FUNCTIONAL PLANT BIOLOGY

Ratnakumar P., Vadez V. (2011) Groundnut (*Arachis hypogaea*) genotypes tolerant to intermittent drought maintain a high harvest index and have small leaf canopy under stress. *Functional Plant Biology* 38, 1016–1023.

http://dx.doi.org/10.1071/FP11145

This is final refereed version (post-print)

Groundnut (*Arachis hypogaea* L.) genotypes tolerant to intermittent drought maintain a high harvest index and have small leaf canopy under stress

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Abstract. Intermittent drought, which varies in intensity, severely limits groundnut yields. Experiments were conducted in lysimeters to assess root development, water uptake, transpiration efficiency, yield components, and their relationships, in twenty groundnut genotypes under well watered (WW) and mild (DS-1), medium (DS-2), and severe (DS-

3) intermittent stress until maturity. Pod yield decreased 70%, 55% and 35% under severe, medium and mild stress, respectively, compared with WW conditions. Pod yield varied among genotypes, and showed highly significant genotype-by–treatment effects. Root length density (RLD) varied among genotypes before and after stress imposition, although RLD did not discriminate tolerant from sensitive lines. Total water uptake and root length density (RLD) under water stress had a significant but weak relationship. Water extraction from the soil profile (total water uptake minus irrigation water), was the highest under the severe stress. Water uptake varied largely among genotypes in all water regimes, but correlated to pod yield under WW conditions only. The relative harvest index, i.e. the ratio of the harvest index under stress to that under WW conditions, was closely related to the pod yield in all three intermittent stresses (R^2 = 0.68 in DS-1; R^2 = 0.65 in DS-2; R^2 = 0.86 in DS-3), and was used as an index of stress tolerance. Under medium and severe stresses, the relative harvest index was negatively related to plant leaf weight (R^2 = 0.79 in DS-2; R^2 = 0.53 in DS-3), but less so under mild stress (R^2 = 0.31). Results suggest that under an intermittent stress, genotypes with lower leaf area may use water more sparingly during drying cycle with less damaging consequences for reproduction and pod development than genotypes having larger leaf area.

Additional Keywords: groundnut, water deficit intensities, water uptake, root characteristics, pod yield, lysimetric system

Introduction

Intermittent water stress occurs in crops that are planted during the rainy season and where gaps in rainfall can expose plants to water stress at any time during the cropping cycle, with possible variation in the timing, the intensity and the duration of the

associated water deficits (Serraj *et al.* 2005). Groundnut typically experiences a range of intermittent stresses and yields are affected when the stress occurs at both vegetative and reproductive phases (Rahmianna *et al.* 2004). Yield is then highly dependent on the stage when the stress occurs and the available water to the crops at that stage (Ratnakumar *et al.* 2009).

From a breeding point of view, knowing whether different stress intensities would affect groundnut yield differently is also critical to set the screening conditions according to those in the targeted environment, and it is of major interest for this present work. Indeed, even if exposed to an intermittent stress that follows the same frequency during the same phenological period, the intensity of the stress can vary a lot depending on how much water is received each time the stress is relieved by rainfall. Therefore, while selecting for lines adapted to an intermittent stress, an important question is whether the genotypic selection, but also possible selection criteria, would differ across a range of stress intensities.

The maximization of water uptake is essential for the growth and production under limited water resource. In several crops, adaptation to drought is closely associated to root development, which provides a better water extraction ability to plants (Jongrungklang *et al*. 2011). Most of the works performed on root so far have relied on the basic and underlying assumption that rooting differences in depth, length density or weight would result in higher water uptake and then in higher yield. Yet, the relation between groundnut root mass, size, density, (Wright *et al*. 1994), profile and water uptake remains understudied and controversial (Passioura 1983; Hamblin and Tennant 1987; Amato and Ritchie 2002). Recent evidence using transgenic lines of wild type JL24 transformed with *At*DREB1A carried out by rd29 drought responsive promoter showed a close relationship between root length density and the water extracted from the soil profile (Vadez *et al*. 2007a). However, other evidence in four breeding materials of groundnut also using lysimeters show poor relationship between root length density and the evapotranspiration (Vadez *et al*. 2008). So, there is first a need to elucidate whether water extraction potential can be inferred from measurements on rooting characteristics (depth and length density in particular). A second issue is whether genotypes having

differences in rooting and water extraction also vary for their adaptation to an intermittent water stress.

Water uptake is crucial during key stages like flowering and grain filling (Boyer and Westgate 2004; Vadez *et al*. 2007b), and small differences in water uptake at these stages can bring large yield benefits in groundnut (Boote *et al*. 1982; Ratnakumar *et al*. 2009) and other crops (Zaman-Allah *et al*. 2011). The decline in the pod yield in groundnut can also be due to a reduction in the shelling percentage, as expressed by the decrease in weight ratio of the seeds and the pods (Nageswara Rao *et al.* 1985; Stansell and Pallas 1985; Rahmianna *et al.* 2004), or to a decrease in the harvest index (Wright *et al.* 1991). Finally, transpiration efficiency has long been used as a major trait to improve the yield under intermittent drought conditions in groundnut (Turner 1986; Wright and Nageswara Rao 1994). A characteristic to all these studies has been to focus on a single trait of the equation Y = TxTExHI, given by Passioura (1977), due to the difficulty to assess all traits with a high degree of precision in the same plants. These components are measured here in the same plants over the entire crop duration and across stress intensities, in a lysimetric system (i.e. long and large tubes that mimic a real soil profile) (Vadez *et al*. 2008), allowing to compare the respective importance of each of the component under differing stress situations.

In summary, a first objective was to test the magnitude of genotype and genotype-by-treatment interaction in the pod and seed yield response to intermittent stress of different intensities by modulating the amount of water added at each re-watering. A second objective was to assess whether rooting characteristics correlate to water extraction and whether both aspects relate to seed yield differences under a range of intermittent stresses in groundnut. A third objective was then to weigh the importance of the water extraction, TE, HI, and other parameters to achieve a high seed yield across a range of stress conditions.

Materials and methods

Plant material and growth conditions

Twenty genotypes were tested and included thirteen breeding lines-cultivars Chico, CSMG 84-1, ICG (FDRS) 10, ICG S 44, ICGS 76, ICGV 00350, ICGV 86015, ICGV

86031, ICGV 91114, ICR 48, JL 24, TAG 24, TMV 2 and seven recombinant inbreed lines (RILs) of a cross between TAG 24 and ICGV 86031, which was previously assessed for mapping drought related QTLs (Krishnamurthy *et al*. 2007; Ravi *et al*. 2011).

The experiments were conducted at ICRISAT, Patancheru (17° 30' N; 78° 16' E; altitude 549 m) during the rainy season 2009 using lysimeters (see below) and a rain out shelter covering the trenches where the lysimeters were set to protect the plants from rainfall. During the crop growing season the maximum and minimum temperatures, humidity data (Suppl. Fig. 1) were collected from a temperature and relative humidity recorder (Gemini Tiny Tag Ultra 2 TGU-4500 Data logger), which was located at the crop canopy level.

Preparation and space arrangement of the lysimeters

The plants were grown in lysimeters, consisting of PVC cylinders (20 cm diameter, 120 cm height) which contained Alfisol collected from ICRISAT farm. 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. However, when water uptake measurements started, the watering of the tubes was done in a way that avoided drainage (see below), so that these tubes fitted the definition of 'lysimeters'. The lysimeters had previously been used for one crop of groundnut and one crop of pearl millet (used as fallow). This allowed usage of an undisturbed soil profile, except superficially at the time of the removal of the previous crops. The legume / cereal crop rotation allowed breaking of the cycle of soil-borne disease, if any. The initial soil filling of the tubes is described in Vadez *et al*. (2008). In short, it had consisted in filling air-dried and sieved soil, about 50 kg in each tube, to ensure a homogenous soil bulk density of about 1.35 Mg m^{-3}. Prior to filling the tubes, the soil was amended with single super phosphate at a rate of 200 mg kg^{-1} soil and muriated potash at a rate of 200 mg kg^{-1} soil. The top 3 cm surface soil was incorporated with 2 g of carbofuran to avoid damage by soil born pests.

The lysimeters were arranged next to one another and the diameter of the tubes was determined in a way that the surface area available to each individual plant in the tubes

corresponded to the area available in the field conditions under sowing density of groundnut (approximately 20-25 plants m^{-2}) (Vadez *et al*. 2008). The lysimeter depth was based on an estimation of rooting depth of groundnut in this type of soil (Alfisol). Before sowing, the lysimeters were watered to field capacity. The tubes were arranged in four trenches of 1.2 m depth and 1.8 m width. Each trench was separated by a 20 cm concrete wall. The top of the cylinders was equipped with a metal collar and rings that allowed the cylinder to be lifted. Weighing of the cylinders was done by lifting the cylinders with a block-chained pulley, and an S-type load cell (Mettler-Toledo, Geneva, Switzerland) was inserted between the rings of the cylinder and the poulie. The scale, of 100 kg capacity allowed repeated measurements and gave an accuracy of 10 g on each weighing.

Sowing, crop management, and treatment imposition

Seeds were sprayed with 90% Etherel solution and air-dried to break dormancy, and treated with fungicide to avoid the seed-borne diseases if any. Prior to planting, soil was wetted to field capacity. Then seeds were planted at a rate of three per cylinder and later thinned to one plant per cylinder. The lysimeters were inoculated with Rhizobium strains NC-29 (IC 7001) using a liquid inoculation method (Brockwell 1982) to ensure better nodulation.

Three seeds of each genotype were sown in the soil on 2nd week of June 2009. After sowing, the cylinders were watered with 500 ml of water immediately after sowing and twice on alternate days with 250 ml until the seedlings uniformly emerged. The plants were thinned to two individuals per cylinder at 7 days after sowing (DAS) and then to a single plant per cylinder at 14 DAS. Plants were maintained under fully irrigated conditions until flowering by regular waterings. The design of the experiment was a randomized block design (RBD) with treatment as the main factor and genotype as sub-factors randomized within each block in five replications. Six blocks were planted, to which the treatments were assigned later on. After thinning, watering was done at regular intervals in all six blocks of plants until the time of flowering.

In Experiment 1, (Exp.1), four blocks were used to impose a set of varying watering regimes. One block was used as a well-watered treatment (WW) and the other three sets were used each as an intermittent drought stress (DS) treatment, applied from flowering

until maturity. All the lysimeters in the WW and DS treatments received 2L of water at flowering to bring up the soil profile at field capacity. Cylinders were then allowed to drain over a period of 48 hours before being weighted. From there on, the three water stress treatments were imposed by stopping irrigation in the three intermittent drought stress blocks, and applying water only at 29, 41, 55, 66 and 79 days after flowering. These re-watering of DS blocks occurred when a majority of plants of the DS-2 treatment had most of their leaves wilted in the early afternoon. To decide on re-watering, a scoring evaluation of the leaf wilting of the DS blocks was done in the early afternoon (1, no wilting; 2, some leaves wilted; 3, most leaves wilted; 4, permanent wilting on some leaves; 5, permanent wilting on most leaves). This followed a similar approach to monitor stress plots in the field where and it allows the application of a stress that usually lead to about 50% pod yield reduction (Hamidou et al, unpubl.). Therefore, at each of 29, 41, 55, 66, and 79 days after flowering, the three intermittent stresses received, respectively, either 2.0 (DS-1), 1.5 (DS-2) or 1.0 L (DS-3) of water. The surface area of the cylinders being 314 cm^2, these drought treatments simulated a 65, 50, or 33 mm irrigation, which allowed comparison to the 40-50mm irrigation that are usually provided in intermittent drought trials in the field. The WW plants were re-watered every week. At each weighing water was added by compensating transpiration losses up to 1kg below the lysimeter's weight at field capacity (weight of cylinder at the beginning of weighing), which corresponded to about 90% field capacity and which avoided water drainage. The week that plants were not weighed, each WW cylinder received half of the water it received the previous week. The cylinders were weighed weekly with a 10g precision hanging scale from the day of treatment imposition (flowering) until four weeks after flowering, and then every two weeks until maturity. Prior to this, the soil surface of the cylinders was mulched with a 2-cm thick polythene beads. Previous assessments (data not shown) indicated that the mulching controlled soil evaporation by over 90% and therefore, tube weight differences were considered to reflect plant water uptake for transpiration. Plant transpiration was calculated from each pair of consecutive weighing.

Experiment 2 (Exp.2) was carried out to assess rooting at the time of treatment imposition (flowering time) and seven weeks after total suppression of irrigation, along with water uptake measurement during the water stress period. In this latter block,

cylinders were weighed every week to measure water extraction from the soil profile. At seven weeks after suppression of irrigation, the plants were cut at the soil surface. Leaves, stems, and roots were dried to constant weight in an oven at 70 ºC and weighed. The soil in the cylinders was washed to collect the whole root system. After removing the soil particles, the roots were gently laid on a table to measure their maximum length as an estimate of rooting depth. The root system was then sliced in portions of 30 cm, to measure the total root length at each of the 30 cm depth of the root system, using image analysis software (WinRhizo, Regent Instruments INC., Canada). Root length density in each 30 cm layer was obtained by dividing root length by the volume of a 30 cm section of the cylinder, assuming roots had colonized the entire volume at each depth.

Harvest of DS1, DS2, DS3, and WW treatment

At maturity, plant were harvested at soil level and individually partitioned into pods, leaves and stems. Pods and seeds were counted for each plant and the seed weight was then recorded. Leaf area was measured in al1 plants. Dry weights of stem, leaf and pod were measured after keeping them to 70^{o}C in a hot air oven for 48 to 72 hours. Transpiration efficiency was calculated as the ratio of the increment of the total aboveground biomass including pod weight by the sum of transpiration values between 28 DAS (flowering) and maturity.

Statistical analysis

Statistical analyses were performed using GenStat 10.1.0.72 by one-way ANOVA and t-test. Differences between mean values of treatments were evaluated using least significant difference (LSD) at a 0.05 significance level. The Residual Maximum Likelihood (ReML) method of Genstat was used to obtain the unbiased estimate of different parameters within each treatment. A two-way ANOVA analysis was also performed to assess the effect of genotype (G), water treatment (T) and genotype-by-water treatment (GxT) interaction for the different traits measured. The correlations between traits were estimated using statistical analysis system (SAS) 9.0.

Results

Biomass and TE and Yield components

The two-way ANOVA showed a highly significant genotypic effect for pod and seed weight, total number of pods, number of immature pods, harvest index (HI) and leaf weight (Suppl. Table 1), and also a significant genotype-by-treatment (GxT) interaction for these traits. However, the magnitude of the genotypic effect was higher than the GxT interaction effects in all these traits. For total leaf area and transpiration efficiency, there was only a genotypic effect. As expected, the treatment effect was highly significant for all growth and yield components.

Interestingly leaf area and leaf weights were similar across all three WS treatments, whereas the stem weight decreased by 20%, 30% and 45% under DS-1, DS-2, and DS-3 respectively. Shoot biomass decreased by about 20% in DS-1, 25% in DS-2, and 35% in DS-3, compared to the WW conditions (Suppl. Table 2). Transpiration efficiency was the highest under WW conditions and decreased with the level of the stress intensity, with a 10% and 50% decrease under DS-2 and DS-3 (Suppl. Table 2). By contrast, the pod weight decreased by about 35% in DS-1, 55% in DS-2 and 70% in DS-3, indicating that pod weight was more sensitive to stress than the production of shoot biomass. The decrease in the seed weight was of the same order of decrease than the pod weight. In fact, the harvest index (HI) was decreased by 15%, 30% and 40% under DS-1, DS-2, and DS-3 respectively, indicating that the stress was increasingly affecting the reproductive processes as the stress intensified. Among twenty genotypes the pod yield varied from 0.7 to 9.3 g plant^{-1} under severe (DS-3), 1.8 to 10.4 g plant^{-1} under medium (DS-2), 2.9 to 14.7 g plant^{-1} under mild (DS-1) intermittent stress, and 4.1 to 21.5 g plant^{-1} under WW conditions. The seed yield varied from 0 to 6.6 g plant^{-1} in DS-3, 0.6 to 7 g plant^{-1} in DS-2, 1.6 to 10.5 g plant^{-1} in DS-1, and 4.1-21.51 under WW conditions.

Under DS-3 conditions, genotypes ICGV 86031, RIL- 228 and RIL-233 showed the highest pod yields and TE, whereas ICGV 86015 had poor pod yield under severe (DS-3) stress conditions. Under DS-2 RIL-233, RIL-175 and Chico had the highest pod yield. Under the mild DS-1 stress ICGV 86031, Chico, JL-24 and RIL-233 had the highest pod yield and TE whereas TMV 2 showed lowest pod yields and TE.

Root characteristics and relationship with water extraction

Rooting depth showed large genotypic differences both at flowering time and after seven weeks of exposure to water deficit (Fig. 1). The group of RILs tended to have on average a deeper root system at flowering time, before imposing the stress (67 versus 78cm). At flowering time, the genotypes TAG-24, ICGV 86031, ICGS 44, RIL-228 and RIL-233 had the shortest root depth while and CSMG 84-1, ICG (FDRS) 10 and RIL-9 had the deepest root among all genotypes. After seven weeks of water stress RIL- 228, genotype ICGV 86031 and ICR48 had the shortest root depth, while RIL-9, RIL-70 and TAG24 had the deepest roots (Fig. 1).

Root length density (RLD) varied substantially among the genotypes across the soil depth. The maximum RLD's were recorded at the 0-30 cm soil profile with all genotypes being above 0.35 $cm.cm^{-3}$ of soil (data not shown). Overall, the genotypes JL 24 and ICGV 91114, RILs-9 and RIL-220 had the highest RLD, and ICG (FDRS) 10, CSMG 84-1, RIL-233 and RIL-75 had the lowest RLD after seven weeks exposure to terminal stress. There was a weak relationship between water extracted after seven weeks of terminal stress and the average root length density (RLD) at that stage (R^2 = 0.31), with a large weight on the regression being played by CSMG84-1 data point on the left hand side (Fig. 2). Similar regressions were attempted between the total water transpired and the RLD at different depth but showed no closer relationships (data not shown). These data suggested that measuring root morphological attribute had little value to infer water extraction results.

The net water extracted from the soil profile was calculated by summing all the transpiration data from the time of treatment imposition until maturity, and deducting all values of water additions. The net water extracted was about 10%, 40%, and 60% higher in the DS-1, DS-2, and DS-3, respectively, than in the WW treatment (Suppl. Table 2).

Water uptake and TE relationship with yield components

Under WW conditions, the pod yield was poorly related to HI and TE (R^2=0.11 and 0.07) but largely related to the total water transpired (R^2=0.55). Under intermittent stress, pod yield was mostly related to the harvest index (R^2=0.82, 0.91, and 0.94 under DS-1, DS-2, and DS-3 respectively), but poorly related to TE (R^2=0.08, -0.06, -0.04 under DS-1, DS-

2, and DS-3, respectively) or the total water extracted (R^2=0.00, -0.09, 0.00 under DS-1, DS-2, and DS-3, respectively). The harvest index under all three intermittent stress treatment was in fact closely related to the harvest index under WW conditions (R^2 = 0.70 in DS-1; R^2 = 0.43 in DS-2; R^2 = 0.38 in DS-3) (Fig. 3). Therefore, a relative harvest index was calculated as the ratio of the harvest index under DS to that under WW conditions, to offset the part of the variation in HI that was explained by the potential HI (HI under WW).

The relative harvest index was closely related to the pod yield in all three intermittent stresses (R^2 = 0.68 in DS-1; R^2 = 0.65 in DS-2; R^2 = 0.86 in DS-3) (Fig. 4), showing that genotypes having the highest yield under intermittent stress were those maintaining high harvest indices when exposed to stress. A possible caveat here is that the relative HI and pod yield are in part related by the sharing of some of their components. However, the scatter diagrams of Figure 4 showed that there was still some variation in pod yield besides those explained by the relative HI. Therefore, these residual pod yield unexplained by the % HI component were computed as the difference between the actual pod yields and the predicted pod yield using the regression equation between pod weight and the relative HI, following previous work (Vadez *et al*. 2007b; 2011). These residuals were then plotted against TE and showed a very tight relationship under the mild DS-1 stress (R^2 = 0.41), whereas there was a non-significant relation in the other two DS treatments (R^2 = 0.15 in DS-2; R^2 = 0.14 in DS-3) (data not shown). These results indicate that beyond maintaining a high harvest index, transpiration efficiency was the second most important criteria under mild stress, *i.e.* with a yield reduction of 40% or less, but not under more severe stress conditions, where the yield reduction was between 50% and 70%.

Further regression work consisted in assessing the possible causes for the drop in HI under DS conditions. The hypothesis was made that under an intermittent stress, i.e. a regime of drying and re-wetting, plants having a smaller canopy use water more sparingly and may stand the gap in watering longer than genotypes having more developed canopy. Therefore, the relative HI under DS was plotted against the leaf weight, as a proxy for leaf area. The relative HI was significantly and negatively related to the leaf weight in all three intermittent stresses. However, the correlation coefficient was relatively weak

(R^2=0.32) and there was no significant relationship with the leaf area (R^2=0.01) under the mild DS-1 stress, whereas it was tightly and negatively related to the leaf weight in the other two DS treatments (R^2 = 0.79 in DS-2; R^2 = 0.53 in DS-3) (Fig. 5), with also a highly significant relationship with the leaf area in the DS-2 treatment (R^2=0.69) (leaf area was not measured in the DS-3 treatment).

Since the relative harvest index was tightly related to pod yield under each stress condition ($0.65<R^2<0.86$), it was taken as a tolerance criteria. Genotypes were then ranked accordingly for the relative harvest index under DS-2, and further comparison was made between the top most tolerant six genotypes to the least tolerant six genotypes (highest and lowest relative HI under DS-2)..

Characteristics of most tolerant lines - Profile of water uptake across water regime

Under WW conditions, the total water transpired by the sensitive lines was about 50% higher than in the tolerant genotypes and so the profile of water uptake from flowering time until maturity of the sensitive lines was above that for tolerant lines (Fig. 6). This agreed with a higher leaf area, leaf weight, pod and seed yield in the tolerant than in the sensitive lines under WW conditions. Under water stress conditions, the total water extracted by the tolerant lines was slightly lower than that in the sensitive lines across the three intermittent stresses, although the pattern of water use by the tolerant and the sensitive lines under DS-2 differed little. Under water stress, leaf area and leaf weight were lower (respectively 10-20% under mild DS-1 and 30-35% under DS-2 and DS-3) in the tolerant than in the sensitive lines. By contrast, pod and seed weight were higher in the tolerant than in the sensitive lines (respectively 70-80 % in mild DS-1; 130-230% in DS-2; 170-290% in DS-3), indicating also that the seed yield advantage of tolerant lines was higher than their pod yield advantage over sensitive lines. In fact, the harvest index (HI) of tolerant lines did not decrease under water stress compared to WW conditions, whereas it decreased dramatically under water stress in the sensitive lines.

Discussion

Dry matter production, pod yield and seed yield of all groundnut cultivars significantly decreased under the three water stress regimes in Exp.1 and showed large genotype-by-

water treatments (GxT) interactions. Although rooting depth and root length density differed between genotypes, there was no distinction of intermittent stress tolerance on the basis of these rooting differences, and this was related to the poor relationship between root length density and water extraction. Pod yield was closely related to the relative harvest index (HI WW / HI WS) ($0.65<R^2<0.86$). It was then found that the relative harvest index, which was taken as a tolerance index, was closely and negatively related to the leaf weight under water stress, and more so under DS-2 and DS-3 ($R^2=0.79$ and $R^2=0.53$) than under DS-1 ($R^2=0.32$). In agreement with these findings, tolerant genotypes had smaller leaf canopy (lower leaf area and weight) under both WW and water stress conditions. Our interpretation is that the smallest water demand by the smaller canopy of tolerant lines led to a more conservative water use between two irrigations. The fact that the harvest index did not decrease under any of the intermittent water stress in the tolerant lines, but decreased dramatically in the sensitive lines, then suggests this more conservative water use from smaller canopy favored the reproductive steps of the tolerant lines.

Large pod and seed yield differences were found under WW and different water stress conditions, with large GxT interactions and no relationship between seed yield under WW and seed yield in each of the three water treatments. These results agree with previous findings on groundnut (Teerayoot *et al*. 2010; Ntare and Williams 1998). These results indicate that screening for drought tolerance in groundnut is specific to the drought conditions that are imposed, which agree with recent results in a wide array of germplasm from the reference collection of groundnut (Hamidou *et al*. under review). The intensity of drought under DS-2 and DS-3 was severe and affected the reproductive process, as shown by an important reduction in the harvest index. This is in agreement with previous report in mungbean showing that water stress during flowering and pod filling stages reduced pod initiation and there by reduced the harvest index (De Costa *et al*. 1999).

Wide genotypic variations in root depths at flowering were not maintained after seven weeks of stress imposition, indicated also by large GxT interaction (Fig. 4). These GxE interactions indicate that genotypic response of root depths and RLD to drought largely depends on how certain genotypes developed roots under water stress. Deep-rooted ness

and faster extraction have been advocated as beneficial in tropical environments where groundnut is grown solely on stored moisture in the dry season (Wright and Nageswara Rao 1994). The superior ability of groundnut to maintain favorable water status during period of water deficits, in comparison to soybean and pigeonpea was related to greater proliferation of roots in the deeper soil layers (Devries *et al*. 1989). However, no discrimination for water extraction was found between tolerant and sensitive genotypes regarding to root length density, at any of the layers that were looked at. The relationship between total water uptake and root length density (RLD) under water stress, although significant, was a weak relationship, indicating that, as in the case of chickpea (Zaman-Allah *et al*. 2011), measuring root morphological attribute has likely little value to assess the water extraction capacity of genotypes (Fig. 3). In fact, Figure 2 indicates that even genotypes with low root length density could extract as much water as genotypes with 50% more RLD, in agreement with the observations of Wright *et al*. (1994) that groundnut genotypes may produce roots in excess of what it requires for water extraction.

It was clear that the relative HI explained the largest portion of genetic variation in pod yield in the different stresses (DS-1: R^2= 0.52; DS-2: R^2 = 0.78; DS-3: R^2= 0.84)**.** The residual yield variations that were not explained by HI were then closely related to the transpiration efficiency under mild DS-1 stress, but not under more severe DS-2 and DS-3. In these stresses, the decrease in the HI of sensitive lines was very severe, whereas HI did not decrease in the tolerant lines, suggesting that drought stress effect on reproduction probably explained most of the pod and seed yield differences under these severe stresses. Under a regime of intermittent stresses, we hypothesized that the most successful genotypes could be those in which water is used in a way that the transpiration of water stressed plants remains relatively high compared to the transpiration of fully irrigated plants. In agreement with this, both pod yield and the relative harvest index, our proxy for stress tolerance here, were significantly and negatively related to the plant leaf weight under medium and severe stresses, but less so under mild water stress (DS-1) (Figure 6). This suggests that under an intermittent stress, *i.e.* a regime of consecutive cycles of drying and re-wetting, genotypes having a lower leaf area and then using relatively less water are likely to suffer less the imposition of the stress than genotypes having larger leaf area.

Conclusion

Large GxT interactions for pod and seed yield across various water regimes clearly shows that the selection of tolerant groundnut lines for breeding purposes need to be done in conditions that are representative of the target region for breeding. This study showed that the relative weight of water uptake, TE and HI in explaining the difference in pod yield varied with the water regime, water uptake driving pod yields under WW conditions, whereas HI drove pod yield differences under water stress, with TE being next to HI under mild water stress but not in more severe stresses. Root depth and length density did not discriminate tolerant from sensitive genotypes and related poorly to net water extraction, suggesting that the measurement of root morphological attributes may not be a research priority for drought research in groundnut. Tolerance to drought was mostly explained by the capacity to maintain a high harvest index under drought in the tolerant genotypes, indicating therefore that, at least under the medium and severe water stress, the reproductive processes were affected. This effect on reproduction in sensitive lines under DS-2 and DS-3 was likely a consequence of having larger leaf canopy. Our interpretation is that larger leaf canopy would lead to higher water use during drying cycles and then more damaging consequences on the reproductive processes in the sensitive lines.

Acknowledgements

The work was supported by a grant from the Bill and Melinda Gates Foundation (Tropical Legumes I project) through the Generation Challenge Program. The authors gratefully acknowledge the technical assistance provided in conducting the experiment by the Mr. Arjuna Rao and Mr. Praneet Yadav, technicians of crop physiology lab, ICRISAT, Patancheru, India.

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Figure Captions

Fig.1. Variation of rooting depth in groundnut genotypes at flowering time and after seven weeks of water stress imposition by withholding irrigation. Data are the means (± SE) of five replicated plants for each genotype. Bar indicates LSD.

Fig.2. Relationship between total root length density (RLD) and total water uptake after seven weeks of water stress imposition (in ml plant^{-1}) in breeding lines and RIL's of groundnut. Data are the means of five replicated plants for each genotype.

Fig.3. Relationship between the harvest index (HI) under three drought stresses (♦ DS-1,□ DS-2 and ▲DS-3) compared to HI under well watered conditions. Data are the mean of five replicated lysimeter- grown plants per genotype of each treatment.

Fig.4. Relationship between pod yield (g plant^{-1}) and the relative harvest index (the ratio of the harvest index under DS to that under WW conditions) under three intermittent stress conditions. Data are the means of five replicated plants for each genotype.

Fig.5. Relationship between the relative harvest index (the ratio of the harvest index under DS to that under WW conditions) and leaf weight (g plant^{-1}) under three different intermittent drought stresses (DS-1, DS-2 and DS-3) in groundnut genotype. Data are the means of five replicated plants for each genotype.

Fig

.6. Profile of total water uptake (g plant-1) in the six top most tolerant and six top most sensitive groundnut genotypes under well watered (WW) and intermittent drought stress (DS). Each data point represents mean (n=5) of five replicated plants, one per cylinder. Arrows indicate the timing of water addition (1500 ml each time).

Supplementary Table 1. Trial means, range of expected means within treatment, and mean sum of squares, least significant differences and probability for genotype effect (G), treatment effect (T) and genotype-by-treatment (GxT) interaction related to leaf area (cm^{-2}), leaf weight (g $plant^{-1}$), stem weight (g $plant^{-1}$), pod and seed weight (g $plant^{-1}$), biomass (g $plant^{-1}$), total number of pods (g $plant^{-1}$), seed weight (g $plant^{-1}$), number of immature pods, harvest index, water extract (kg $plant^{-1}$) and transpiration efficiency (TE g kg^{-1}).

		Leaf Area	Leaf wt	Stem wt	biomass	Total number of pods	Number. of Immature pods	Pod wt	seeds wt	HI	Water extract	TE
	Mean	1413	8.9	10.1	26.7	15.3	4.7	7.9	5.1	0.28	4.27	2.0
	Max	1769	12.6	14.8	34.3	23.8	7.2	12.7	8.8	0.45	5.97	2.4
	Min	1081	5.6	5.2	19.1	9.7	3.1	4.4	2.7	0.14	1.65	1.7
G (*df*:19)	LSD	310***	1.5***	1.5***	3.6***	3.7***	2.1***	2.0***	1.5***	0.05***	1.23***	0.2***
T (*df*:3)	LSD	138 **	0.7***	0.7***	1.6***	1.6***	0.9***	0.9***	0.7***	0.02***	0.21**	0.1**
G x T (*df*: 57)	LSD	621*	3.1**	3.1***	7.2***	7.3***	4.2***	4.1**	3.1**	0.10**	1.65*	0.5*

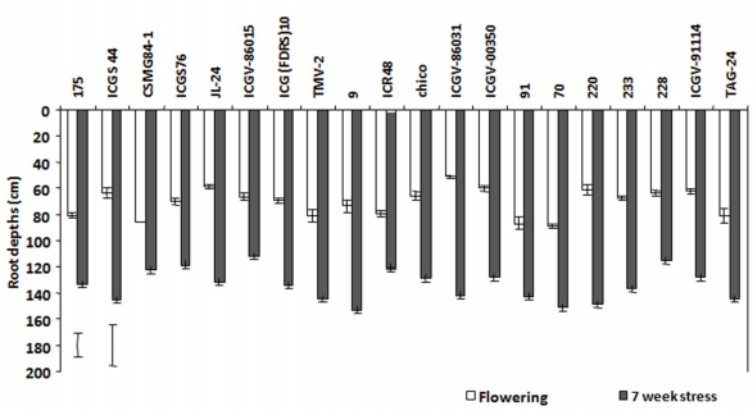
*, **, *** Significant at P= 0.5, 0.01 and 0.001 respectively

Supplementary Table 2. Pod and seed dry matter (g plant^{-1}), harvest index (HI), water extracted (kg plant^{-1}), transpiration efficiency (TE g biomass kg^{-1} water transpired), and total transpiration (kg plant^{-1}) of 20 genotypes under WW and intermittent drought stress (DS-2) conditions. The six most sensitive genotypes (lowest relative harvest index) are highlighted in bold font and the six most tolerant genotypes (highest relative harvest index) are underlined. Total transpiration of the DS-2 treatment (not shown) is equal to the sum of the net water extracted plus water applied at 29, 41, 55, 66 and 79 days after flowering (10000, 7500, and 5000 g plant^{-1}, respectively). Data are mean values of five plants (*n* = 5).

	Well-watered						Water stress (DS2)				
Entries	Pod wt	Seed wt	HI	Net water extracted	TE	Total Tr.	Pod wt	Seed wt	HI	Net water extracted	TE
ICGS76	**19.3**	**12.3**	**0.37**	**4.40**	**2.30**	**26.68**	**1.8**	**0.7**	**0.07**	**5.48**	**2.01**
ICR 48	**18.7**	**12.3**	**0.36**	**4.29**	**2.28**	**26.28**	**3.4**	**1.2**	**0.15**	**5.23**	**1.69**
228	**13.5**	**8.6**	**0.34**	**3.78**	**1.92**	**23.24**	**3.8**	**2.0**	**0.15**	**5.36**	**1.87**
ICGV-00350	**21.5**	**14.2**	**0.41**	**4.49**	**2.41**	**26.02**	**4.5**	**1.4**	**0.17**	**5.78**	**2.01**
JL-24	**15.5**	**11.1**	**0.32**	**4.03**	**2.30**	**24.15**	**4.0**	**1.9**	**0.15**	**5.19**	**1.94**
ICGV-86015	**10.9**	**7.0**	**0.27**	**3.54**	**1.86**	**22.52**	**3.2**	**1.9**	**0.14**	**3.35**	**1.98**
ICGV-86031	15.0	9.3	0.31	4.29	2.40	23.60	3.9	1.6	0.16	5.34	2.01
ICG (FDRS)10	13.5	8.1	0.27	4.05	2.35	23.10	4.0	2.0	0.15	4.26	2.11
ICGV-91114	11.9	7.4	0.31	3.27	2.03	21.03	4.8	2.6	0.21	4.98	1.75
233	18.5	12.7	0.50	3.23	2.50	18.23	10.4	6.8	0.37	5.03	2.53
TAG-24	9.4	6.1	0.43	1.65	2.18	12.31	6.5	4.4	0.34	3.72	1.81
CSMG 84-1	4.1	2.0	0.28	3.43	2.27	10.32	5.1	2.5	0.23	5.39	1.81
TMV-2	9.8	6.7	0.23	3.94	1.99	23.35	4.2	2.6	0.18	4.65	1.87
Chico	14.3	10.2	0.48	2.71	2.12	17.73	8.0	5.4	0.39	4.82	1.91
70	15.0	9.6	0.42	3.61	2.07	20.49	8.7	5.8	0.37	4.83	2.2
220	11.5	7.6	0.35	3.40	1.93	17.73	7.9	4.4	0.34	5.00	2.06
ICG S 44	9.7	6.8	0.3	2.49	2.11	17.34	5.7	3.4	0.28	5.13	1.7
91	10.3	6.8	0.39	2.06	2.06	14.44	8.7	6.0	0.39	3.99	2.14
175	10.7	7.4	0.44	2.42	1.85	13.66	9.7	7.0	0.46	4.20	2.07
9	10.9	7.1	0.42	2.68	2.22	14.39	8.5	5.9	0.46	4.02	1.72

Significance	0.001	0.001	0.001	0.001	0.40	0.001	0.001	0.001	0.001	0.135	0.001
Grand mean	13.2	8.7	0.36	3.38	2.16	19.83	5.8	3.5	0.26	4.83	1.95
LSD	6.1	4.8	0.113	0.81	0.53	5.51	2.4	1.9	0.09	0.96	0.38
CV	38.9	43.5	24.8	24.2	19.3	21.9	33	45.3	28.5	19.8	15.3

Figure 1: Variation of rooting depth in groundnut genotypes at flowering time and after seven weeks of water stress imposition by withholding irrigation. Data are the means (± SE) of five replicated plants for each genotype. Bar indicates LSD.



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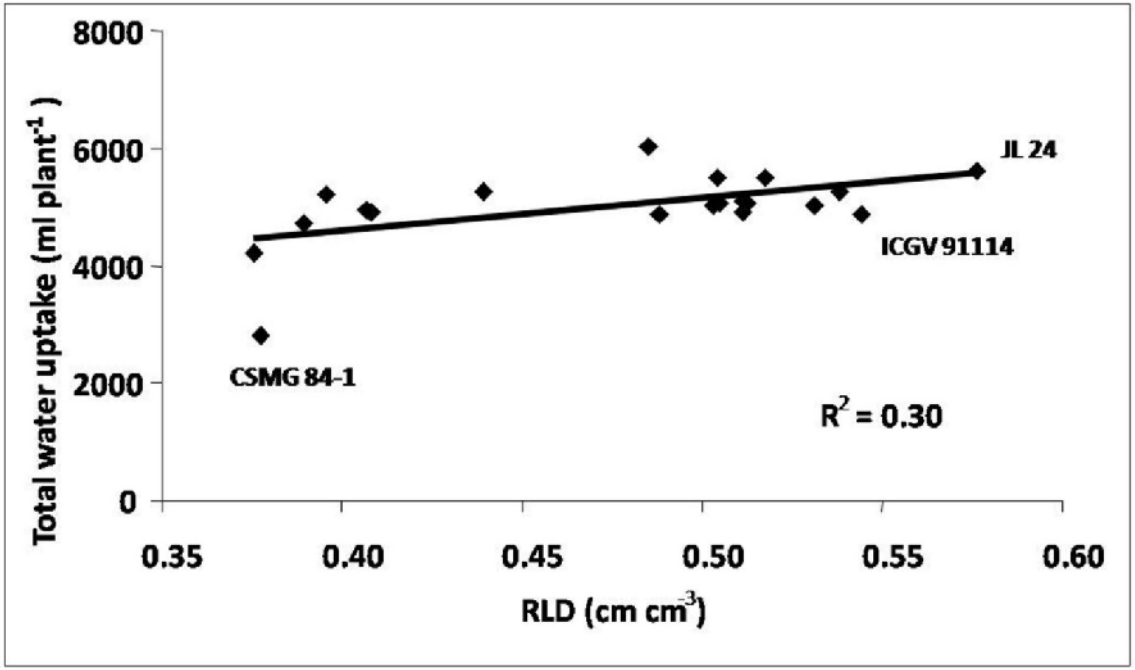

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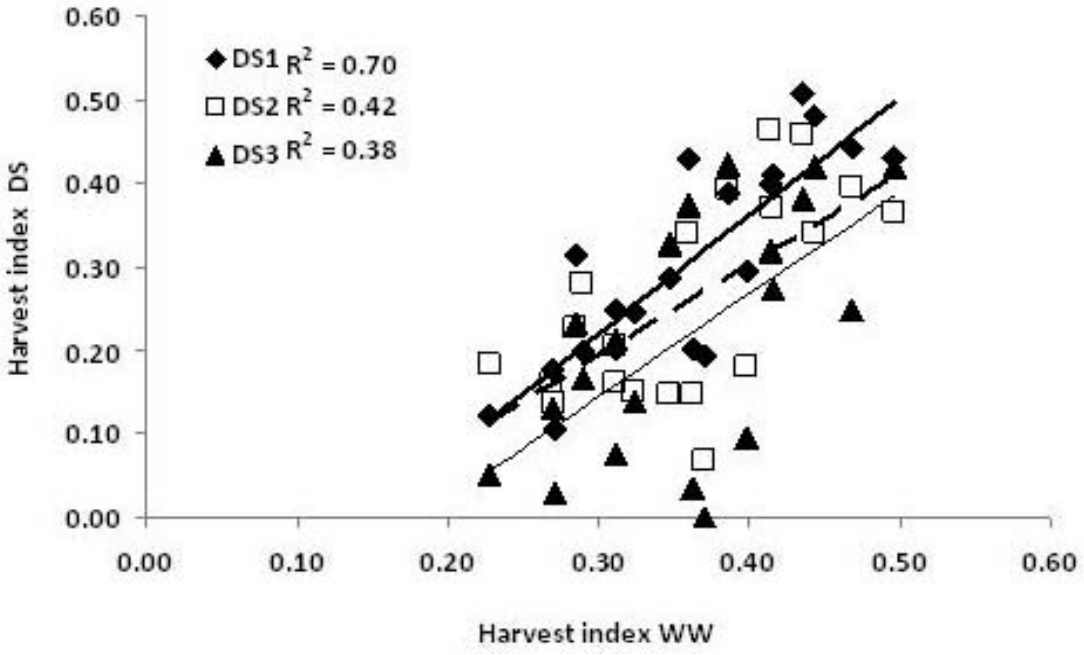

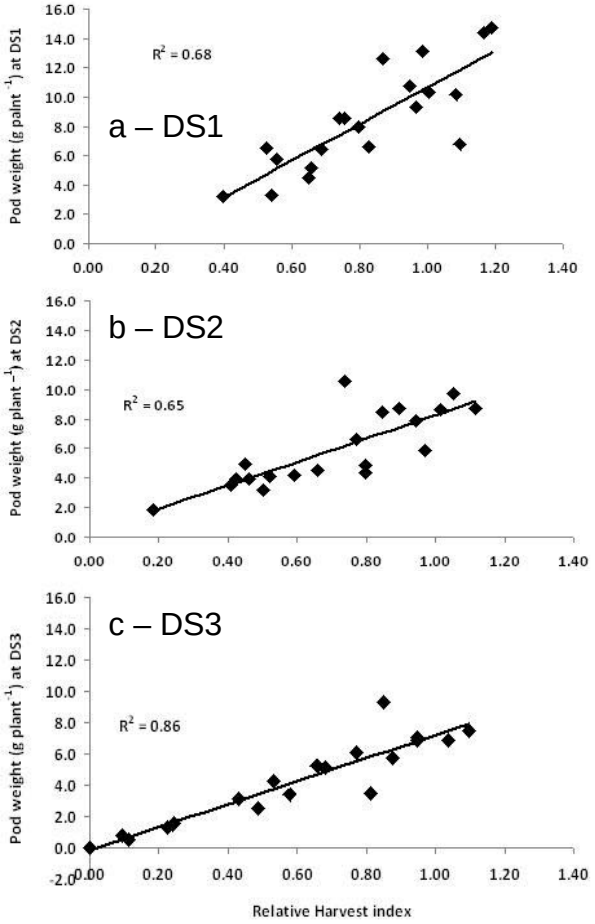

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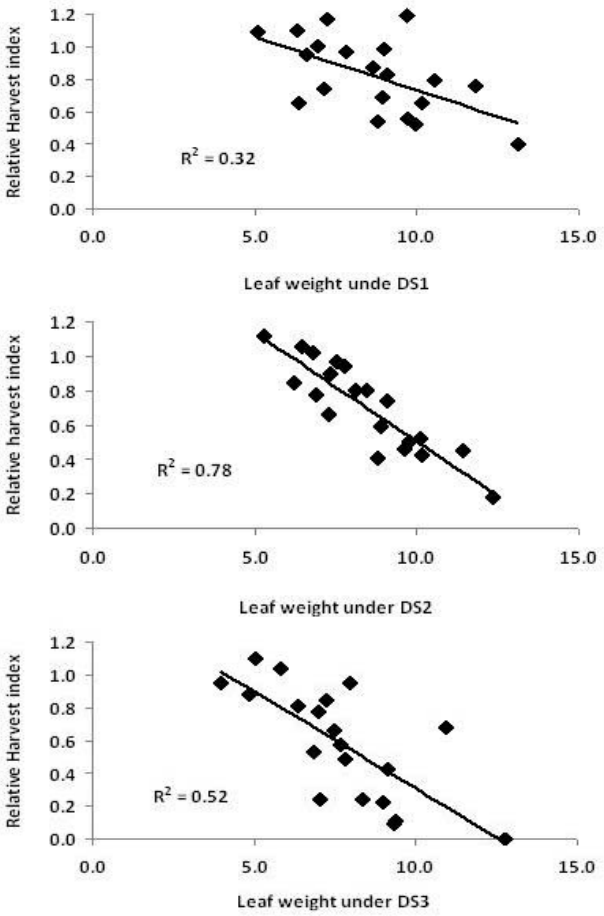

Figure 5: Relationship between the relative harvest index (the ratio of the harvest index under DS to that under WW conditions) and leaf weight (g plant^{-1}) under three different intermittent drought stresses (DS-1, DS-2 and DS-3) in groundnut genotype. Data are the means of five replicated plants for each genotype.

Figure 6: Profile of total water uptake (g plant-1) in the six top most tolerant and six top most sensitive groundnut genotypes under well watered (WW) and intermittent drought stress (DS). Each data point represents mean (n=5) of five replicated plants, one per cylinder. Arrows indicate the timing of water addition (1500 ml each time).