1 plants that were initially obtained from the cross involving 7 ICGV 86031 plants × RD2-8-3-1-2 (Table: 4.29). Similarly, 128 BC1F0 seeds were produced from the backcross (BC1) between ICGV 86031 and the 29 PCR positive F1 plants that were obtained from the cross involving 6 ICGV 86031 plants × RD2-8-3-1-5. 24 BC1F0 seeds from first batch and 38 BC1F0 seeds from the second were sown for BC2.

**BC2** - Screening of all the BC1F0 plants before carrying out BC2, showed presence of the transgene in only 14 out of 62 BC1F0 plants; 3 from the first batch and 11 from the second batch of BC1F0 population (Table 4.30). The BC2 population resulting from backcross of the PCR positive BC1F0 progeny with the concurrent parent ICGV 86031 resulted in the production of 19 BC2F0 seeds from first batch and 60 BC2F0 seeds from the second batch of plants.

**BC3** - All the 79 BC2F0 seeds from the PCR positive BC2F0 parents were sown for carrying out the third successive backcross resulting in the BC3 population (Table 4.31). PCR screening of the BC2F0 progeny detected the transgene in a total of 17 plants including four from first batch and 13 from the second batch progeny. BC3 involving the backcross of the PCR positive BC2F0 progeny with the concurrent
parent ICGV 86031 resulted in the production of 59 BC3F0 seeds from first batch and 97 BC3F0 seeds from the second batch of plants.

**Comparative morphology:** Following the generation of BC3 population, the BC2F0 progeny carrying the transgene *rd29A:DREB1A* showed almost all the morphological features (90%) of ICGV 86031 with no detectable resemblance to JL 24 (Table 4.32).

**RT-PCR studies to detect the induction of DREB1A gene in the introgressed F1 progeny** - RNA was isolated from young leaves of mature F1 plants. The integrity of the isolated RNA was confirmed by the presence of two bands of rRNA (28S and 18S) on resolving the total RNA on 1% TBE agarose gel. Of the 24 tested F1 hybrid plants, one plant showed an amplification of the expected 499 bp *DREB1A* fragment (Fig. 4.41).