

Recent Advances in Marker-Assisted Selection for Drought Tolerance in Pearl Millet

Rachid Serraj^{1,2}, C. Tom Hash¹, S. Masood H. Rizvi¹, Arun Sharma^{1,3},
Rattan S. Yadav⁴ and Fran R. Bidinger¹

¹International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

E-mails: C.Hash@cgiar.org, M.Rizvi@cgiar.org, F.Bidinger@cgiar.org;

²Joint FAO/IAEA Division, Wagramer Strasse 5, A-1400, Vienna, Austria. Email: r.serraj@iaea.org;

³The Pennsylvania State University, 103 Tyson Building, University Park, PA 16802-4200, USA. Email: aus9@psu.edu;

⁴Institute of Grassland & Environmental Research (IGER), Aberystwyth SY23 3EB, United Kingdom.

Email: rattan.yadav@bbsrc.ac.uk

Abstract : Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the staple cereal of the hottest, driest areas of the tropics and subtropics. Drought stress is a regular occurrence in these regions, making stress tolerance an essential attribute of new pearl millet cultivars. Recent breeding research has mapped several quantitative trait loci (QTLs) for components of grain and stover yield *per se*, as well as yield maintenance, under terminal drought stress conditions. We report here the evaluation of these QTLs as possible selection criteria for improving stress tolerance of an elite hybrid cultivar. Initial evaluations, based on hybrids made with topcross pollinators bred from lines selected directly from the mapping population, indicated an advantage to the QTL-based topcross hybrids. This advantage seemed to be related to a particular plant phenotype that was similar to that of the drought tolerant parent of the mapping population. Subsequent evaluations were based on testcross hybrids of drought tolerance QTL introgression lines in the background of the drought-sensitive parent of the mapping population, H 77/833-2. These introgression lines were bred by limited marker-assisted backcrossing of a putative major drought tolerance QTL into H 77/833-2 from the mapping population's drought tolerant parent. Several of these QTL introgression lines had a significant positive general combining ability for grain yield under terminal stress and significantly out-yielded testcross hybrids made with the original recurrent parent both in unrelieved terminal drought stress and in gradient stress evaluations.

Key words : Drought, Marker-assisted breeding, *Pennisetum glaucum*, QTL introgression lines, QTL mapping.

The general complexity of drought stress is aggravated in the semi-arid tropics and subtropics by highly unpredictable rainfall, and by high temperatures, high levels of solar radiation, and low soil fertility. The resulting large variability in the nature and occurrence of drought stress and our insufficient understanding of its complexity have made it generally difficult to identify the physiological traits required for improved crop performance under drought stress conditions. This has consequently limited the use of a trait-based approach in plant breeding to enhance crop drought tolerance. Pearl millet is generally known for its superior ability, relative to other cereals, to withstand periods of water-limited conditions and still produce biomass and grain (Bidinger and Hash, 2004). Even for this crop, however, the constant challenge is to reduce yield gaps between research plots and farmers' fields, in order to assure sustained food security for resource poor farmers. Specifically the task is two fold: (1) to identify plant responses that will minimize yield losses to the patterns of stress with the greatest effects on crop yields, or plant traits

that will allow some measure of continued plant function despite such stress, and (2) to evaluate ways of incorporating these responses/traits into new cultivars to both increase overall productivity under stress and to reduce spatial and temporal variation in productivity.

The most relevant way of addressing this challenge is through a holistic approach that integrates appropriate matching of crop phenology with the prevalent patterns of moisture availability, with physiological dissection of selected drought tolerance traits and the application of molecular genetic tools to integrate these into cultivars with high economic yield potential in the target environment, and implementation of agronomic practices that improve soil moisture utilization. This paper summarizes the recent progress made at ICRISAT-Patancheru in using new molecular tools to breed more drought tolerant experimental cultivars of pearl millet as one component of such an approach.

1. Characterization and molecular mapping of terminal drought tolerance

Genetic improvement of stress tolerance in pearl millet has concentrated on the flowering and grain-filling stages of crop growth as stress in this period has the greatest effect on grain yield (Mahalakshmi *et al.*, 1987). Statistical procedures have been developed to partition crop yield under stress into effects of stress escape, yield potential and stress response (Bidinger *et al.*, 1987), and emphasis has been on identification and selection for traits related to stress tolerance rather than yield potential or drought escape. A series of empirical selection experiments using panicle harvest index as a tolerance criterion resulted in small (5%) but significant gains in grain yield in selected materials under terminal stress (Bidinger *et al.*, 2000; unpublished data).

Emphasis in more recent research has been on the identification and evaluation of QTLs associated with grain yield and with stress tolerance under conditions of unrelieved post-flowering (*i.e.*, terminal) drought stress. Two mapping populations based on hybrid parental lines with known differential responses to terminal stress were created, genotyped and phenotyped under managed field stress conditions (Yadav *et al.*, 2002, 2004). Both exercises consistently detected a major putative QTL for grain yield under

terminal drought stress conditions (and/or the ability to maintain grain yield under such stress conditions) on pearl millet linkage group 2 (LG2), along with a small number of minor QTLs for both grain and stover yield and their components, under stress, that were identified in some but not all phenotyping environments. In most but not all cases, the favorable allele was contributed by the drought tolerant parent of the mapping population. Analysis of co-mapping of QTLs for individual yield component traits and for grain yield under stress, suggested a genetic basis for the observed association between the ability to maintain grain yield under stress and the ability to maintain both panicle harvest index (primarily grain filling) and harvest index under terminal stress, as well as confirming the benefits of drought escape achieved through early flowering (Yadav *et al.*, 2002, 2004). Our current research emphasis is on evaluation of the putative QTLs as selection criteria in the genetic improvement of drought tolerance. Results from the first mapping population, based on the cross between elite terminal drought stress-sensitive pollinator H 77/833-2 and PRLT 2/89-33, a terminal drought tolerant breeding line derived from the ICRISAT Bold Seeded Early Composite (BSEC), are described below.

Table 1. Summary of comparisons of the drought QTL-based and field performance-based pollinators. Data are means of twelve topcross hybrids made with each topcross pollinator, evaluated in non-stress, terminal stress and post-flowering line-source irrigation environments (numbers of comparisons in brackets) at ICRISAT-Patancheru, dry seasons of 2001–2003.

Moisture Environment / Crop trait	QTL topcross hybrids	Field topcross hybrids	LSD (P = 0.05)
Non-stressed (3)			
Flowering (d)	39.1	41.3	0.19
Biomass (g m ⁻²)	777	845	15.6
Harvest index (%)	49.6	45.9	0.55
Grain yield (g m ⁻²)	381	393	7.7
Terminal stress (5)			
Flowering (d)	41.4	43.5	0.15
Biomass (g m ⁻²)	581	619	11.4
Harvest index (%)	41.7	38.3	0.60
Grain yield (g m ⁻²)	245	239	5.6
Line source (8)			
Flowering (d)	35.4	38.1	0.12
Biomass (g m ⁻²)	537	562	7.9
Harvest index (%)	49.5	43.8	0.5
Grain yield (g m ⁻²)	268	255	5.1

Table 2. General combining ability (GCA) effects for grain yield (g m^{-2}) of selected nearly near-isogenic introgression lines, their recurrent parent H 77/833-2, and donor parent PRLT 2/89-33, across 12 individual environments in India, 2003–2004. S04 and S03 designate summer dry season drought nursery environments of 2004 and 2003 at ICRISAT-Patancheru; K03 and K04 designate rainfed rainy season environments of 2003 and 2004. Irrigated control = fully irrigated non-stress treatment; Late-onset stress = mild terminal drought stress treatment receiving final irrigation 1 week after flowering; Early-onset stress = severe terminal drought stress treatment receiving final irrigation 1 week prior to flowering. Patancheru 1 = ICRISAT, Patancheru (RL 24 field); Patancheru 2 = ICRISAT, Patancheru (RP 6 field); Jodhpur = CAZRI, Jodhpur, Rajasthan; Nagaur = RAU RRS, Nagaur, Rajasthan.

Environments	ICMR 01029	ICMR 01031	ICMR 01038	H 77/833-2	PRLT 2/89-33
S04Irrigated control	31.9**	12.4*	- 11.5	7.2	20.1**
S04 Late-onset stress	10.0*	13.6**	1.4	- 8.6	18.2**
S04 Early-onset stress	9.1*	- 2.3	1.7	1.7	- 7.8*
S03 Irrigated control	4.6	4.3	- 15.7*	- 6.8	26.6**
S03 Late-onset stress	3.8	- 21.0**	8.2*	- 7.9*	11.3*
S03 Early-onset stress	21.7**	- 1.5	1.2	- 17.0**	- 4.8
K03 Patancheru 1	25.7**	- 0.2	8.0	- 1.1	16.5*
K03 Patancheru 2	- 4.8	15.0**	- 13.0*	30.9**	18.7**
K03 Jodhpur	5.8	5.7	- 6.9	- 1.3	23.0*
K03 Nagaur	3.0	5.1*	- 11.0*	8.0	22.5**
K04 Patancheru 1	22.8**	17.8*	7.5	11.5	14.1
K04 Patancheru 2	21.7*	23.0*	- 25.7*	40.9**	34.6**

Significance level ** < 0.01; * < 0.05

2. Evaluation of drought tolerance QTL effects on phenotype

A simple initial evaluation of the putative drought tolerance QTL on LG2 as a selection criterion was made by comparing hybrids made with a topcross pollinator bred from progenies selected from the original mapping population for presence of the tolerant allele at the target QTL vs. hybrids made with a topcross pollinator bred from progenies selected for field performance in the field trials used to phenotype the original mapping population (Bidinger *et al.*, 2005). A set of 24 topcross hybrids (12 male-sterile lines \times 2 topcross pollinators) was evaluated in 21 field environments, which included both non-stressed treatments and drought-stressed treatments during the flowering and grain filling stages. Hybrids of the QTL-based pollinator were significantly, but modestly, higher yielding in a series of both severe and moderate terminal drought stress environments, but at the cost of a lower yield in the non-stressed evaluation environments (Table 1). This particular pattern of adaptation in the hybrids of the QTL-based pollinator was consistent with their general phenotype – earlier flowering, limited effective basal tillering, lower biomass and a higher harvest index – which resembled the phenotype of the drought tolerant parent of the original mapping population, and which appear to provide advantages under conditions of unrelieved post-flowering drought stress. The results thus confirmed the effectiveness of the putative drought tolerance QTL on LG2 as a selection criterion, but

suggested that it may enhance drought tolerance by favoring a phenotype that permits grain filling to escape from the most severe terminal stress conditions, rather than by improving drought tolerance *per se* at a more basic physiological level.

3. Field evaluation of drought tolerance QTL near-isogenic lines (NILs)

A more rigorous evaluation of the putative drought tolerance QTL is currently underway, using nearly near-isogenic versions of H 77/833-2, into which overlapping segments of pearl millet LG2 from the donor parent PRLT 2/89-33 have been introgressed by limited marker-assisted backcrossing. BC_4F_3 progenies from 19 selected BC_4F_2 plants homozygous for various portions of the LG2 target region were crossed to each of five different cytoplasmic male-sterile hybrid parental line testers in the background of 843A [the female parent of popular extra-early maturing hybrid HHB 67, which has the same H 77/833-2 used in the mapping population as its male parent (Kapoor *et al.*, 1989)], and the resulting hybrids were evaluated under a range of moisture regimes (non-stressed controls and early-onset, and late-onset terminal drought stress). The hybrids exhibited a large variation in grain and stover yield component expression, as well as yield responsiveness to the moisture regimes, but there was a consistent grain yield advantage for hybrids carrying alleles from the drought tolerant donor parent PRLT 2/89-33 in the vicinity of the target QTL. Several of the QTL introgression lines had significant positive

general combining ability (GCA) for grain yield under terminal drought stress conditions (Table 2), which was associated with a higher panicle harvest index. Interestingly this superior grain yield performance of the introgression line hybrids was often accompanied by increased biomass yields and reduced grain harvest indices instead of the reduced biomass yield and increased grain harvest index that appears to contribute to the higher grain yield potential and superior terminal drought tolerance of hybrids of donor parent PRLT 2/89-33 and the QTL-based topcross pollinator. Thus these product hybrids have potentially greater value for dual-purpose use (grain and stover), under both water-limited and non-limited conditions, than hybrids of either the donor parent or recurrent parent used in breeding their pollinators.

A line-source gradient stress experiment was conducted to further assess the performance of selected QTL-NIL pollinator lines from the general combining ability trial. The 20 trial entries were comprised of testcrosses (on four seed parents) of the two pollinators with the highest GCA values for grain yield under stress (ICMR 01029 and ICMR 01031), one pollinator with a negative GCA for grain yield under stress, and the two mapping population parental lines from which the introgression lines were bred. The line-source sprinkler irrigation system produced a perfectly linear soil moisture gradient, which was paralleled by crop performance in this trial. The results confirmed the previous findings of yield advantage of ICMR 01029 and ICMR 01031 compared to parents H 77/833-2 and PRLT 2/89-33. Leaf gas exchange was measured with a LCA 4 leaf gas analyzer (ADC Bio-Scientific Ltd. UK), in three different moisture regimes (rows 2, 10 and 18 of the 20-row soil moisture gradient, which corresponded to non-stressed control, intermediate and severe drought stress environments). In each of these three moisture regimes photosynthetic rates were significantly correlated with grain yield ($r^2=0.80$), suggesting the existence of genotypic differences in the response of leaf gas exchange to soil moisture availability, or differences in the ability of the genotypes to access soil moisture that in turn contribute to the observed differences in leaf gas exchange and grain yield, which could help us to better understand the mechanisms of plant water relations in these genotypes and to devise more effective screening protocols for selection of drought tolerant hybrid parental lines, hybrids and

open-pollinated varieties in pearl millet.

Acknowledgements

This document is an output from projects funded by the Plant Sciences Research Programme of the UK Department for International Development (DFID), administered by the Centre for Arid Zone Studies (CAZS) for the benefit of developing countries. The views expressed are those of the authors and not necessarily those of DFID.

References

- Bidinger, F.R., Serraj, R., Rizvi, S.M.H., Howarth, C., Yadav, R.S., Hash, C.T. 2005. Field evaluation of drought tolerance QTL effects on phenotype and adaptation in pearl millet [*Pennisetum glaucum* (L.) R. Br.] topcross hybrids. *Field Crops Res* In press.
- Bidinger, F.R., Hash, C.T. 2004. Pearl millet. In 'Physiology and Biotechnology Integration for Plant Breeding' (Eds HT Nguyen, A Blum). : 225-270. (Marcel Dekker: New York)
- Bidinger, F.R., Chandra, S., Mahalakshmi, V. 2000. Genetic improvement of tolerance to terminal drought stress in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]. In 'Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments'. A Strategic Planning Workshop held at CIMMYT, El Batan, Mexico, 21-25 June 1999. (Eds JM Ribaut, D Poland) : 59-63. (International Maize and Wheat Improvement Center: Mexico).
- Bidinger, F.R., Mahalakshmi, V., Durga Prasada Rao, G. 1987. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. II. Estimation of genotype response to stress. *Australian Journal of Agricultural Research* 38 : 49-59.
- Kapoor, R.L., Kakkar, P.S., Khairwal, I.S., Baniwal, C.R., Nijhawan, D.C., Yadav, H.P. 1989. Bajra hybrid HHB 67—a major break through. *Haryana Farming* 18 : 17-21.
- Mahalakshmi, V., Bidinger, F.R., Raju, D.S. 1987. Effects of timing of stress in pearl millet. [*Pennisetum americanum* (L.) Leeke]. *Field Crops Res* 15 : 327-339.
- Yadav, R.S., Hash, C.T., Bidinger, F.R., Cavan, G.P., Howarth, C.J. 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. *Theoretical and Applied Genetics* 104 : 67-83.
- Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M., Howarth, C.J. 2004. Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and testers background. *Euphytica* 106 : 265-277.