

The Scope of Gene Technologies in Improving Drought Tolerance in Crops

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Introduction

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is one of the 15 Future Harvest Research centres, led by the Consultative Group on International Agriculture Research (CGIAR). Headquartered in India with two regional hubs in Niger (for West Africa) and Kenya (for East/Southern Africa) and mandate for the semi-arid tropics, ICRISAT deals with five mandate crops commonly grown in the region: Chickpea, groundnut, pigeonpea, pearl millet and sorghum. For obvious reasons, drought is at the center of the research and development agenda of ICRISAT. Its major objective is to develop resilient crops to low and erratic rainfall.

Water deficit is the most prominent abiotic stress, which limits severely crop yields and opportunities to improve livelihoods of poor farmers in the

semi-arid tropics. For instance, it is estimated that drought is responsible for \$ 520 million loss per year in groundnut only (Subbarao et al., 1995). Therefore, major efforts are needed to improve the tolerance of crops to water deficit, and there is now increasing hope that this would be possible. For instance, from the estimated 3.7 million tones loss annually in chickpea from water deficit, about 2.1 million tones could be recovered from crop improvement efforts and similarly half of the loss in groundnut (Johansen and Nigam, 1994).

Yet, drought is a complex issue, which involves a number of agronomic, edaphic and climatic aspects. Although drought can be broadly characterized by its timing, duration, and intensity, the breeding of drought tolerant varieties through conventional breeding remains difficult, in particular because of the large genotype by environment interactions. This is in part because yield is the combination of different traits whose relative importance usually varies a lot in different types of environment. A common success has been achieved across the different mandate crops by breeding early maturing varieties, which are able to escape the drought and mature before the water deficit becomes too severe.

Yet, further improvements under water deficit are still needed. A trait-based approach has been considered to dissect yield under drought into its different components. In that approach, we try to follow a simple model defined by (Passioura, 1977), where yield (Y) is defined as $T \times TE \times HI$, where T represents how much water is taken up by roots, TE (transpiration efficiency) represent how efficiently the transpired water is converted into biomass, and HI (harvest index) represents how the biomass is converted into grain. To identify contrasting parents for these traits, we also screen large numbers of representative germplasm utilizing representative subsets of germplasm (Upadhyaya and Ortiz, 2001). A trait-based approach has yielded some success for some abiotic stresses (Hall, 1992; Condon et al., 2002; Sinclair et al., 2000). However, some of these traits are not easily measurable and remain difficult to pyramid in a similar background. Recent advances in molecular genetics now allow identifying quantitative trait loci (QTLs) related to those traits. Linked to molecular markers that can be easily pinpointed, these QTLs could now be pyramided, and this would make the molecular breeding of drought tolerant crops possible (Ribaut et al., 1996).

In certain crops, a transgenic approach has been used as an attempt to speed up the process of molecular introgression of putatively beneficial genes. This approach could be a valid one in those crops that have received little attention from molecular studies and which do not have sufficient set of tools to undertake molecular breeding. This is for instance the case in groundnut, the lack of genetic polymorphism, a bottleneck during the evolution of peanut, makes it difficult to breed through molecular approaches. However, a single-gene transgenic approach could be criticized because abiotic stress tolerance involves very likely many genes. So, it has been suggested that a wiser approach could be the use of transcription factors, i.e. major “switch” that trigger a cascade of genes in response to a given stress (Chinnusamy et al., 2004). This approach has been undertaken at ICRISAT.

The objective of this paper was to make an update drought research at ICRISAT, to highlight the major achievements of the past years, and to give an insight of the major current research orientations, in particular in marker-assisted selection (MAS). In this review, we will not address drought escape mechanisms.

Marker Assisted Breeding in Pearl Millet

Pearl millet is a well-adapted plant to semi-arid areas and is subjected to post-flowering stress, for which the major focus is currently on terminal drought tolerance. Yet, improvement of its performance under drought conditions remains possible. It was found that the performance of pearl millet under water deficit were in part explained by the yield potential under water deficit and the drought escape mechanism. Therefore, a drought resistance index (DRI) was calculated by Bidinger et al. (1987) to identify source of tolerance to drought. The criterion that was the most related to the DRI was the panicle harvest index (PNHI), and this criterion is now used to screen tolerant accessions.

Crosses have been made between genotypes having high and low PNHI and the genotyping and phenotyping of testcross hybrids of the progenies have led to the identification of QTLs for terminal drought tolerance in multi-location trials. A major QTL has been identified on linkage group 2 of pearl millet. The existence of that QTL has also been confirmed using another cross between tolerant/sensitive genotypes (Yadav et al., 2004). In

short, that QTL contributes to a larger number of florets setting grains on the panicle, and to a better grain filling, both contributing to a higher PNHI (Figure 1). That QTL is currently the major focus of research. It has been introgressed in the background of terminal drought sensitive genotype H77/833-2 and after several round of backcrosses, several introgression lines such as ICMR 01029 or ICMR 01031, have been identified with superior terminal drought tolerance (Hash et al., 2005).

Recent work has tested some of the QTL introgression lines, along with contrasting parents and tends to conclude that more profuse rooting in the deeper soil layer may be a major underlying factor to that QTL (Vadez et al., 2005). Current efforts are now being put on pyramiding that QTL on LG2 with downy mildew resistance QTL. Efforts are also being made to further characterize the role of roots in that QTL, and possibly to identify QTLs for root traits in pearl millet.

Marker Assisted Breeding in Sorghum for Staygreen

Sorghum is a dual-purpose (grain + straw) crop adapted to the semi-arid tropics and subtropics. Sorghum is increasingly grown during the post-rainy season and is therefore dependent on stored soil moisture, for which it is commonly exposed to terminal drought conditions, usually starting around flowering. The extension of leaf greenness in sorghum, i.e. the stay-green trait, has been described as an important secondary trait involved in yield improvement under terminal drought stress (Borell and Hammer, 2000). The maintenance of green leaves is related to a delay of nitrogen remobilisation from leaves, which maintains photosynthetic activity during grain filling. Under terminal drought conditions, a sustained photosynthesis allows a continued supply of carbohydrate to the developing grains. It was recently shown that staygreen expression was correlated to the 100-seed weight, a good proxy for grain filling (Bidinger, personal communication). Staygreen is evaluated by following the pattern of leaf senescence under terminal drought conditions after flowering. In short, several plants are tagged in each plot and the top 6 leaves are scored weekly for their percentage of green leaf area (GLA). The comparison of the pattern of GLA retention allows identifying staygreen from senescent materials (Figure 2).

Several QTLs have been found for staygreen from different crosses (Crasta et al., 1999; Haussman et al., 2002) from which 4 major QTLs have been identified. At ICRISAT, the sorghum molecular breeding group has been focusing on staygreen QTLs stg1, stg3, stg4, and stgB, using the donor parent B35. Introgression lines have been produced from two cycles of backcrossing and MAS selection for different combinations of the staygreen QTLs described above. Because B35 has poor adaptation to tropical environment, the introgression has been hampered by a lot of “linkage drag” and those lines have not been able to outperform the elite drought sensitive parents R16 and ISIAP Dorado. However, 2 of the introgression lines already produce larger forage quantities than and have better forage quality (higher N content and better digestibility).

The current focus of work is therefore to remove the linkage drag associated with the use of B35 as a donor parent. To do so, two more backcrosses assisted by markers for the 4 QTLs have been performed to recover most of the genome of the recurrent parent R16 on the most promising introgression lines. These materials will be tested in the forthcoming season at ICRISAT (2006-07). To exclude from the introgressed QTLs those genes that are tightly linked to the QTL and which confer poor agronomic or quality characteristics, more markers are needed in the vicinity of the targeted QTL. In parallel, we have tested whether root traits would have any underlying role in the staygreen trait. We have evaluated the root growth of 2 staygreen donor parents, B35 and E36-1 and two sensitive parents R16 and ISIAP Dorado, in 2.0 m long, 18 cm diameters PVC cylinders. We have found that under water deficit both staygreen parents had roots about 60 cm deeper than senescent materials whereas under well-watered conditions, the rooting depth of all genotypes did not differ (Vadez et al., 2005).

Mapping Root Traits in Chickpea

Chickpea is exclusively grown during the post-rainy season, which coincides with the cool days of the year. As major chickpea cultivation areas are rain fed environments, Chickpea exclusively depends on store moisture and, therefore, faces terminal drought conditions. Roots have been a major focus of research for over a decade at ICRISAT. It has been

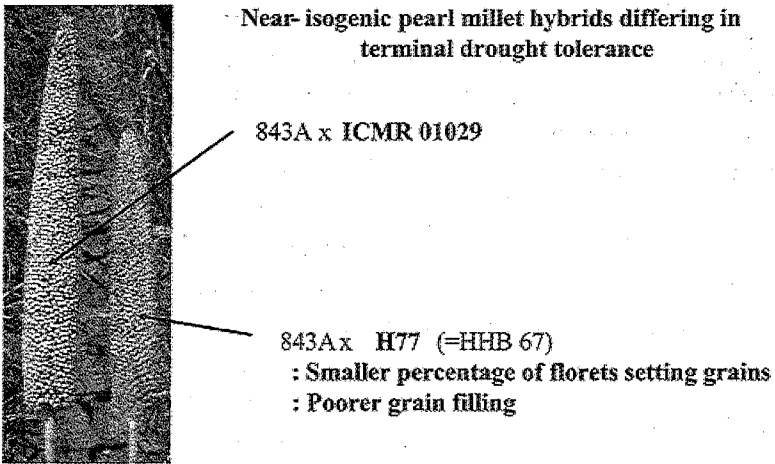


Figure 1. Typical difference in the panicle of a terminal drought tolerant genotypes ICMR 01029 x 843 A: A higher number of florets setting grains and a better grain filling, both ontributing to higher PNHI (panicle harvest index).

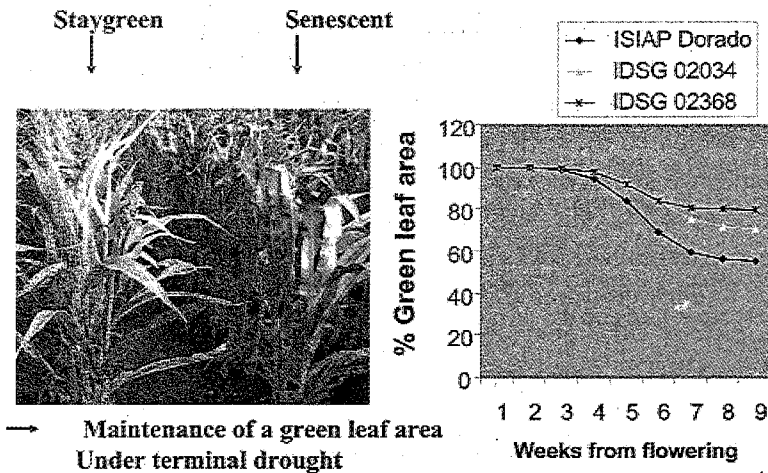


Figure 2. Typical appearance of the stay green trait in field conditions under terminal drought, i.e. a delayed leaf senescence, and a typical pattern of green leaf area (GLA) retention over time between introgression lines and senescent parent ISIAP Dorado.

found that deep and more profuse rooting was a direct contributor to the seed yield under terminal drought (Kashiwagi et al., 2006). However, the methods used to assess root traits have only improved in the past few years (Kashiwagi et al., 2005 & 2006), where roots are now assessed in 1.2 m long, 18 cm diameter PVC cylinders. Roots used to be dugged out from field conditions, using the monolith method, an extremely time-consuming method. A good agreement between field and cylinder data has been found (Kashiwagi, 2006). Using the PVC cylinder method, the range of genetic variation for root trait has been recently explored using a large representative set of genotypes, including the mini-core collection of ICRISAT (10 per cent of core collection, 1 per cent of entire collection) (Upadhyaya and Ortiz, 2001). From that screening, more contrasting genotypes for root traits have been identified and new mapping populations developed from parents showing more root contrasts than those used previously (Figure 3) (Kashiwagi et al., 2005).

The current focus of research is now to phenotype the different populations. From the initial population between Annigeri (shallow roots) and ICC4958 (deep and profuse roots, a QTL accounting for over 30 per cent of the variation in root length density has been found (Chandra et al., 2004). The phenotyping and genotyping of two recent populations is under way (ICC1882 x ICC4958 and ICC283 x ICC8261) and there is good scope to find more QTLs for root traits in chickpea. Next step toward MAS breeding in chickpea would be to start their introgression into locally adapted varieties.

A Transgenic and Marker-Assisted Approach in Groundnut for TE

Groundnut is usually grown under rain fed conditions and is often exposed to erratic rainfall pattern, which exposes the crop to intermittent drought spells at every stage of crop development. For that reason, the major focus of research is to develop varieties that are able to efficiently use erratic amounts of water, having therefore high transpiration efficiency (TE). Genetic variability for TE has been found in groundnut (Wright et al., 1994). Unfortunately, an attempt to integrate the TE traits into varieties using a trait-based approach has been only equally successful

than following a conventional approach. This has been mostly because of the difficulty to pyramid high TE and high HI in a similar background, i.e. genotypes having high TE usually had low harvest index (HI) (Wright et al., 1991).

Yet, contrasting parents have been identified and mapping populations developed. One such population has been phenotyped for TE and shows good and consistent segregation across seasons. Our current major limitation for mapping QTLs linked to TE trait is to genotype that population with a sufficient number of polymorphic markers. Groundnut is one such crop that probably results from a single event of hybridization between two wild ancestors. This has isolated groundnut from its wild progenitors and has created a genetic bottleneck characterized with the lack of genetic polymorphism between most of the cultivated germplasm. In collaboration with EMBRAPA (Brazil), we are currently trying to re-synthesize groundnut from its wild progenitors, as an attempt to re-introduce genetic polymorphism. Alternatively, we are also trying to develop more markers for cultivated groundnut, in particular exploring new molecular techniques to generate novel types of polymorphic markers such as DArTs.

Since the molecular breeding approach in groundnut is bound on the success of finding a sufficient number of markers, a transgenic approach has been attempted in groundnut to introgress genes putatively involved in drought tolerance. Instead of using a single gene, the genetic transformation laboratory of ICRISAT has introgressed a transcription factor, DREB1A, using rd29 as a stress responsive promoter, into JL24, a popular groundnut variety in India. The testing of 5 events in T3 generation, after an initial screening of 14 events, has revealed that most transgenics had higher TE than the non transformed parent under well watered conditions, and that one event had higher TE than the non transformed parent across moisture conditions (Table 1) (Vadez et al., 2005). These results are very encouraging because the range of variation for TE between the transgenics and the parent was higher than what has been found with a RIL population. Further, the fact that transgenic events showing such large phenotypic contrast, while being isogenic for one inserted gene, provides great opportunities to re-investigate the mechanisms underlying high TE in groundnut.

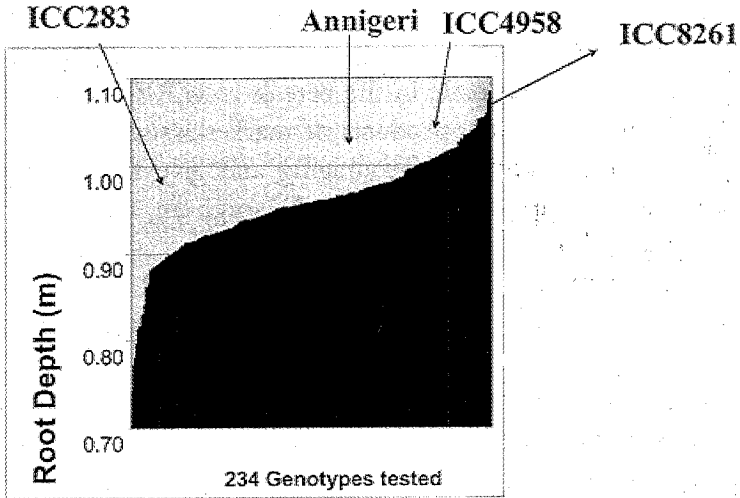


Figure 3. Range of genotypic variation for root traits in 234 genotypes, including the mini-core collection. Data show the genotypes involved in the newly developed mapping populations.

Table 1. Transpiration efficiency (TE, in g biomass kg⁻¹ water transpired) in 5 transgenic events and the untransformed parent JL 24, under well-watered (WW) and drought stressed conditions (DS). Pooled data from 2 replicated experiments.

	TE (WW)	TE (DS)
JL 24	2.05 ^b	4.29 ^b
RD 19	4.31 ^a	4.99 ^b
RD 12	5.13 ^a	4.63 ^b
RD 20	3.19 ^b	4.52 ^b
RD 2	4.09 ^a	6.12 ^a
RD 11	4.96 ^a	5.59 ^a

Conclusion

Although major progresses have been made to breed drought adapted varieties, mainly by breeding for earliness, efforts need to be made to further improve drought tolerance and thereby increase and stabilize yield

in drought-prone areas. The advent of molecular breeding now gives the possibility to identify molecular markers for traits contributing to drought tolerance. The progresses made in the cereals at ICRISAT, in particular pearl millet for which the first products of marker-assisted selection have been produced, is particularly encouraging and give hope that similar success could be reached in other crops. In sorghum, hope is high that same success would occur in the next few years.

In the legumes, molecular breeding is lagging behind because chickpea and groundnut have not received as much attention as the cereals. Molecular breeding of root traits should be possible in the next few years in chickpea, once several other major QTLs for root traits are identified from the newly developed mapping populations. The situation is a little more remote in groundnut where the lack of markers still limits the possibility to map traits of interest. Recent progresses in developing synthetic amphidiploids, i.e. re-synthesized groundnut, should help circumvent the lack of genetic diversity, and could possibly reintroduce beneficial alleles in the background of cultivated groundnut. Current efforts are also being made to develop a new generation of more polymorphic markers to fully saturate the genetic maps of groundnut. There, the transgenic option might be a technical alternative, provided the choice of insert is judicious and evaluation of event made with care and sense.

In the end, results obtained in the cereals at ICRISAT give evidence that MAS breeding is possible. Yet, what appears from all crops is that the introgression of beneficial QTL is likely to always be accompanied by lots of linkage drag. The solution to this is to develop more markers in the vicinity of targeted QTLs, which is always going to be a difficult, time-consuming, and costly challenge.

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