

Root research for drought tolerance in legumes: *Quo vadis?*

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ABSTRACT

Improving crop productivity under conditions of abiotic constraints in field is one of the major concerns in many areas of the world where legumes are grown. The legumes are generally grown under water-limiting conditions and as a result, these crops often encounter drought situation that reduces productivity to a large extent. Among many factors that are associated with drought tolerance in legume crops, root traits have been considered to be the most important attributes enabling the plant to mine water efficiently from deeper soil layer under dry environments. Most of the methods used to evaluate roots are time consuming, that provide valuable information about the root morphology but they do not reflect the dynamic characteristics of roots and root systems. Considerable amount of genetic variability with respect to root traits involving length, dry weight, root length density (RLD), etc., has been observed. Apart from the observed morphological variation in roots which has specific significance of adaptation, their functional aspects involving direct water uptake and their related kinetics are equally important. A large lysimetric system has been developed at ICRISAT to make progress in this direction. Compared to other legumes, root traits in chickpea has been thoroughly investigated and consequently, preliminary breeding works have been initiated using root traits. Root measurements in plants grown in cylinders showed almost similar relationship with depth and RLD determined in the field and thus have been used to explore diversity for these traits in chickpea. The possible diversity for root traits has been analyzed in wild relatives and transgenics as well. Using this method, root depth and RLD are being phenotyped in several recombinant inbred lines (RIL) populations. A major putative QTL for RLD was identified in a population involving a profuse rooting parent ICC 4958 and the contrasting Annigeri. These QTLs will be a faster and easier replacement technique against time-consuming phenotyping of roots and will be the way forward to introgress superior root characteristics. Efforts are also being made to use wild relatives of *Cicer* for inheriting some adaptive traits. In chickpea, preliminary work is being carried out to test the response to drought stress of DREB1A and P5CSF transgenic events using a lysimetric system. The work demonstrated a modest increase in water uptake but not yield under water stress in one transgenic event of DREB1A and one P5CSF event. In groundnut, DREB1A triggers native genes that might be involved in root development. In this review, the progress made so far on roots in legume crops has been elucidated which might explore possibilities of breeding genotypes to inherit efficient root system in legumes.

Dr. Vincent Vadez is a French national working as a Senior scientist and leading the Crop Physiology Laboratory at ICRISAT, working on the plant response to water deficit in mandated crops of ICRISAT such as groundnut, chickpea, pearl millet and sorghum. His research at ICRISAT



focuses on traits that are closely related to the yield architecture and that can have a benefit under water limited environments. Major focus of his studies is on transpiration efficiency (TE, i.e. the amount of biomass produced per unit of water transpired) with a specific goal to obtain QTL for TE that would then be introgressed into locally adapted germplasm. Presently, challenges of his research team are to explore the genetic diversity for the trait TE towards the development of suitable mapping populations, to understand the interaction between TE and the environmental conditions and to assess the potential of introgressing such traits from wild *Arachis* relatives. His current interest is to focus on water uptake by roots, where the goal is also to obtain QTL for an increased and efficient water uptake towards yield increase under water limited environments. Based on the intensive research done for the past several years, his group is presently attempting to understand the relation between “physical” root traits (e.g. depth, length density, volume, etc.) and functional root traits (water uptake, pattern of uptake over time under stress), and their relation to yield. The diversity and QTL mapping of such traits are being explored.

INTRODUCTION

Drought is one of the most common abiotic stresses reducing the yield of many crops including legumes. Improving the tolerance of crops under water limited environments is prerequisite if agricultural production is to keep pace with the expected demographic increase. Beyond productivity, resilience of crops to water limited environments, i.e., the capacity to yield even under very harsh conditions will be increasingly important. Extensive literatures are available describing roots as the potential trait to improve yield and resilience under drought. Yet, very little achievements have been made specifically in root based breeding. This could be

due to inherent difficulties in assessing root traits in a large set of genotypes that can be meaningful for plant breeders. Indeed, most studies on roots involve cumbersome methods which are time-consuming and the desired precision level with respect to measurement of rooting differences is hardly maintained, thereby limiting their application in breeding in spite of the fact that significant progress has been made in this direction (Kashiwagi *et al.* 2006). Therefore, there is a need for massive refinement and quantum leap in methodologies for assessing root traits in diverse germplasm to increase the throughput in a way that fulfills the needs of the breeding community.

Breeding for roots not only requires efficient and improved methods of screening but also in-depth knowledge about the specific function of the roots extracting water from soil from the deeper layers under water limited conditions. Indeed, most of the earlier workers attempted to relate the observed rooting differences among genotypes with higher water uptake, which may contribute towards higher yield. However, the relation between rooting behaviour and water uptake remains controversial (Passioura 1983, Hamblin and Tennant 1987, Amato and Ritchie 2002). Water uptake is considered to be a crucial factor during key stages like flowering and grain filling (Boyer and Westgate 1984), and small differences in water uptake at these stages can bring large yield benefits (Boote *et al.* 1982). The measurements of roots carried out so far do not allow answering these questions. Differences in water uptake could be measured and used in breeding but prior to that, methods are needed for direct and precise measurement of genotype's seasonal pattern of water uptake.

Water uptake by roots would also depend on the pattern of water loss by the shoot. In fact, differences in water uptake at key stages could be due to a sparing use of soil moisture earlier on when the soil is wet. Therefore, work on water uptake by roots should not disregard the role of shoot in controlling plant water loss, in particular under well-watered conditions. How such traits vary in plant species, and the way they contribute to water availability at key stages leading to better yield under drought conditions, have not virtually been tested.

This review describes the progress made in roots in few legumes and limitations of the current systems being applied to assess roots, and will address the fundamental questions whether observed root morphological differences actually translate into differences in water uptake. We will finally propose a set of improvements that are needed for such kind of studies and then try to focus upon the new lysimetric system that is set up at ICRISAT.

Review and Update in Root Research

Chickpea

In South Asia, chickpea is mostly grown during the

post-rainy season in deep clay soil, thus, facing water deficit in later part of the growth cycle. Under terminal drought, breeding for root traits is a logical approach. The work on roots in chickpea has been a major effort at ICRISAT for the past 20 years (Saxena 1984, Johansen *et al.* 1997, Krishnamurthy *et al.* 1999, Kashiwagi *et al.* 2006). Their work could successfully establish better adaptation of plants with deep root and high root length density (RLD) under terminal drought conditions. However, having deeper and more profuse roots often may not suffice the requirement of chickpea to contribute more yields. For example, the chickpea genotypes K 1189 and ICC 898 with fairly profusely roots had shown poor yields under terminal drought, mostly because of their long duration. As such, the putatively beneficial effect of roots on terminal drought yield was overridden by the dominating effect of crop phenology. Also, the testing of a mapping population developed between two elite parental lines of chickpea with varying root volume showed that the differences in RLD would not always translate in yield increase over locations (Serraj *et al.* 2004). This was especially the case in locations of northern latitudes where the season is longer and the evaporative demands lower (Krishnamurthy *et al.* 2004). Therefore, roots are only one of the components among all others, which influence overall performance of chickpea under terminal drought conditions.

Groundnut

Roots are expected to play an important role in adaptation to drought in the light-textured and deep soils of the South West US (Ketrting *et al.* 1982, Pandey *et al.* 1984), where relation between root depth and pod yield has been established (Robertson *et al.* 1980, Boote *et al.* 1982). However, such observation was based upon few studies involving only few genotypes. There appears to be some controversy on the response of root to stress. Root growth was reported to increase upon water deficit (Allen *et al.* 1976), in particular rooting depth (Lenka and Misra 1973, Ketrting and Reid 1993). By contrast, Robertson *et al.* (1980) did not find any RLD differences at shallow soil depths between well-irrigated and water stressed conditions. Similarly, Meisner and Karnok (1992) found that root growth decreased upon water deficit, though not as much in the deeper layer where water was still available. In fact, Ketrting and Reid (1993) found that groundnut was able to establish both a deep and laterally spreading root system fairly early during the growing cycle, providing adaptation to drought occurrence during and later in the season. In summary, root characteristics appear to vary in groundnut but the dynamics of root growth under water deficit are still unclear. The above works were performed under different soil conditions, with an expected strong effect on the root growth and response to stress. Based on this, it is still unclear which root trait could contribute to better yield under water limited environment in groundnut, and in which soil, environment, and stress type.

Pigeonpea

Virtually nothing is published on roots in pigeonpea under water stress, except few reports (Arihara *et al.* 1991). It is simply assumed that pigeonpea is deep-rooted, which imparts tolerance to drought during pod setting and grain filling process of the crop cycle that are taking place under residual moisture. Yet, pigeonpea is a crop where a lot of questions on roots are still without an answer, and where more work is to be done for characterizing the root traits to exploit their full potential for breeding programme which demands inheriting drought tolerance characters for better adaptability under water-limiting conditions. Are roots involved in the drought resilience of pigeonpea, or is it an adaptation of the shoot system? Are hybrids better endowed with roots? Have short duration pigeonpea “lost” their roots and is there a prospect to “breed them back”? Like chickpea, the large variations in flowering time across the pigeonpea germplasm would require a comprehensive consideration of both phenology and roots.

Progress in Breeding

Some breeding efforts have been made in chickpea (Serraj *et al.* 2004) where massive investments in labour have been made to measure roots in the field. Since field-based data are frequently associated with poor heritability that undermine the use of these traits for breeding, simpler systems have been designed for assessing variation in root traits, which consist of growing plants in 1.2 m tall and 16 cm diameter cylinders, and measuring RLD at every 15 cm depth interval at 35 days after sowing (Kashiwagi *et al.* 2005). Cylinder measurements show good agreement with depth and RLD as determined in the field and have been used to explore the diversity for these traits in chickpea (Kashiwagi *et al.* 2006, 2007). Using this method, root depth and RLD are being phenotyped in several RIL populations. A major putative QTL for RLD was identified in a population involving a profuse rooting parent ICC 4958 and the contrasting Annigeri (Chandra *et al.* 2004). These QTLs provide faster and easier replacement against time-consuming phenotyping of roots and will be the way forward to introgress superior root characteristics in superior background.

Although critical for deciding breeding strategies, the available information about the genetics of root characteristics is still limited, except for some reports on heritability estimates (Krishnamurthy *et al.* 2004, Kashiwagi *et al.* 2005). In chickpea, a major contribution of additive gene effects and additive \times additive gene interactions in root dry weight and root length density has been reported (Kashiwagi *et al.* 2007). In addition, the consistent direction of the gene effects toward increasing root growth was also observed. Similar results were reported in common bean about gene components that control the expression of root dry weight and root surface area (Araujo *et*

al. 2004). Since, the root characteristics in both the legume crops including chickpea and common bean showed additive \times additive epistasis, an advised selection procedure to exploit their inter-allelic interaction would be to delay selection to later generations and generating larger populations for selection, as shown earlier (Upadhyaya and Nigam 1998). By contrast, early-generation selection would be less effective.

No effort has been made to breed for roots in groundnut and pigeonpea. In fact, there is no prior knowledge on the range of genetic diversity for root traits in these crops and no knowledge about a putative relation between differences in rooting and drought adaptation. Such work should be done first before deciding whether focus on breeding for roots in these crops is needed.

Any Scope from the Wild Relatives

There are few scattered and non-convincing reports advocating the use of wild relatives of chickpea to improve the overall tolerance to drought (Singh *et al.* 1998, Toker *et al.* 2007). Unfortunately, there is virtually no proof of this concept demonstrating an actual improvement in the tolerance of cultivated chickpea. One of the difficulties to assess the relevance of wild germplasm in improving tolerance of cultigen is the difficulty to compare performance of wild and cultivated species. Wild chickpea, for instance, are usually long duration, showing poorer vegetative growth than the cultivated type. Wild peanut also have long duration, sometimes behave like perennials, are capable of vegetative multiplication. In any case, most wild relatives have poor agronomic characteristics, making comparison on the basis of yield virtually irrelevant. Therefore, their comparison to the cultigens can only be done on the basis of traits known to have a relation to a better adaptation to water limitation, and on the knowledge of the environment where such trait can be beneficial.

In chickpea, efforts have been made to characterize roots using different accessions of *Cicer* species (Krishnamurthy *et al.* 2003). The root system of wild relatives was found to be less deep than that of the cultivated ones (Krishnamurthy *et al.* 2003). Therefore, it was concluded that wild chickpea had limited potential for improving the tolerance of the cultigen through their root system. However, re-analysis of these data showed that the root/shoot ratio of the wild relatives was among the highest, and superior to most cultivated ones (Fig. 1). This would imply that each gram of shoot tissue, *i.e.*, each surface unit of leaf area, is supported by a larger length of roots compared to the cultivated species, which could have a real importance under certain drought conditions. Work would then be needed to determine under which water-limited agronomic environments, such a higher partitioning to roots is needed to achieve better yield. In such case, wild relatives could become a potential donor of genes for that particular phenotype.

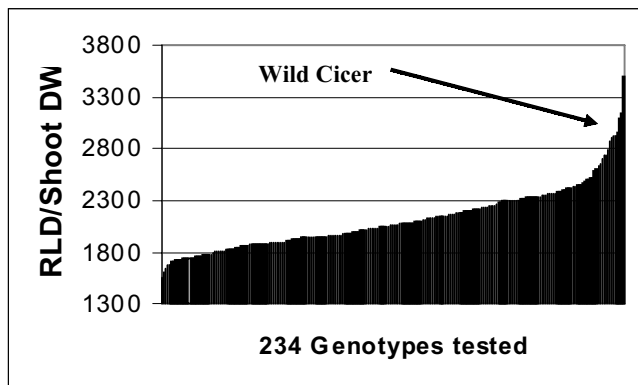


Fig. 1. Root length density (RLD) per shoot dry weight ratio ($\text{cm cm}^{-3} \text{g}^{-1}$) in 234 genotypes of chickpea (*Cicer arietinum* L.), including some accessions from wild *Cicer* species. Data are means of three replicated plants. See Krishnamurthy *et al.* (2003) for details on the root extraction procedure.

In case of groundnut and pigeonpea, the evaluation of rooting characteristics in the wild relatives has not been undertaken.

Any Scope from the Transgenics

There is only one report on transgenics in which an *Arabidopsis* gene related to the vacuolar H^+ pyrophosphatase (AVP1) led to an increased root growth under water deficit conditions (Park *et al.* 2005), which is hypothesized to be related to a modification in the auxin fluxes.

In chickpea, preliminary work is being carried out at ICRISAT to test the response to drought stress of DREB1A and P5CSF transgenic events, using a lysimetric system by which water uptake is assessed rather than root morphological characteristics (see below). So far, only a modest increase in water uptake under water stress conditions has been found in one transgenic event of DREB1A and one P5CSF event. However, the observed differences did not lead to any significant yield increase (our unpublished data).

Contrary to chickpea, a recent study carried out at ICRISAT shows the involvement of *DREB1A* transcription factor driven by a stress responsive promoter from the *rd29* gene of *Arabidopsis thaliana*, on the development of groundnut roots under drought stress conditions (Vadez *et al.* 2007a). These transgenic plants of groundnut variety JL 24 were grown in 1.2 m long and 16 cm diameter cylinders under well-watered conditions for 30 days before withdrawing irrigation in half of the plants. Forty days after stopping irrigation in the drought treatment, the root growth was found to dramatically increase in the transgenics, whereas roots remained unchanged in the non-transgenic plants. This resulted in a higher water uptake from the soil (Fig. 2). This work suggests that *DREB1A* triggers native genes of groundnut that might be involved in root development, and needs further investigations.

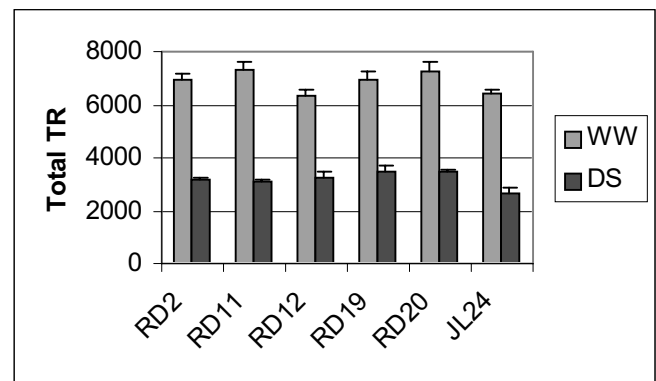


Fig. 2. Total evapo-transpiration over a 40-days period (g plant^{-1}) in 5 transgenic events and a wild type parent (JL 24) grown in PVC cylinders of 16 cm diameter and 1.20 m long. Plants were exposed to well-watered (WW) by compensating water loss or to drought stress (DS) by saturating the soil profile at 30 days after sowing and preventing further irrigation. Data are means (bars indicate SE) of 5 replicated cylinders with one plant in each cylinder.

Usual Assumptions on Roots and Limitations of Current Methods

Current assumptions

An increase in rooting depth has often been equated to increased drought tolerance (Miller 1916, cited by Kashiwagi *et al.* 2006, Jordan *et al.* 1983, O'Toole and Bland 1987). Jones and Zur (1984) reported that an increased soil volume explored would increase crop yield under water-limited environments. There is some theoretical analysis from modeling showing that increasing the root depth of maize to the rooting depth of sorghum would contribute to yield increase in most dry years (Sinclair and Muchow 2001). There is no such work in legumes, although rooting depth is one of the three traits that potentially contribute to drought tolerance among the 16 traits reviewed by Ludlow and Muchow (1990). Although in these studies, the type of drought imposed was not fully described, it is understood that roots would have an essential role under terminal drought conditions, *i.e.*, for those crops grown on residual soil moisture after the end of the rains, and where drought stress usually occurs after flowering. Whether roots contribute during intermittent drought still needs investigation, as there is virtually no published data on the topic. Such work would be needed in the case of groundnut and pigeonpea that are usually rainfed crops, both in Africa and Asia and can face episodes of drought throughout the cropping cycle.

Limitations to available studies on roots and the methods used

Although there has been a number of studies on roots in different crops, the lack of use into breeding programmes suggest some limitation to the works that were performed: (i) most of these studies assessed roots in a very "static" manner,

i.e., destructive samplings at one or several points in time, giving virtually no information on the “dynamics” of root characteristics, and it is not clear what particular root trait, or what particular aspect of root growth would contribute to a better adaptation to water deficit; (ii) all the studies on roots used limited number of genotypes except few (Kashiwagi *et al.* 2006), giving poor idea on the range of variations available to breed; This drawback is mostly explained by the difficulty in studying roots, thus, requiring a simplification of the methods used to evaluate a larger number of lines; (iii) some of the reported work clearly show that the putative relation between rooting traits and drought tolerance is often overridden by escape mechanisms related to genotype’s phenology (Blum *et al.* 1977, Kashiwagi *et al.* 2006). Therefore, the contribution of roots to tolerance can only be truly tested once genetic variation in root traits is found within groups of genotypes sharing a similar phenology; (iv) Above all, most studies on roots published so far have relied on a fundamental assumption that increased root length density would equate with higher water uptake (see below) and therefore, on yield.

What Progress is Needed in Root Research?

Test whether differences in root length density relate to differences in water uptake

How much water is taken up would obviously relate somewhat to the RLD, but this link is still unclear because of the lack of data comparing the two parameters. Several authors concluded that RLD and water uptake is related (Passioura 1983, Monteith 1986, Lafolie *et al.* 1991). This view is challenged by other studies showing poor relation between water uptake and RLD across several cereals and legumes (Hamblin and Tennant 1987, Dardanelli *et al.* 1997, Katayama *et al.* 2000, Amato and Ritchie 2002). Even after considering legumes and cereals separately, legumes appear to have higher rate of water intake per unit root length compared to cereals, which would be explained by more abundant metaxylem vessels, which decrease their axial resistance to water flow. In any case, whether the RLD differences in chickpea relate to differences in water uptake or not need to be tested in view of its possible impact on yield. In fact, the lack of relation between water uptake and RLD agrees well with our data on groundnut (Fig. 3), where we run regression with root dry weight. By contrast, we found good relation between water uptake and root dry weight in *DREB1A* groundnut transgenics, where higher water uptake of transgenic plants under water deficit was well related to higher RLD below the 40 cm depth (Vadez *et al.* 2007a – Fig. 4). Our interpretation is that the root hydraulic conductance of groundnut varies likely across genotypes, which would explain that similar weights of roots, assumed to be related to proportional RLD, could lead to totally different water uptake. The good relation between RLD and water uptake found in transgenics would probably mean that the genetic transformation with *DREB1A* did not affect root hydraulics.

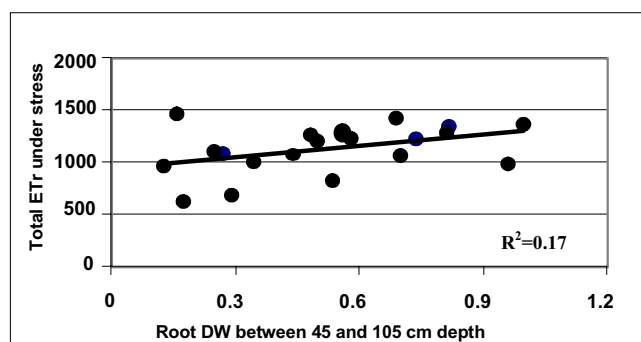


Fig. 3. Lack of a clear relationship between root dry weight (g plant⁻¹) within the 45-105 cm depth and the sum of the evapotranspiration, Etr (g plant⁻¹) when plants were undergoing water stress (after evapotranspiration of drought stressed plants fell below 50% of that in control plants). Each data point represents a replicated cylinder with one plant per cylinder. The 20 data points are 4 genotypes and 5 replications per genotype.

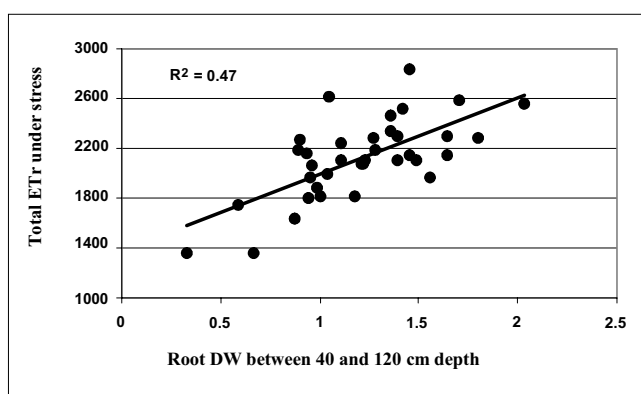


Fig. 4. A fairly clear relationship between root dry weight (DW) within the 45-120 cm depth (in g plant⁻¹) and the sum of the evapotranspiration when plants were undergoing water stress (after evapotranspiration of drought stressed plants fell below 50% of that in control plants). Each data point represents a replicated cylinder with one plant per cylinder. The 20 data points are 6 genotypes (5 transgenics events and one wild type) and 6 replications per genotype. See Vadez *et al.* (2007) for details on the experimental procedure.

The need to assess root “dynamically”

Following the above debate on root length density and its putative relation to water uptake, it has also been shown that a small length of roots in deep layers where water is plentiful would be sufficient to fully supply water to the plant when the top soil is dry (Gregory *et al.* 1978, Sharp and Davies 1985). This would logically offset the linear relation between water uptake and RLD. This hypothesis suggests that the assessment of RLD over the entire profile would be less relevant than the assessment at specific depth. Kashiwagi and colleagues showed that the overall RLD was related to seed yield in two experimental years and that RLD at depth was significantly related to seed yield under harsher conditions

only, although the overall RLD was also significantly related to seed yield in that year.

In any case, there are clearly some controversies over the water uptake and RLD relationship and several of our datasets on germplasm (unpublished) shows that root dry weight and water uptake are little related. Again, the benefit of deeper / more profuse rooting system remains to support enhanced/ sustained water uptake, in particular for key developmental stages. The assessment of roots at a given time during the lifetime of the crop is unlikely to deliver the precise information that would be needed to truly assess the potential of roots. As suggested by other authors, water uptake should be the prime target as suggested previously (McIntyre *et al.* 1995, Dardanelli *et al.* 1997) and such water uptake should be assessed *in-vivo* and repeatedly in plants exposed to stress, in conditions that mimic the field conditions, in particular with regards to having a soil profile, and compare to well-watered plants. In that respect, our preliminary data (Fig. 5) indicate that different genotypes appear to deplete the soil profile water of the cylinder in a different manner after irrigation has been suppressed. In Fig. 5, genotype TMV2 appears to take up higher amount of water than TAG24 during the initial 8 days after irrigation was withheld. In the forthcoming 10 days (9-19 days after withdrawing irrigation), TAG24 took up more water on almost everyday than TMV2. Such figure indicates that the kinetics of water uptake vary among genotypes.

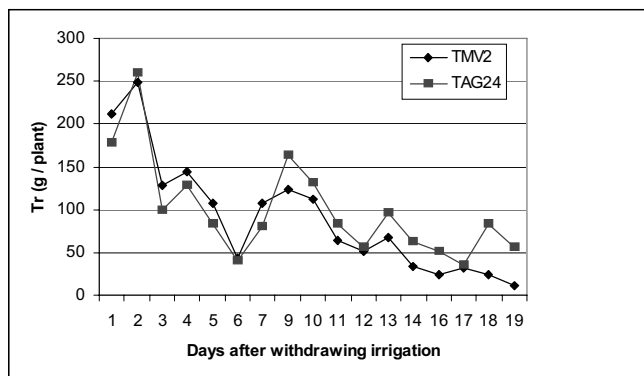


Fig. 5. Profile of transpiration, Tr ($g\ plant^{-1}$) in two groundnut genotypes grown in 1.2 m cylinders (16 cm diameter) after saturation of the soil profile and after irrigation has been withheld. Data are the mean of 5 replicated plants, one per cylinder.

The need to understand comprehensively root water uptake and shoot water loss

A better water uptake by roots at key stages like the grain filling period could be related to a sparing use of water by the shoot at earlier stages when the soil is wet. This type of behavior would permit saving of water in the soil profile and make it available for the later stages of the cropping cycle, *e.g.* during reproduction or grain filling. Although a more

moderate water use would be related to less photosynthetic activity and therefore potential growth, this characteristic could be of value where water is limited. In fact, a modeling study showed that imposing a maximum rate of transpiration per day would contribute to water saving, increase in transpiration efficiency, and lead to yield benefit in most years in sorghum (Sinclair *et al.* 2005). There are possibly two ways in which maximum rate of transpiration can be achieved: (i) by having a lower stomatal conductance; (ii) or by limiting stomatal conductance when the vapor pressure deficit (VPD) is high.

In relation to mechanism (i) as indicated above, recent work on the *ERECTA* gene shows a role both on the regulation of photosynthesis, but also on the stomatal conductance (through stomatal density) (Masles *et al.* 2005). This would potentially lead to a limitation in the maximum rate of transpiration. We recently found that a major difference among pearl millet genotypes having similar phenology but differing in terminal drought tolerance was indeed due to a lower rate of water loss per unit of leaf area (transpiration over a period of 2 days divided by leaf area) in terminal drought tolerant genotypes (unpublished data). These differences were measured under well-watered conditions, indicating that this trait is constitutive. These results have been observed very consistently across experiments, either on whole plants taken at different stages or on detached leaves over short periods of time. This index, which reflects the leaf stomatal conductance over a period of time, would save water and make it available for the later stages of the crop cycle, in particular grain filling. In fact, the QTL for terminal drought tolerance that was identified was a QTL for yield and for a high panicle harvest index, which indirectly assesses grain filling in plants. Water saving from this lower stomatal conductance would decrease the proportion of water used before anthesis and make it available for grain filling. This would fit well an old hypothesis by Passioura (1983) that a higher proportion of water loss after anthesis would contribute to better grain filling under water stress conditions.

In relation to mechanism (ii) as mentioned above, the transpiration of certain genotypes of soybean has been shown to no longer increase, or to increase at a lower rate at vapor pressure deficit (VPD) above 2.0 KPa (Sinclair *et al.* 2008). This trait would limit soil moisture use when the VPD is high and when carbon fixation has a high water cost. Similar and additive to the above trait, it would make more soil water available for grain filling. We have observed before that certain species such as pearl millet in semi-arid conditions do maximize transpiration even if the vapor pressure deficit (VPD) is high (above 2.5 KPa) (Squire 1979). In the work reviewed by Bidinger and Hash (2004), no attention was paid to possible genetic variations in this strategy. Our recent data fully agree with the above reported data on soybean and show that pearl millet genotypes differ in their transpiration response to VPD (Vadez

et al. 2007b), with the terminal drought tolerance QTL donor having a considerably slowed transpiration at high VPD, whereas genotypes not holding this QTL would have transpiration responding linearly to VPD increases above 2.0 KPa.

The way forward to progress in root research

Basic considerations

Given these limitations and to remove the “static” approach used so far, we propose that our future approach on roots should focus on root functionality rather than morphology. We mean to say the focus be given to measuring water uptake under water deficit, in a “dynamic” and precise way, in a large range of genotypes representative of the species’ diversity. This should carefully consider the phenology of genotypes, and determine the relation between a given pattern of kinetics/volume of water uptake and the performance under stress (based either on biomass or yield). Further, the evaluation of water uptake under stress should be compared to a fully irrigated control. Once contrasting genotypes are identified, the root developmental and morphological patterns could be investigated thoroughly to understand the genetics of development patterns.

The lysimetric system set up at ICRISAT

Such lysimetric system is shown in Fig. 6 and 7. It takes into account the above considerations. Additionally, we have paid utmost attention to develop a system that mimics the field conditions as closely as possible. The cylinders on Fig. 6 are 1.20 m long and 20 cm in diameters (thereafter called “small tubes”). The dimensions of the tubes have been

determined in a way that the volume of soil available to each individual plant grown in each tube corresponds to the volume of soil available under current sowing density of groundnut and chickpea (approximately 25 – 30 plant /m²). A large scale lysimetric system has been set up with over 2300 cylinders. The major purpose is to translate the capacity to precisely measure water uptake differences into a throughput that can fit the need of breeding program (Fig. 7). Another system is being developed for more loosely planted crops such as pigeonpea. In that system, the tubes are 2.0 m long and 25 cm diameter (thereafter called “long tubes”) and 1200 are available.

The soil packing has been done using soil sieved in particles smaller than 1 cm. This allowed us to control the bulk density to approximately 1.4, which is standard value for Alfisol. To ensure that moisture is available in all parts of the cylinders, 40 kg of dry soil were initially filled in each cylinder. We checked that the soil level was similar in all tubes. A previous assessment of the water needed to fill the profile before drainage determined that the soil water holding capacity of the soil was approximately 20%. Therefore, 8 liter of water was added to the first 40 kg of soil. An additional 10 kg of dry soil were filled to each cylinder soon after the water had penetrated the profile and watering with 2 L was done subsequently. At that stage, the cylinders were almost filled at the desired level, *i.e.* approximately 5 cm from the top. A top up using dry soil was done to ensure that all cylinders would be filled at the same level. This top up varied between 500g and about 1 kg, *i.e.* less than 1% variation across cylinders, for which all the cylinders had a very similar bulk density, close to 1.4.

At planting, the soil is wetted and seeds planted at a rate of 2/cylinders, later on thinned to 1 plant per cylinder. Watering is done at regular interval. This system is most suited to tailor a number of drought regimes to a range of crops (Fig. 7). In our preliminary experiments, we have imposed the last



Fig. 6. Lysimetric system newly used at ICRISAT to evaluate plant water uptake in PVC tubes (1.2 m long, 20-cm diameter), filled with Alfisol at a bulk density of about 1.4. The length and diameter of the tubes are designed so that the soil volume available to each plant is equivalent to the soil volume available under field conditions at current sowing densities (for groundnut and chickpea). The system is used here for the evaluation of transgenics in P2 facilities.



Fig. 7. Overall view of the lysimetric system developed at ICRISAT. The tubes are 1.2 m long with 20 cm diameter. They are filled with Alfisol at a set bulk density of 1.4. Each trench can accommodate approximately 700 cylinders. It takes about one day to 4 persons for weighing about 600 cylinders.

irrigation (to saturate the soil profile) at about one week after flowering. At that stage, low density polyethylene beads were applied to all cylinders (600 ml per cylinder to have a bead layer of approximately 2 cm). The purpose of the beads was to limit soil evaporation and our data show that about 90% of the soil evaporation is prevented. Therefore, the regular weighing of the cylinders provides essentially transpiration data. The beads also allow pegging in the case of groundnut.

CONCLUSION

Although some work has been attempted to exploit the potential of roots to improve the adaptation of crops to water limiting environments, it is little in comparison to the number of studies on other plant parts. So far, the methods used to evaluate roots are time consuming and provide information that, though valuable, does not reflect the dynamic characteristics of roots and root systems. In this review, we advocate that more focus should be given on the functionality of roots rather than their morphology, in particular by looking at direct water uptake measurements and their related kinetics. Additionally, we argue that the capacity of roots to take up water should also be looked at in the context of the capacity of shoot to limit their water loss, in a comprehensive manner. To progress in that direction, a large lysimetric system has been developed at ICRISAT.

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