DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit

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High water uptake, efficient conversion of water into biomass and high harvest index are the key components of the yield architecture (Passioura 1977), in particular under conditions where water deficit is common. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been involved for many years in the development of groundnut (*Arachis hypogaea*) breeding lines having high water use efficiency (WUE), assessed by its major component, evapotranspiration efficiency (TE), because WUE has been identified as a major contributor to pod yield under intermittent water deficit conditions (Wright et al. 1993, 1994). However, a trait-based approach using surrogates of TE as selection criteria to select genotypes with high TE has not proved more successful than a yield-based approach where lines are selected based on yield under water deficit (Nigam et al. 2005). Partly it might be because of the insufficiently strong correlation between TE and its surrogates (Krishnamurthy et al., in press). It may also be explained by a reported negative link between TE and the harvest index (HI), ie, genotypes with high TE usually had low HI (Wright et al. 1991). These results demonstrate that each component of the yield architecture cannot be addressed independently from the other.

In a previous work (Bhatnagar-Mathur 2006), we found that the DREB1A, an ABA-independent transcription factor, introduced into the groundnut variety JL 24, appeared to confer water-economizing capacity in the resulting transgenic plants when compared to their non-transformed parent. Several transgenic events appeared to have consistently higher TE than the wild type (WT) across different water regimes. Although these data are very encouraging, one should be cautiously certain about the fact that improved TE in these lines is not at the cost of other yield architecture components.

In any case, DREB1A certainly appeared to confer some drought avoidance mechanism, by improving WUE. Here we were interested to test whether DREB1A could also induce drought avoidance through a better water capture. It is known that under water stress, plants tend to increase their root/shoot ratio. So, in this work, we tested whether *DREB1A* gene driven by stress inducible rd 29A promoter could have an effect on root growth under water deficit.

Measurement of root traits, though better and more easily done in a controlled cylinder system than in the field, remain a time consuming exercise, with large error component. Root traits are usually evaluated by destructive sampling at set dates (Kashiwagi et al. 2006). Although this approach has been suitable to reveal large variation in root traits, the root data thus generated inform neither about actual water uptake, nor about the kinetics of water uptake. In fact, there are contradictory reports about the relation between root length, density and actual water uptake. In the end, water uptake under water deficit matters more than roots. Therefore, we were interested to assess whether DREB1A transgenics would show an effect of the transgene on water uptake under water deficit. If so, the next step would be to assess how these putative differences in the evapotranspiration pattern under water deficit conditions relate to the rooting pattern, and to root trait in particular. To do so, we used long (1.2 m) and large (16 cm diameter) PVC tubes, a system where plants would have a large and deep volume of soil to explore, which would mimic the soil profile.

Therefore, the purpose of this work was two-fold: (i) report on the use of this lysimetric system; and (ii) assess the changes in the evapotranspiration response and in the rooting pattern upon exposure to water deficit in five transgenic events and their WT parent.

Materials and methods

The lysimetric system used was previously set up using groundnut genotypes, and showed that large differences could be found in the evapotranspiration profile and rooting pattern upon exposure to water deficit (data not shown). *DREB1A* transgenic groundnut plants in their T_4 generation, carrying the *DREB1A* gene under the control of stress-inducible promoter from the gene *rd29A* were grown in 1.2 m long and 16 cm diameter PVC tubes,

mimicking closely the field conditions in terms of soil volume available to each plant.

Five transgenic events (RD 2, RD 11, RD 12, RD 19 and RD 20 that were also used in previous dry-down trials in the greenhouse) of the variety JL 24 were assessed along with their non-transformed parent (WT). Eighteen PCR (polymerase chain reaction) positive plants per genotype were grown for 30 days and at 30 days after sowing (DAS), 6 plants per genotype were harvested to assess root depth and root dry weight in 15 cm layers. The remaining 12 cylinders per genotype were saturated with water, following which 6 plants were maintained under well-watered (WW) conditions while the other 6 were left with no further irrigation [ie, water stressed (WS)].

Cylinder weight was recorded on a regular basis, usually every 3 days. Water loss in WW plants was adjusted to the cylinder weight 3 days after imposing the treatment to maintain WW cylinders close to field capacity. The process of weighing the cylinders was relatively simple and rapid. Evapotranspiration was calculated as the difference in cylinder weight between consecutive weighings. The evapotranspiration data were normalized against controlled plants for each genotype, ie, each individual plant's evapotranspiration was divided by the

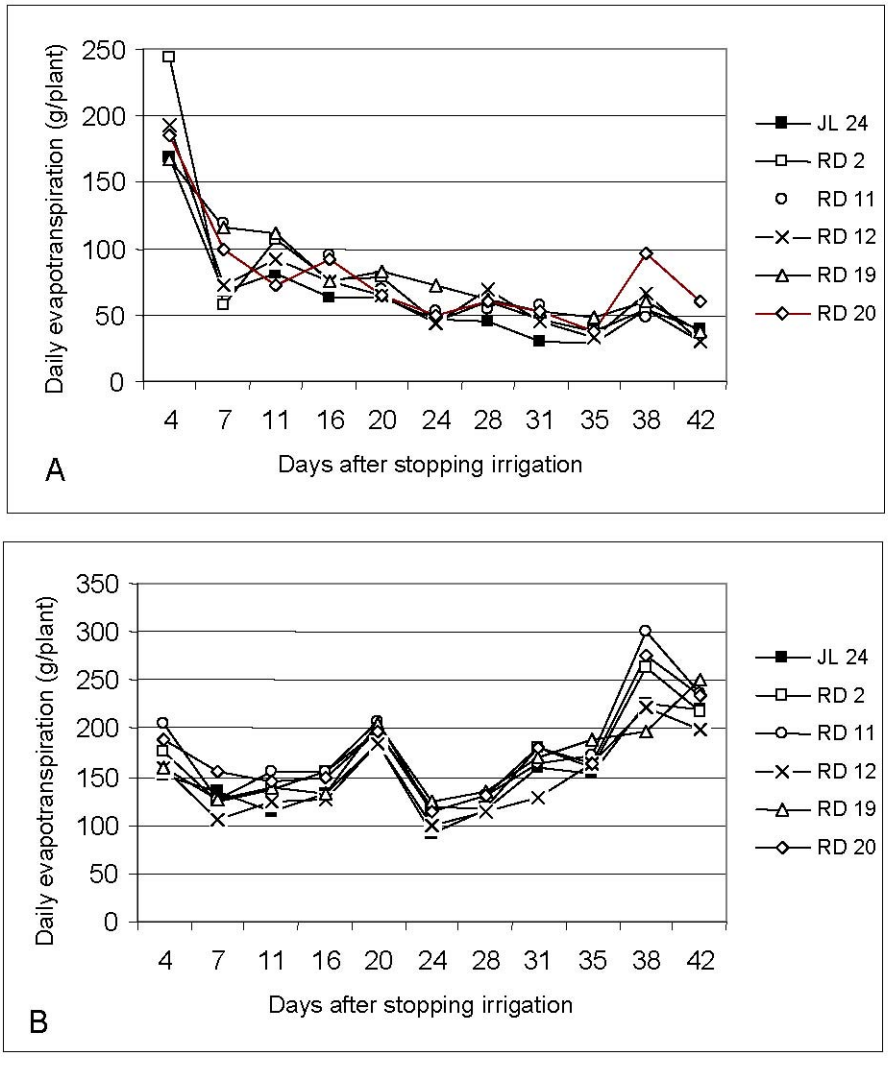

Figure 1. Transpiration profile in five transgenic DREB1A::rd29 events and wild type JL 24, under water stress (A) and well-watered conditions (B). (Data are means of 6 plants per genotype and treatment.)

Table 1. Cumulative evapotranspiration (Tr) over the entire experimental period, or when FTSW (fraction of transpirable soil water) was below 0.68 (from 12 to 42 days after imposing the treatments), on five transgenic DREB1A::rd29 events and wild type JL 24, over a period of 38 days after imposing water stress and under well-watered conditions[1].

Genotype	Cumulative Tr (g)	Tr <68%	Shoot dry weight (g)
Water stress			
RD 2	2780 ± 32	2120 ± 13	12.07 ± 1.55
RD 11	2847 ± 128	2113 ± 72	13.87 ± 1.35
RD 12	2643 ± 126	2040 ± 121	13.30 ± 1.74
RD 19	3055 ± 180	2375 ± 136	13.06 ± 1.83
RD 20	2957 ± 128	2287 ± 120	14.92 ± 1.28
JL 24	2328 ± 209	1788 ± 159	11.45 ± 2.02
Well watered			
RD 2	6608 ± 267	5887 ± 245	17.44 ± 2.63
RD 11	7022 ± 285	6233 ± 272	16.50 ± 2.37
RD 12	5857 ± 277	5220 ± 274	16.82 ± 3.92
RD 19	6630 ± 291	5933 ± 231	17.11 ± 1.93
RD 20	6897 ± 381	6057 ± 353	20.90 ± 3.09
JL 24	6043 ± 83	5333 ± 61	16.96 ± 2.19

1. Data are means (±SE) of 6 plants per genotype and treatment.

mean evapotranspiration of WW plant. Then a second normalization was done by dividing normalized values by the initial value for each individual plant. These normalizations allowed for the comparison of the relative profile of evapotranspiration of WS plants with regard to their respective controls at the beginning of the experiment, when control and WS plants were still very similar in biomass. Later on, these normalizations were not used because WS plants had a very different development than controls, and then we preferred to use the profile of evapotranspiration with no normalization.

At the end of the experiment, cylinder weight was taken one last time. Plants were then harvested. Shoot and pods were harvested and separated. Roots were extracted gently by washing the soil from both ends of the cylinders after removing the end cap. Total root depth was measured by stretching the entire root system. Then, the root system was divided into 15-cm portions, which were cut, bagged, dried and weighed. Because similar harvest happened at the time of treatment imposition, we could measure TE as the ratio between biomass increase during the experimental period and the evapotranspiration during the experiment. Here, we assumed that the evaporation component was similar across all genotypes.

Results

Normalized evapotranspiration (NTR), ie, the evapotranspiration under WS conditions relative to WW control, dropped to about 50–60% of control following 12 days after the imposition of drought stress. Thereafter, NTR of most of the transgenic plants except one remained above that of the WT. The daily transpiration rate in transgenics was above JL 24 for most of the time from 7 days after withdrawing irrigation, more particularly in RD 12, RD 19 and RD 20 (Fig. 1A). By contrast, the profile of transpiration under WW conditions was essentially the same in all the genotypes (Fig. 1B). Overall, the total evapotranspiration under water deficit was higher by 14 to 31% in all transgenic plants when compared to the WT. By contrast, the total evapotranspiration under WW conditions was within a close range in all genotypes with evaporation being 0–16% higher in the transgenic events than in JL 24. For instance, cumulative evapotranspiration of the event RD 12 (during the 12–42 days after stress imposition) was about 300 g higher than that in WT, whereas under WW conditions, cumulative evapotranspiration of the event RD 12 was 200 g lower than JL 24. This trend was true in all the tested transgenic plants (Table 1).

A remarkable finding was that the root dry weight of all six genotypes was within a very narrow margin under WW conditions (1.48–1.63 g, with WT having 1.61 g). By contrast, under WS conditions, while the root dry weight of WT remained unchanged (1.73 g), that of all the transgenics dramatically increased to a range of 2.27 to 2.65 g, a 30% overall increase (Fig. 2A). We found that under WW conditions the root/shoot ratio was similar and slightly larger in WT than in the transgenics. By contrast, under water deficit, the root/shoot dry weight ratio dramatically increased in the transgenics and became higher than in WT (Fig. 2B).

Indeed, under WS conditions, all the transgenics had more profuse rooting in deep soil layers when compared to the WT. Figure 3B shows how under WW conditions, there was virtually no difference between the transgenics and the WT in the pattern of root distribution over the different soil depths. By contrast, under WS conditions, there were large differences in this pattern where all the tested transgenics had much deeper and more profuse rooting than the WT (Fig. 3A). In fact, there was a good relation between the root dry weight within the 40–120 cm soil depth and the total evapotranspiration ($r^2 = 0.91$). Consequently, shoot dry weight was 20–40% higher than JL 24 under water deficit, whereas shoot dry weight was similar in all genotypes (except RD 20) under WW conditions. Also, we found that drought stressed JL 24 had no pods, whereas all transgenic plants did have few pods. In that system, we also found that TE under WS conditions was about 20% higher in RD 2, RD 11 and RD 20 than in JL 24, whereas it was only slightly above that of JL 24 in the other two events.

Discussion

A remarkable finding in the present study was that DREB1A clearly induced a root response under water deficit conditions. This response enhanced root growth under water deficit, in particular in the deep soil layers. Consequently, water uptake under water deficit was enhanced, up to 20–30% in some transgenics compared to the WT. And this water uptake was well related ($r^2 = 0.91$) with the root dry weight below the 40 cm soil depth.

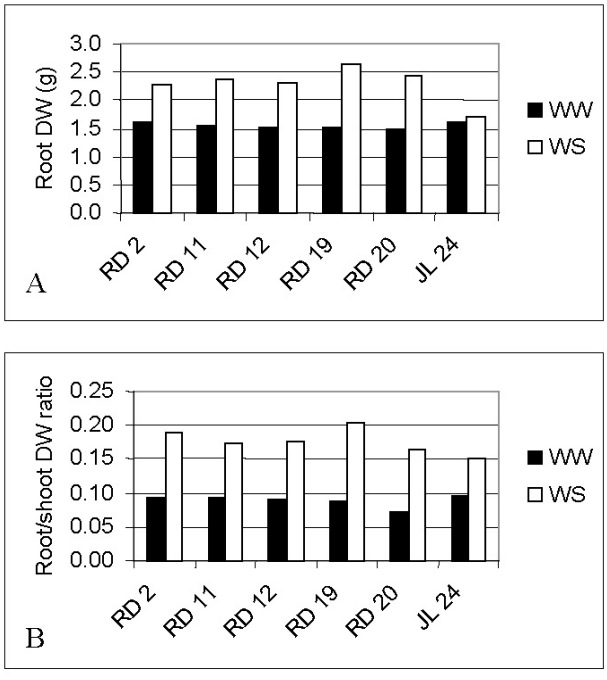

Figure 2. Root dry weight (DW) (A) and root/shoot DW ratio (B) in five transgenic DREB1A::rd29 events and wild type JL 24, at 35 days after imposing water stress (WS) and under well-watered (WW) conditions. (Data are means of 6 plants per genotype and treatment.)

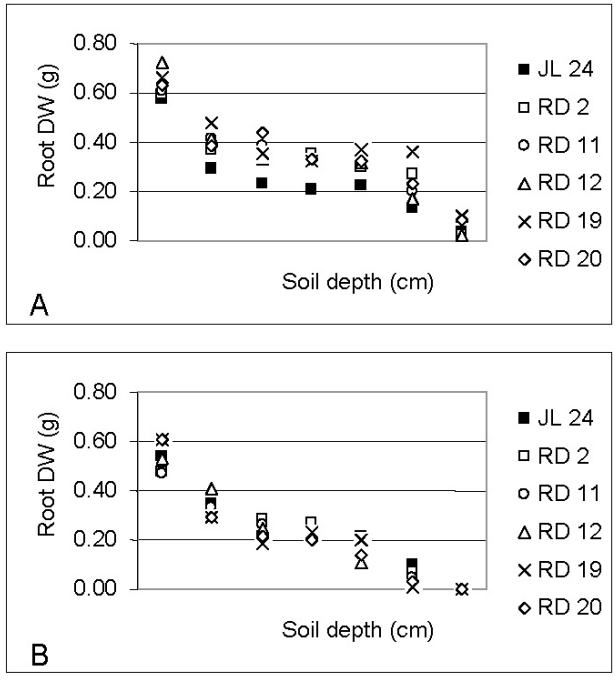

Figure 3. Distribution of root dry weight (DW) over different soil depths in five transgenic DREB1A::rd29 events and wild type JL 24, at 35 days after imposing water stress (A) and under well-watered conditions (B). (Data are means of 6 plants per genotype and treatment.)

Finally, it appeared that the putative effect of DREB1A on root under WS conditions was due to an effect on the root/shoot ratio, which was dramatically increased under water stress in all transgenic lines.

This is the first ever report of DREB1A transcription factor having such an impressive effect on the root growth under water deficit. It is not unexpected, as DREB1A appears to be a major "switch" for a cascade of genes that are activated under water deficit. Interesting work would be needed to compare the root transcript profile of the WT with that of the transgenics under water stress.

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Citation: V Vadez, S Rao, KK Sharma, P Bhatnagar-Mathur **and** M Jyotsna Devi. (2007) DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit. Journal of SAT Agricultural Research 5(1).