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***DREB1A* promotes root development in deep soil layers and increases water extraction under water stress in groundnut**

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Keywords

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ABSTRACT

Water deficit is a major yield limiting factor for many crops, and improving the root system has been proposed as a promising breeding strategy, although not in groundnut. Present work was carried out mainly to assess how root traits are influenced under water stress in groundnut, whether transgenics can alter root traits, and whether putative changes lead to water extraction differences. Several transgenics events, transformed with *DREB1A* driven by *rd29* promoter, along with wild type JL24, were tested in a lysimetric system that mimic field conditions under both water stress (WS) and well-watered (WW) conditions. The WS treatment increased the maximum rooting depth, although the increase was limited to about 20% in JL24, compared to 50% in RD11. The root dry weight followed a similar trend. Consequently, the root dry weight and length density of transgenics was higher in layers below 100cm depth (Exp.1) and below 30 cm (Exp.2). The root diameter was unchanged under WS treatment, except a slight increase in the 60-90 cm layer. The root diameter increased below 60 cm in both treatments. In the WW treatment, the total water extraction of RD33 was higher than in JL24 and other transgenic events and was somewhat lower in RD11 than in JL24. In the WS treatment, the water extraction of RD2, RD11, and RD33 was higher than in JL24. These water extraction differences were mostly apparent in the initial 21 days after treatment imposition and were well related to the root length density in the 30-60 cm layer ($R^2 = 0.68$), but not to the average root length density. In conclusion, water stress promotes rooting growth more strongly in transgenic events than in the wild type, especially in the deep soil layers, and this leads to an increase in the water extraction. This opens a scope for tapping these characteristics toward the improvement to drought adaptation in deep soil conditions, or toward a better understanding of genes involved in rooting in groundnut.

Key words: Water extraction, drought, root length density, breeding

INTRODUCTION

Water stress is the most important abiotic factor limiting crop production in groundnut, causing an estimated 520 M US\$ losses annually (Sharma & Lavanya 2002). Breeding efforts to improve the adaptation of groundnut to drought have been undertaken, mostly focusing on trying to improve groundnut's water use efficiency (Hubbick *et al.* 1986; Wright *et al.* 1994; Krishnamurthy *et al.* 2007). A previous report on groundnut transgenic events of a popular groundnut variety (JL24), using *At rd29::DREB1A*, showed an increase transpiration efficiency (TE) in several events (Bhatnagar-Mathur *et al.* 2007; Devi *et al.* 2011). However, the possibility of using *DREB1A* transgenics in groundnut to improve the rooting capacity of groundnut to extract water from the soil profile to support transpiration has not received attention.

Few studies have been done on roots in groundnut and most report rooting differences under various water regimes (Ketring *et al.* 1982; Pandey *et al.* 1984; Robertson *et al.* 1980, Boote *et al.* 1982). Preliminary data indicate that *DREB1A* events of groundnut increased evapotranspiration and the root/shoot ratio (Vadez *et al.* 2007) under water deficit, although detailed results on water extraction to support transpiration are lacking. More recent data in groundnut using a lysimetric system that mimics field conditions indicated that the pattern of water extraction was also critical in explaining yield differences under intermittent drought (Ratnakumar *et al.* 2009). So, here we assess whether transgenic events differ for water extraction to support transpiration and for the profile of water extraction, using a lysimetric system where soil evaporation was strictly controlled by mulching the soil surface.

Whether root length density and water extraction are closely related is still a matter of debate. Several authors concluded that root length density and water uptake are related (Passioura 1983; Monteith 1986, Lafolie *et al.* 1991). By contrast, other studies show poor relationships between water uptake and RLD across several cereals and legumes (Hamblin & Tennant 1987; Dardanelli *et al.* 1997; Katayama *et al.* 2000, Amato & Ritchie 2002; Zaman-Allah *et al.* 2011). In groundnut, poor relationships between root dry weight and evapotranspiration in groundnut varieties was reported (Vadez *et al.* 2008), or between root length density and water extracted to support transpiration in breeding materials (Ratnakumar & Vadez 2011). However, closer relationship between root dry weight and evapotranspiration in *DREB1A* groundnut transgenics was found

(Vadez *et al.* 2007). This raises the question whether the nature of the genetic material used in such studies, either with large genetic variation in germplasm or breeding material, or near isogenic in the case of *DREB1A*, matters for the relationships that were found. In the latter report root length density was not assessed, root extraction was done in plants having gone beyond permanent wilting, and only evapotranspiration was measured. Here precise data on root length density and water extraction are generated following more recent studies (Ratnakumar *et al.* 2009; Ratnakumar & Vadez 2011) to ascertain a possible link between rooting and water extraction differences.

The objectives of the work were: (i) to assess whether *DREB1A* events of groundnut do extract higher amount of water from the soil profile; (ii) assess the kinetics of water extraction over time; (iii) assess root attributes, especially root length density and maximum rooting depth, and assess their relationship with water extraction.

MATERIALS AND METHODS

Soil filling and growth conditions in the lysimeters

Plants were grown in lysimeters, i.e. PVC tube of 20 cm diameter and 1.2 m length, filled with a mixture of Alfisol and sand (1:1 w:w) to facilitate root washing. A PVC end plate was placed on top of four screws at the bottom of the cylinders, 3cm from the very bottom, to prevent the soil from seeping through. The endplate did not fit the cylinder tightly and allowed water drainage. The Alfisol used to fill the tubes was collected on the ICRISAT farm and was sieved in particles smaller than 1 cm, before mixing with the sand, to ensure homogeneity of the bulk density in the soil profile and across cylinders. The soil that was used to fill up the lysimeters had been fertilized with diammonium phosphate and muriated potash, both at a rate of 200 mg kg⁻¹ soil. It was also complemented with sieved and sterilized farm manure at a rate of 2:50 to prevent micro-nutrient deficiency. A total of 48 kg of soil was filled in each cylinder and watered to field capacity, following procedures previously described (Vadez *et al.* 2008). Cylinders weighed between 58 and 60 kg.

The top of the cylinders was equipped with a metal collar and rings that allowed the lifting and weighing of the cylinders with a S-type load cell (Mettler-Toledo, Geneva, Switzerland). The scale, of 100 kg capacity allowed repeated measurements and gave an accuracy of 10 g on each weighing. The lysimeters were separated from one another by a distance of approximately 2 cm so that the planting density of groundnut was about 21 plants per square meter, a density very similar to the field planting (20-25 plant m⁻²). Therefore, soil volume available and plant spacing provided growth conditions that were very similar to the field environment. The tubes were arranged in a P2 glasshouse at ICRISAT and arranged in strips of four cylinders width.

Plant material and experiments

Six transgenic events (RD2, RD11, RD12, RD19, RD20, and RD33) along with the wild type parent JL24 were tested. Each event was a single copy insert of *DREB1A*, driven by rd29 promoter from *Arabidopsis thaliana*. A detailed description of the materials can be found in Bhatnagar-Mathur et al. (2007). The events tested here were part of a larger generation of events that underwent an initial selection in pots (Bhatnagar-Mathur et al., 2004) and then an assessment of transpiration efficiency (Devi et al., 2011). Three seeds were planted in each cylinder, and then thinned to two seedlings per cylinder at 7 days after sowing (DAS). PCR were carried out on each plant within the first 2 weeks in order to eliminate PCR-negative plants. Thereafter, only one PCR positive plant was kept in each cylinder from 14 DAS.

Two experiments were carried out between the August-October period and the December-February period. The conditions in the glasshouse were controlled in a similar way with day/night temperature at 20/28°C. Relative humidity oscillated between about 60 and 80% during the day. The purpose of Exp.1 was to provide information on the root dry weight and depth, on the profile of plant water extraction to support transpiration under both a water stress (WS) imposed by stopping irrigation at flowering (36 days after sowing) and a well watered (WW) treatment, and on the total volume of water extracted from the soil profile. The purpose of Exp.2, carried out in a different season but with similar environmental control, was then to generate precise data on the root

characteristics at different depth (root length density in different soil layers, surface area, diameter, maximum rooting depth), with the objective of assessing the relationship between these rooting traits and the water extraction. The protocol used was similar to the one of Exp.1, with a WS treatment imposed at flowering (34 DAS) and compared to a WW treatment. Roots were also assessed prior to imposing the stress, in an additional treatment maintained under WW conditions until 34 DAS (WW-Flowering) using an additional set of lysimeters that were harvested at 34 DAS.

Treatment imposition and water extraction measurements

Prior to planting, the cylinders were irrigated to reach field capacity. All plants were kept under fully irrigated conditions until treatment application around flowering time (36 and 34 DAS in Exp.1 and Exp.2). Until then, cylinders received 500 mL twice a week. Prior to flowering, the surface of the cylinder was covered with a 2-cm layer of plastic beads to prevent soil evaporation. At flowering, all cylinders received a 1-L irrigation to bring the soil profile to field capacity. The cylinders were allowed to drain the excess water for 36 hours before the cylinder weighing started. The initial cylinder weight corresponded to the weight at field capacity. The cylinder weighing was then done twice a week, with a frequency of either 3 or 4 days between each weighing, for 7 and 6 weeks after treatment imposition in Exp.1 and Exp.2 respectively. The water stress (WS) cylinders received no more water until harvest at 49 and 42 days after treatment imposition in Exp.1 and Exp.2 respectively. After each weighing, the WW treatment received water to compensate water losses in excess of 1L from the cylinder weight at field capacity. This allowed keeping the WW plant fully irrigated while avoiding the possibility of water drainage at the bottom of the cylinder.

Measurement of root parameters

At harvest, the aboveground parts were severed at the hypocotyls level and shoot were dried in a forced-air oven at 70°C for 3 days. Before extracting the roots, the soil was re-saturated with water to facilitate root washing. The screws supporting the end-plate at the

bottom of the cylinders were taken off to allow gentle hose-washing of the soil to separate the entire root system. Root washing was completed within 2 days after harvest and scanning was done in the subsequent 2-3 days. Once the root system was extracted, it was gently laid on a table to assess its maximum length. In most cases, the roots had reached the bottom of the cylinders and the roots were curling at the bottom. In such case, the roots that grew beyond 120 cm were considered in separate sections.

In Exp.1, the root system was divided in 20 cm (0-20; 20-40; 40-60; 60-80; 80-100; 100-120; 120-140; 140-160; 160-180; 180-200) sections, which were put in a paper bag and dried in a forced-air oven at 70°C for 3 days. In Exp.2, root washing followed the same procedure until the root system was divided into 30 cm sections (0-30cm; 30-60cm; 60-90cm; 90-120cm; 120-150) from the top, put in a plastic bag, and kept in the refrigerator at 4°C. Scanning of each section took place in the next 2 days following cylinder washing. Each root system section was spread out on transparent plexiglass trays containing tap water. The roots were gently spread to occupy all space available in the tray. Scanning of the tray was done with a WinRhizo scanner with 2,400 dpi. Analysis of the images was done with WinRhizo software (Regent Instruments, Chemin Sainte Foy, Quebec, Canada). The parameters included the total root length of each root system section, root diameter within different categories, root surface area. After scanning the roots were put in a paper bag and dried in a forced-air oven at 70°C for 3 days.

Statistical analysis

In both the experiments, the treatments used six replications per genotypes, arranged in a factorial design with treatment (WW-Flowering, WW, WS) as main factor and genotypes as sub-factor and randomized within each main factor in 6 replications. One-way ANOVA was carried out to test for genotypic differences within treatment, using Genstat. Correlation analysis was done using Excel.

RESULTS

Root development in transgenic events under WW and WS conditions

In Exp.1, maximum root depth varied between 80 and 150 cm under WW conditions and between about 120 and 180 cm under WS, reflecting a significant treatment effect ($P < 0.001$). For individual genotypes, the increase in root depth under WS conditions was not significant in the wild type JL24 but it was in all events except RD2 and RD19. In the WS treatment, the maximum root depth was not significantly different between JL24 and any of the events (Fig. 1a). In Exp.2, the maximum root depth varied between 80 and 110 cm under WW conditions and between about 120 and 140 cm under WS. There also a highly significant treatment effect was found, whereby all genotypes showed a significant increase in the maximum root depth due to WS conditions. In the WW treatment, the maximum root depth was lower in RD11 and RD12 than in JL24, RD2 and RD33 ($P < 0.01$). By contrast in the WS treatment, the maximum root depth was higher in RD20 and RD33 than in JL24 and RD19 (Fig. 1b). Overall, the root depth was somewhat lower in Exp.2 than in Exp.1, although the genotypic trends remained the same. There is no clear explanation for these seasonal differences and we can only speculate about slight differences in the day time duration, since the temperature and relative humidity were controlled at about equivalent levels in both seasons.

The root dry weight data followed similar trends. In Exp.1, root dry weight varied between about 1 to 2.5 g plant⁻¹ in WW conditions and between 1.5 and 3.7 g plant⁻¹ in WS conditions, resulting in highly significant WS treatment effect ($P < 0.001$). In the WW treatment root dry weight was higher in RD33 than in JL24, RD12 and RD20, whereas in the WS treatment, root dry weight was higher in RD33 than in RD12, but none of the events had a different root dry weight than JL24 (Fig. 1c). In Exp.2, the root dry weight varied between 1.8 and 2.8 in the WW treatment and between 3.3 and 5.7 in the WS treatment, showing also a highly significant WS treatment effect ($P < 0.001$) and a clear increase in the root dry weight in the WS treatment in all genotypes. In the WW treatment none of the transgenics was different from JL24, whereas in the WS treatment the root dry weight was higher in RD33 than in JL24 (Fig. 1d).

In sum, the WS treatment in both experiments increased the maximum rooting depth and promoted root growth across all genotypes, although to a higher extent in several

transgenics, for example RD33 that had the capacity to grow longer and/or larger roots than the wild type JL24.

Root dry weight and root length density at different depth

In Exp.1, the entire root system was separated in 20-cm segments to evaluate the root biomass at different depth. In the WS treatment, there were no significant root dry weight differences among genotypes in layers above 100 cm depth. By contrast, significant differences in the root weight were found in the 100-120 cm and 120-140 cm layers, with several transgenic events having larger root dry weight in these layers than JL24, in particular RD20 and RD33 (Table 1). In the WW treatment, there was no significant difference in the root weight between genotypes in any of the root layers.

In Exp.2 the entire root system was also separated in segment of 30-cm and the root length density of each segment was evaluated, assuming that each segment would correspond to a volume of soil of 30-cm depth and 20-cm diameter (WinRhizo calculated to total root length). Across the genotypes, the RLD was higher in the WS treatment than in the WW treatment in all layers except in the 0-30 cm layer. The average RLD was also higher in the WS than in the WW treatment. In the WS treatment, there was no significant genotypic differences in the average root length density and on the root length density of the 90-120 cm and 120-150 layers. By contrast under WS conditions there were significant genotypic differences in all the other layers (Table 2). In the WW treatment, the root length density varied between genotypes in the 0-30 cm and 90-120 cm layers and on the average value across layers. JL24 had a higher average RLD and RLD in the 0-30 cm layer than RD11.

Given the significant differences in rooting depth and root development under WS in some transgenics, the root diameter, a possible important component of root penetration in soil, was assessed. Differently from other root parameters evaluated above, there was no treatment effect on the root diameters in the 0-30 cm, 30-60 cm and 90-120 cm layers, and only a treatment effect leading to a slight increase (about 0.05mm) in root diameter in the WS treatment in the 60-90 cm layer (Fig. 2). In the WW treatment, there were no significant genotypic differences in any of the root layers. In the WS treatment, the root

diameter varied significantly only in the 60-90 cm layer where the root diameter was higher in RD11 and RD33 than in JL24 (Fig. 2b). It also appeared that the average root diameter increased substantially at depths below 60 cm, especially in the WS treatment, but also in the WW treatment. The average root diameter in the 60-90cm layer was also significantly related to the RLD in that layer ($R^2 = 0.49$), indicating a higher root diameter likely helped root penetration from this depth downwards.

Water extraction

In Exp.1 in the WW treatment, the cumulated water extraction in the first 21 days after treatment imposition was significantly higher in RD33 than in RD2, RD12 and RD19. Overall, the total water extraction of RD33 was higher than that in RD12 and RD19 (Table 3). In the WS treatment, the cumulated water extraction in the first 21 days after treatment imposition of JL24 was lower than in all transgenic events except RD20. Then in the period from 21 to 49 days after treatment imposition, the cumulated water extraction during that period was indeed similar in JL24, RD2, RD11, and RD33, but was lower in RD12 and RD19. Overall, the total water extraction in the WS treatment was significantly lower in JL24 than in RD2, RD11, RD20 and RD33 (Table 3).

In Exp.2 in the WW treatment, the cumulated water extraction in the first 21 days after treatment imposition was significantly higher in RD2 than in RD11, RD12, RD19, and RD20. Then in the period from 21 to 42 days after treatment imposition, the cumulated water extraction of RD33 was higher than in JL24 and all other events except RD2 (Table 3). Overall, the total water extraction of RD33 and RD2 was higher than JL24 ($P < 0.1$) and than the other events ($P < 0.001$). In the WS treatment, the cumulated water extraction in the first 21 days after treatment imposition of JL24 was lower than RD2, RD11, and RD33. Then in the period from 21 to 42 days after treatment imposition, the cumulated water extraction during that period was similar in all genotypes. Overall, the total water extraction in the WS treatment was not significantly different among the genotypes, although RD2, RD11, and RD33 had values that were about 10% higher than JL24 (Table 3) ($P < 0.1$).

The profile of water extraction for Exp.1 and Exp.2 are presented in the supplementary Fig. 1 and in Fig. 3, respectively. Overall, it illustrates that in the WW treatment of Exp.2, the water extraction was similar in all genotypes until about three weeks after treatment imposition, except for a higher water extraction in RD33. Thereafter, RD33 had a higher extraction than JL24 in both experiments, whereas RD11 had lower water extraction than JL24 (Fig. 3a), and RD2 and JL24 were similar. In the WS treatment, JL24 had a lower water extraction in the first 21 days after treatment imposition than in RD2, RD11 and RD33 (Fig. 3b) and also in Exp.1 (Suppl. Fig. 1). Then after 21 days of treatment imposition the water extraction profile of JL24 was not significantly different from the transgenics.

In sum, RD2, RD11, and RD33 were transgenic events that had higher water extraction in the WS treatment than JL24. However, in the WW treatment, RD33 also had a higher water extraction than JL24 whereas RD11 had a lower water extraction.

Relationships between water extraction and root parameters

In Exp.2, in the WS treatment, the average root length density was not significantly related to the total water extracted from the soil profile ($R^2 = 0.24$, ns). By contrast, the root length density in the 30-90 cm layer was significantly and positively related to the total water extracted from the soil profile ($R^2 = 0.68$, $P < 0.01$) (Fig. 3a). Similar regression were carried out with the total root dry weight and with the root dry weight in the 60-90 cm layer, which showed also a significant positive relationship ($P < 0.05$ and $P < 0.01$ respectively), indicating that in this case, measuring the root dry weight in the different layers was sufficient.

DISCUSSION

Here we showed that a water stress treatment promoted root growth in transgenic events more than in the wild type and this was in part related to promotion of root growth in deep layers. This led to a higher water extraction in three transgenic events than in the wild type JL24 under WS conditions, and this increase was accountable to 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.

Higher root growth in the transgenics

The stimulation of root dry weight under WS conditions confirmed earlier studies in groundnut (Allen *et al.* 1976), although another study showed that root growth decreased upon water deficit (Meisner & 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 & Misra 1973; Narasimham *et al.* 1977; Ketring & Reid 1993). Here it was found that several transgenic events had higher root growth especially in the deep soil layers. It confirmed earlier results in smaller tubes, based on a late assessment of root dry weight (Vadez *et al.* 2007). This earlier report and results here are one of the first times when a genetic transformation reports an increase in the root biomass, except for the transformation with vacuolar H₂-pyrophosphatase (H₂-PPase) AVP1 which increased root growth in tomato (Park *et al.* 2005), and a study reporting an enhanced root growth in transgenics tobacco (Werner *et al.*, 2010). Modeling studies that have shown the benefit of improving crop rooting depth (Sinclair & Muchow 2001; Hammer *et al.* 2009). The capacity for deep rooting and water extraction in these transgenics open 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.

Relationship between root length density and water extraction

Despite the remaining controversy about the relationship between root length density and water extraction, the results presented here showed a clear relationship between either root length density or root dry weight in the deep layer (60-90 cm) and water extraction. This confirmed earlier results with these transgenic events (Vadez *et al.* 2007), and also from transgenic tomato with vacuolar H⁺-pyrophosphatase (H⁺-PPase) AVP1 gene, where an increased root dry weight also led to an increase in water extraction. These results are somewhat different to others in groundnut, 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 & 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 , lower in groundnut than in other crops, providing 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 (Fig. 4), 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 groundnut. This would agree with the report of JL24 having a relatively poor maximum root depth and among the highest root length density (although lower than 0.50 cm cm^{-3}) (Ratnakumar & Vadez, 2011), and here with the relatively heterogeneous distribution of roots in the different soil layers (Table 1). For instance, the root dry weight of JL24 below 60 cm was about half that above 60 cm, whereas this root weight was about the same across the layers in RD11 or RD33 (Table 1). Similar results could be seen for root length density (Table 2). Therefore, our interpretation is that the effect of *DREB1A* on roots under WS conditions was to alter the distribution of the root system to make it more uniform across the soil profile, therefore leading to increasing the RLD at each level closer to a value allowing maximum water extraction rate.

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Figure captions

Fig. 1. Maximum root length (cm) under well-watered (WW, white bars) and water stressed (WS, black bars) conditions in Exp. 1 (a) and Exp. 2 (b), and total root dry weight (g plant^{-1}) under well-watered (WW, white bars) and water stressed (WS, black bars) conditions in Exp. 1 (c) and Exp. 2 (d), in six transgenics events transformed with *DREB1A* and carrying prefix 'RD' and the wild type JL24. Data are means (error bar indicates standard error) of six replicated plant per treatment and genotype. LSD bars for each treatment are indicated above JL24 treatment bars, when significant.

Fig. 2. Root diameter at different depths (0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm) under well-watered (WW, a) and water stress (WS, b) conditions, in six transgenics events transformed with *DREB1A* and carrying prefix 'RD' and the wild type JL24. Data are means (error bar indicates standard error) of six replicated plant per treatment and genotype. In the WW treatment, absence of bars in the 90-120 corresponded to an absence of roots at that depth. LSD bars for each treatment are indicated above JL24 treatment bars, when significant.

Fig. 3. Transpiration profile (g plant^{-1}) as a function of days after treatment imposition in Exp. 1 under well-watered (WW, a) and water stress (WS, b) conditions in transgenics events transformed with *DREB1A* (RD2, RD11, RD33), representative of the variation among genotypes, and wild type JL24. Data are means of six replicated plant per treatment and genotype.

Fig. 4. Relationships between the root length density (cm cm^{-3}) in the 60-90 cm layer (closed symbols) or the average root length density in the entire soil profile (open symbols) and the total water extracted to support transpiration (a). Relationships between the root dry weight in the 60-90 cm layer (closed symbols) or the total root dry weight in the entire soil profile (open symbols) and the total water extracted to support transpiration (b). Data are means of six replicated plant per treatment and genotype.

Figure 1

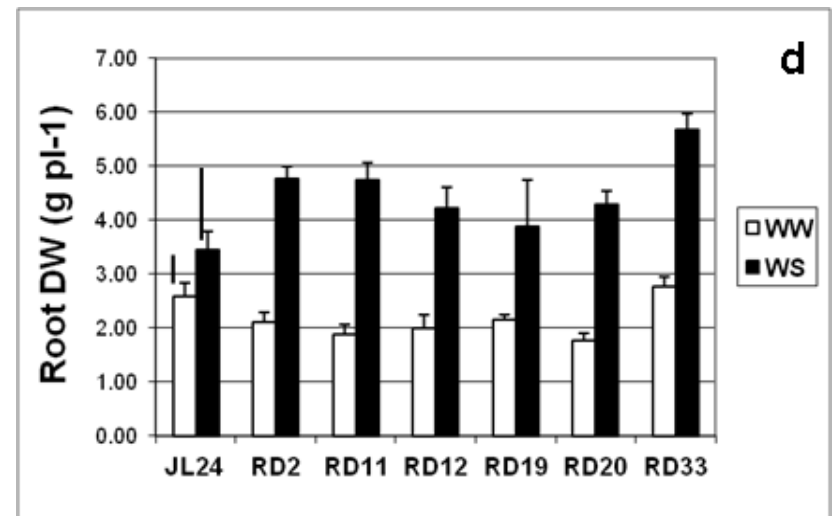
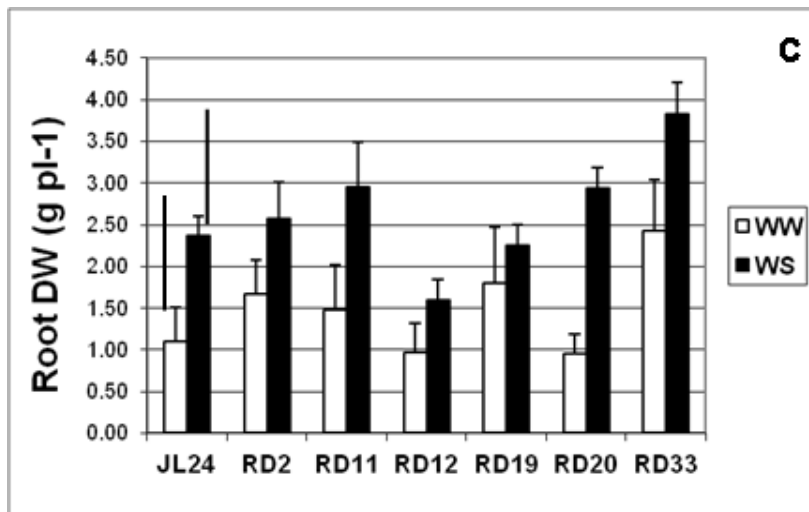
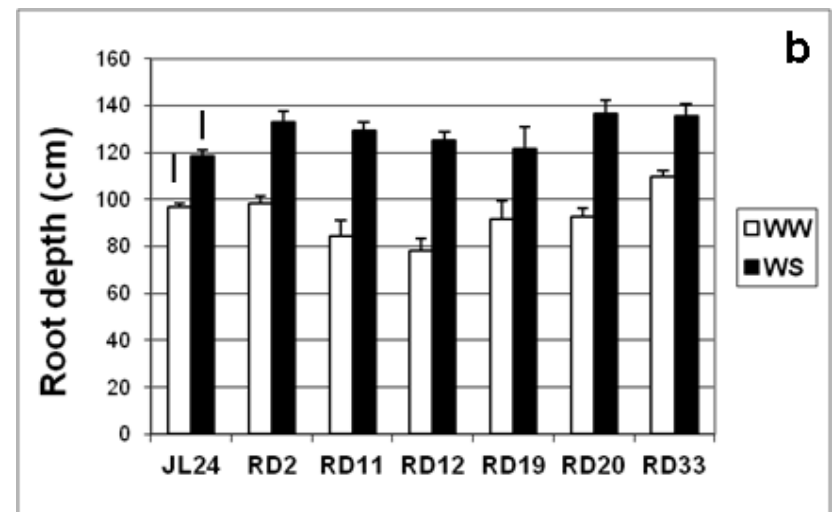
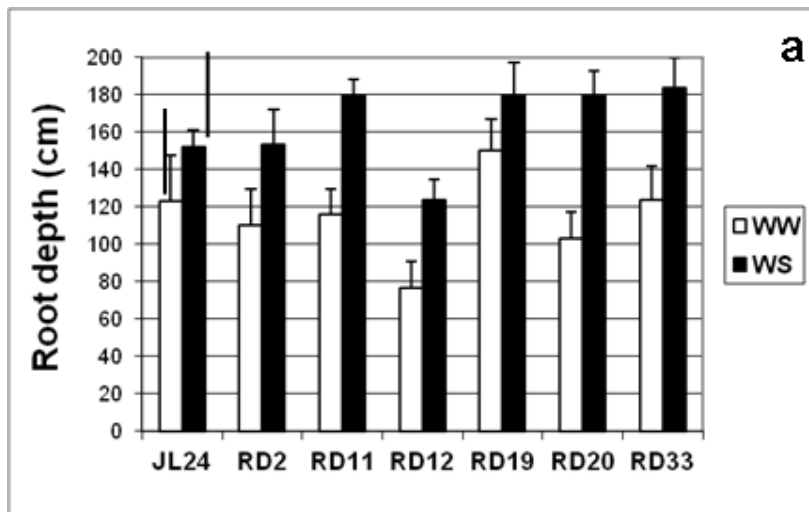


Figure 2

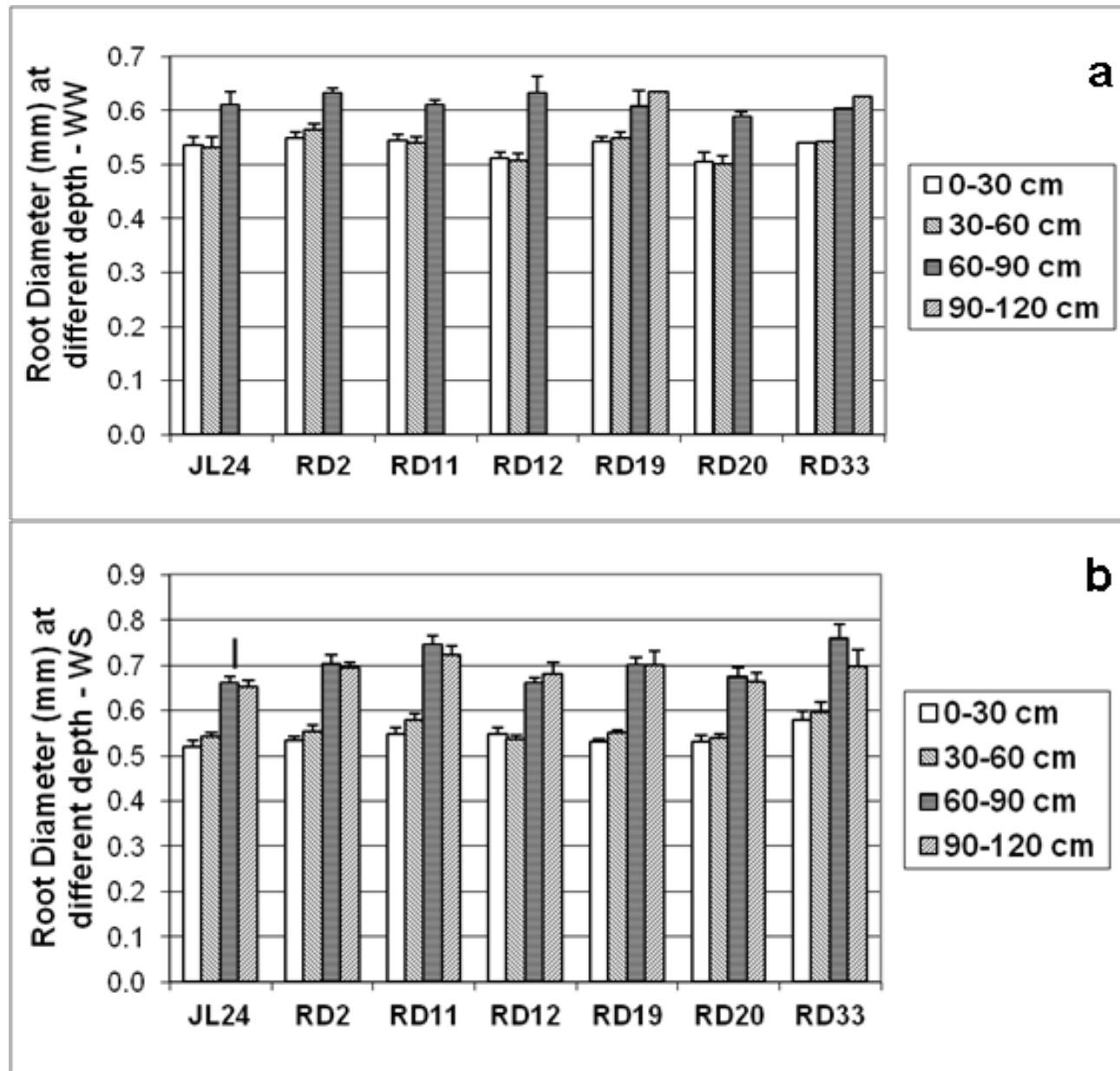


Figure 3

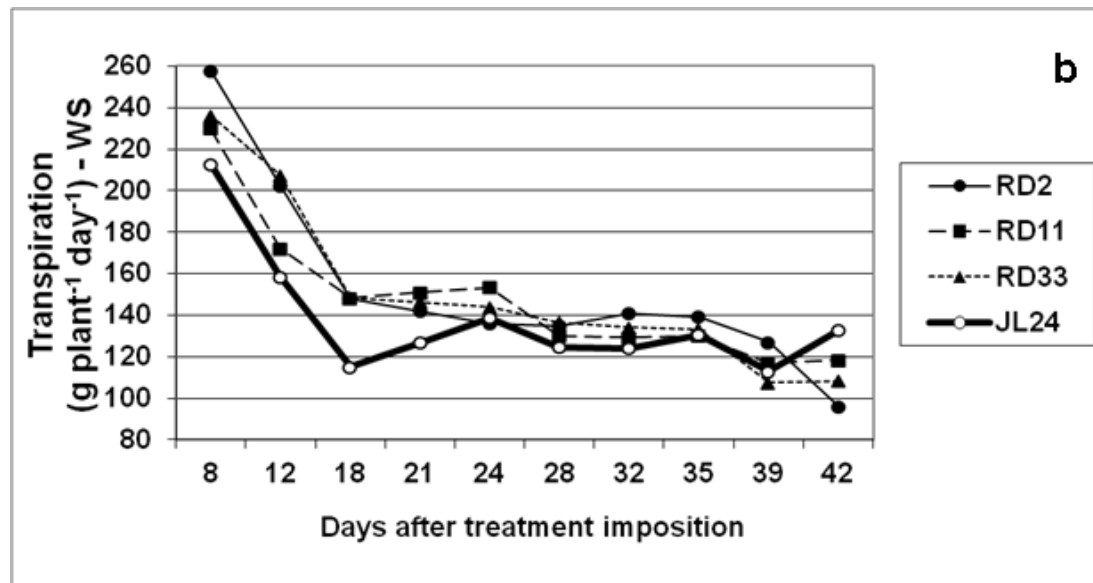
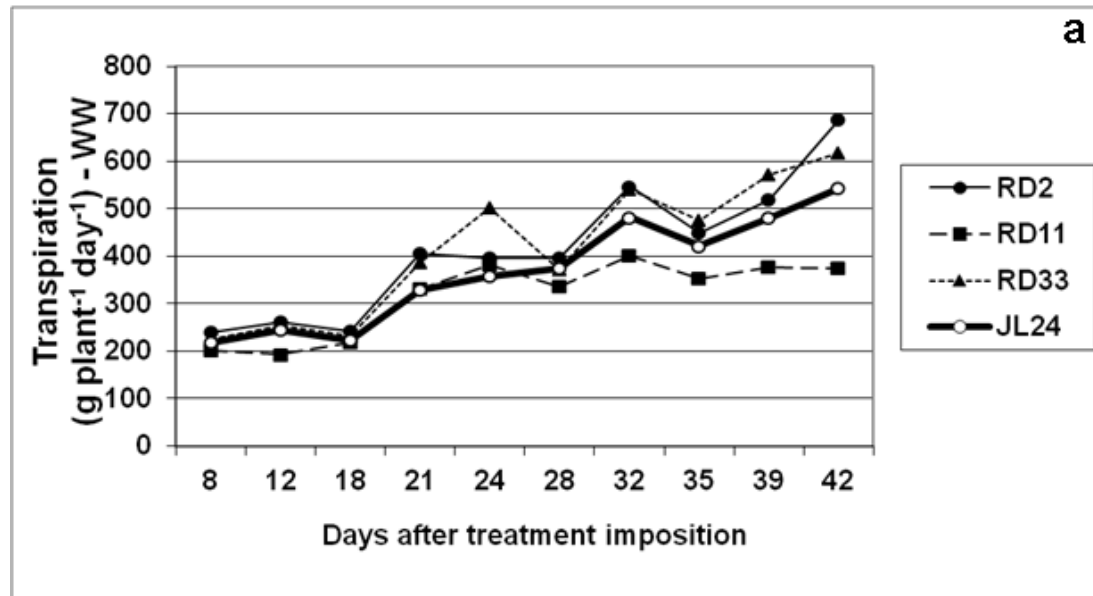
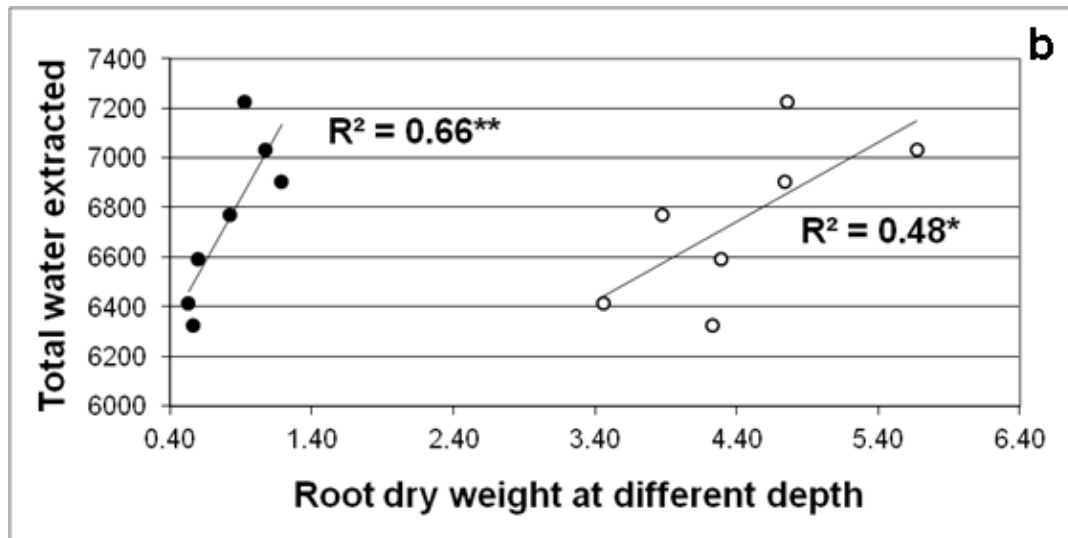
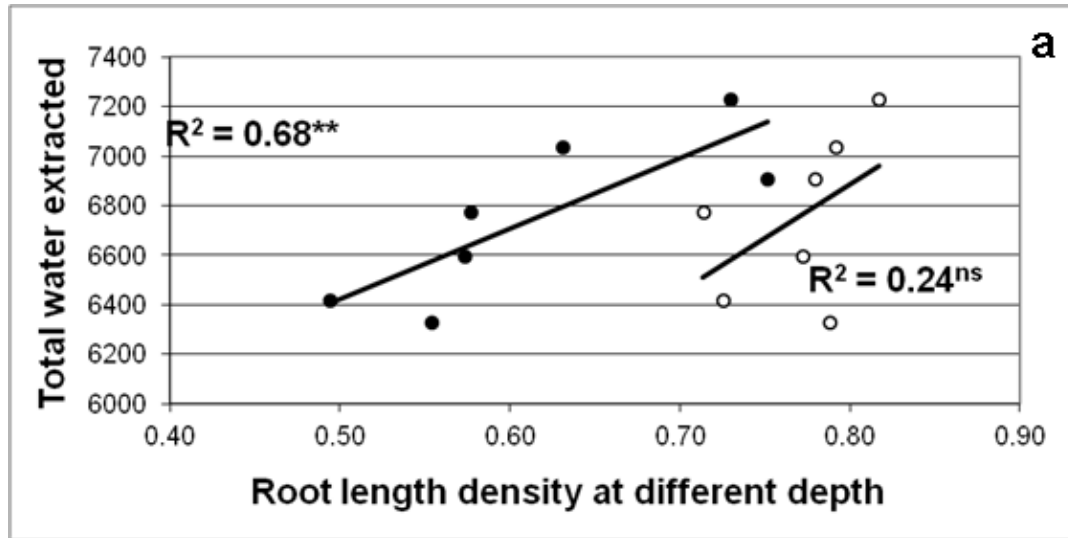
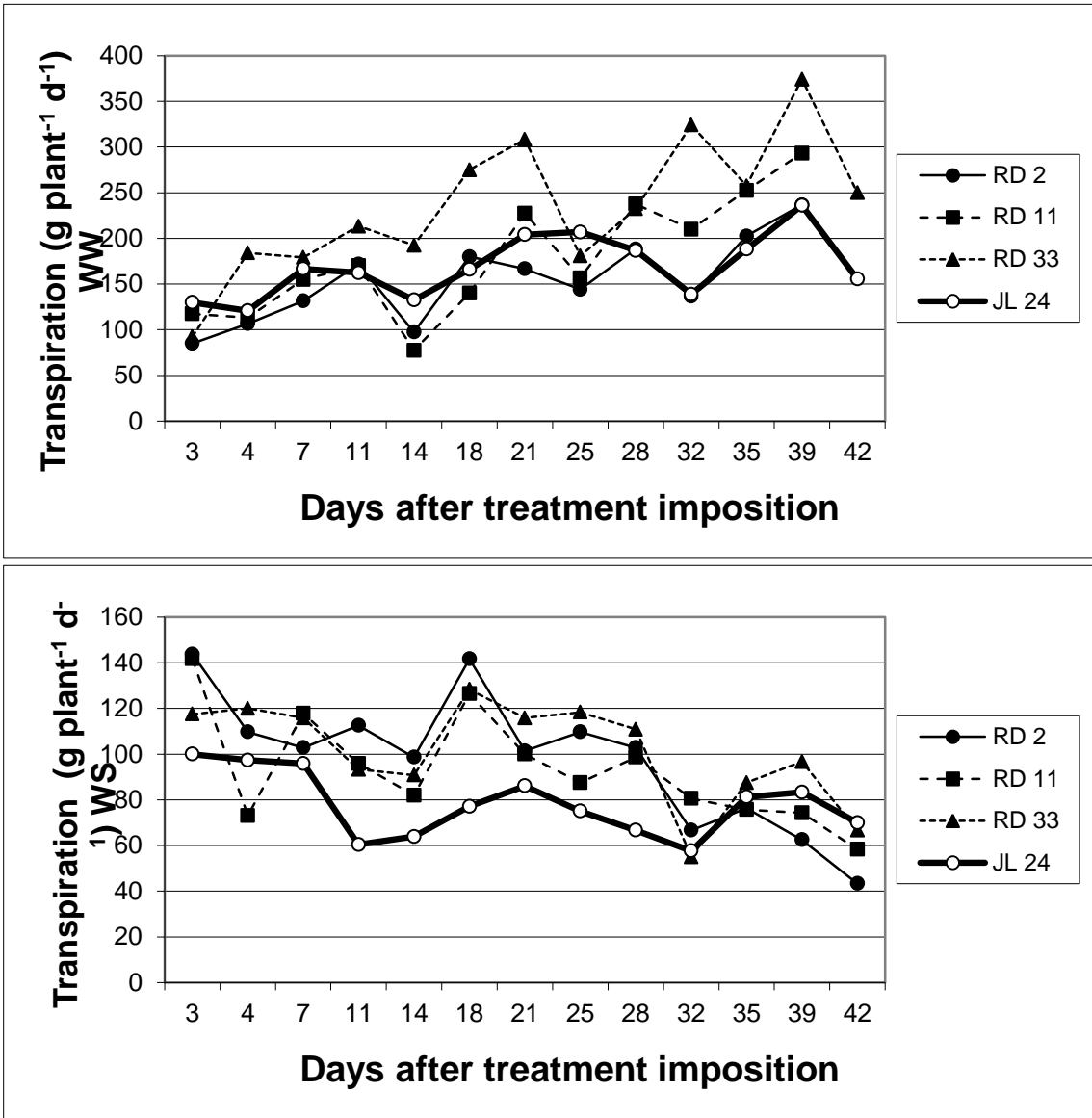


Figure 4



Suppl. Figure 1



Suppl. Fig 1. Transpiration profile (g plant⁻¹) as a function of days after treatment imposition in Exp. 2 under well-watered (WW, a) and water stress (WS, b) conditions in transgenics events transformed with *DREB1A* (RD2, RD11, RD33), representative of the variation among genotypes, and wild type JL24. Data are means of six replicated plant per treatment and genotype.