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Pearl Millet Molecular Marker Research

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(summarised by Clare Stirling)

ABSTRACT

Ten years ago, when most of the world still viewed molecular marker technology as an untested luxury, a collaborative John Innes Centre-ICRISAT project was funded by the DFID Plant Sciences Research Programme to develop molecular techniques for the breeding of crops for developing countries.

The first major milestone was achieved in 1993 with the creation of the first genetic map of the pearl millet genome. Now more than 600 molecular markers have been created, detailed linkage maps produced and quantitative trait loci (QTLs) linked to the map.

MilletGenes, the first UK plant genome database, was initiated with DFID funding and has now been incorporated into the BBSRC-funded UK CropNET Programme. The database is publicly available and is being used by ICRISAT and NARS scientists to assist their MAS (marker-assisted selection) breeding programmes.

The first pearl millet marker-assisted backcrossing program to transfer downy mildew resistances and drought tolerance genes into commercial hybrid parental lines in India has been completed.

Based on past experience a major pearl millet downy mildew epidemic could result in a grain loss worth £7.8 million.

About £2.6 million worth of additional grain yield could be expected annually from a 10% increase in grain yield of the best new versions of the popular hybrid HHB 67.

INTRODUCTION

Grain of pearl millet [*Pennisetum glaucum* (L.) R. Br.] provides the staple food of millions of poor people in Africa and South Asia (FAO and ICRISAT, 1996). In addition it provides a critically important source of fodder for the livestock of these people. Pearl millet is primarily grown in some of the most hostile rainfed agricultural environments in the world – where it is too hot and/or too dry for reliable cultivation of any staple food crop. Because of these stressful production environments, the grain and fodder yields of pearl millet are low compared to other crops grown in more favourable regions. Despite its importance to the people and livestock of arid and semi-arid Africa and South Asia, pearl millet has received very little research investment compared to higher-value crops of more favourable agricultural environments. None-the-less, modest research investments have been made to develop the molecular genetic tools needed to support conventional plant breeding of pearl millet in developing countries. Much of this research investment has been made by ICRISAT and a team of UK-based collaborators supported by the Plant Sciences Research Programme.

Improving pro-poor public goods

Pearl millet seed is tiny and because of this, farmers need only a very small amount to sow their crop, for example one kilogram typically has over 100,000 seeds. This means that although hybrid seed is relatively more expensive than local seed, it still presents a profitable option, even for poor farmers. This is reflected by the fact that single-cross hybrids are now the most widely grown pearl millet cultivars in India and grown by all categories of farmers. There are many pearl millet hybrids from private-sector programmes, but public sector-bred hybrids, such as HHB 67 from Haryana Agricultural University, is one of the most extensively grown. This particular hybrid was released in 1989 and has many traits that farmers appreciate, including early maturity that allows it to escape end-of-season droughts.

Pearl millet farmers in India have no public-sector alternative to HHB 67 in this maturity class, and all private-sector hybrids mature later than HHB 67. However, its popularity makes it vulnerable to an epidemic of downy mildew. In the past, every highly popular single-cross pearl millet hybrid in India has ultimately succumbed to this disease. When this happens, farmers not only suffer the direct losses caused by the epidemic, but they lose the management options associated with growing their most preferred hybrid. The hybrid seed industry also faces losses as it takes time to gear up seed production of the next best alternatives, and for farmers to then identify which of these best match their needs.

RESULTS

Creation of the first genetic map of pearl millet

A major milestone was the creation of the first genetic map of the pearl millet genome in 1993. This map provided the foundations for studying the genetic basis of drought tolerance and downy mildew resistance. The outputs of this research have been stored in the MilletGenes database, which is based at the John Innes Centre (JIC). MilletGenes was initiated with DFID-PSP funding and has now been incorporated into the BBSRC-funded UK CropNet Programme. The database contains information on probes, DNA polymorphisms, gel images, genetic maps and QTL data. ICRISAT and National Agricultural Research Systems (NARS) scientists are now using this database to assist their MAS breeding programmes.

Locating the position of markers for downy mildew resistance and drought tolerance on the linkage map

Quantitative trait loci (QTLs) have been successfully mapped for both downy mildew resistance and drought tolerance of grain yield.

Downy mildew: Pathogen populations from western and central Africa and Asia have been studied and no single QTL has been found to be effective against all populations. This indicates that pathotype-strain specificity is a major mechanism of downy mildew resistance. Interestingly, not all of the QTLs resistance were inherited from 'resistant' parents – quite often the susceptible parent possessed QTLs that contributed to resistance. Resistance at most QTLs was inherited in a dominant or over-dominant manner so heterozygotes were as resistant, or more resistant, than genotypes homozygous for the allele conferring resistance.

Drought tolerance: Mapping of drought tolerance QTLs in pearl millet began as a spin-off from a project primarily aimed at identifying QTLs for high-temperature tolerance of seedlings. QTLs have now been successfully mapped for a wide range of phenotypic traits associated with drought tolerance (Fig. 1).

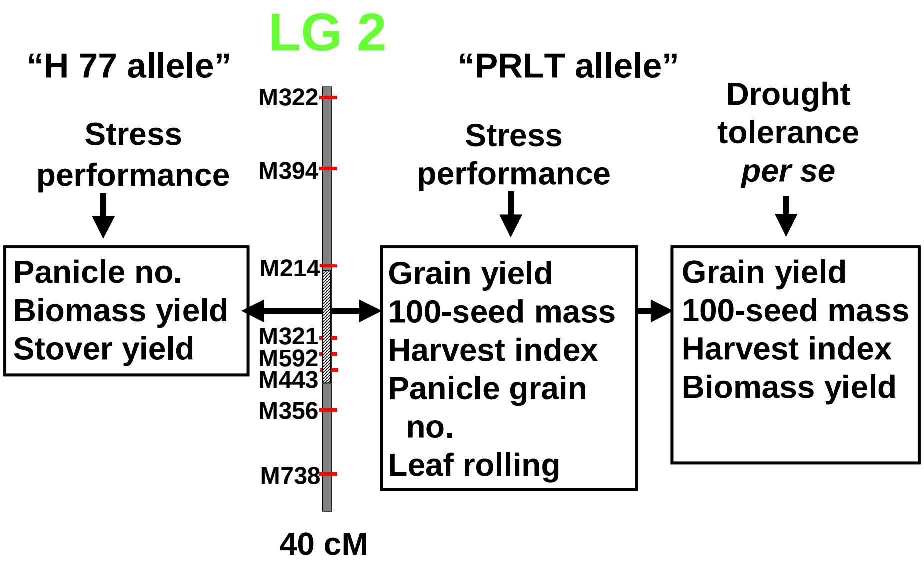

Figure 1: **QTLs for pearl millet leaf rolling, biomass yield, straw dry yield, panicle number, panicle grain number, 1000-seed mass, harvest index and grain yield in three drought experiments for a portion of linkage group (LG) 2.**

Development of downy mildew-resistant and drought-tolerant hybrids

Downy mildew: Marker-assisted backcross transfer of mapped downy mildew resistance QTLs from 'resistant' sources to the genetic background of an economically important hybrid seed parent maintainer line, 843B and from source ICMP 451 to another economically important hybrid pollinator line H77/833-2 has been achieved. The first homozygous products of these marker-assisted breeding programmes have now been developed and tested.

IMPROVED DOWNY MILDEW RESISTANT HYBRIDS

Resistance QTLs have been successfully transferred into elite hybrid lines such as HHB 67, a hybrid widely cultivated in India. Promising improved versions of this hybrid have now been produced and are currently being evaluated.

New Hybrid	Pedigree		
ICMH 01102	843A	x	ICMR 01007
ICMH 01122	ICMA 99022	x	ICMR 01007
ICMH 01144	ICMA 99022	x	ICMR 01004

control, conventional backcross, marker-assisted backcross

Drought: Marker-assisted selection has proven a successful tool in the improvement of drought tolerance of pearl millet, a trait notoriously difficult to breed for using conventional methods. Using marker-assisted selection, parental lines have been developed for the creation of improved hybrids and this work has advanced to the phenotypic evaluation of advanced marker-assisted backcross (MABC) products. Initial data indicates that grain and biomass yield of these MABC products are superior to controls across a range of moisture regimes. Under severe early-onset terminal drought stress, the best hybrids of the drought QTL introgression line ICMR 01029 have higher grain and stover yields than the original HHB 67 (Fig. 2).

Additional Outputs

Screening data from the work on drought tolerance and downy mildew resistance has been used to examine the potential of mapping QTLs for components of grain and stover yield. Marker-assisted backcrossing for target QTLs has produced unexpected benefits. For example, a strong relationship has been found between QTLs for flowering date and stover yield. From a breeding point of view, the most interesting stover yield QTL detected so far is that from 863B in linkage group 3, which segregated independently from flowering time QTLs. This offers the potential to improve stover yield potential of some hybrids, without delaying their maturity, by marker-assisted backcross transfer of part of the linkage group 3 from 863B into the genetic background of 841B. The male-sterile line counterpart of this new version of 841B could then be used to produce higher stover yield versions of the hybrids currently produced on 841A. Another trait that has been targeted for QTL mapping and MAS is improved ruminant nutritional quality of the stover.

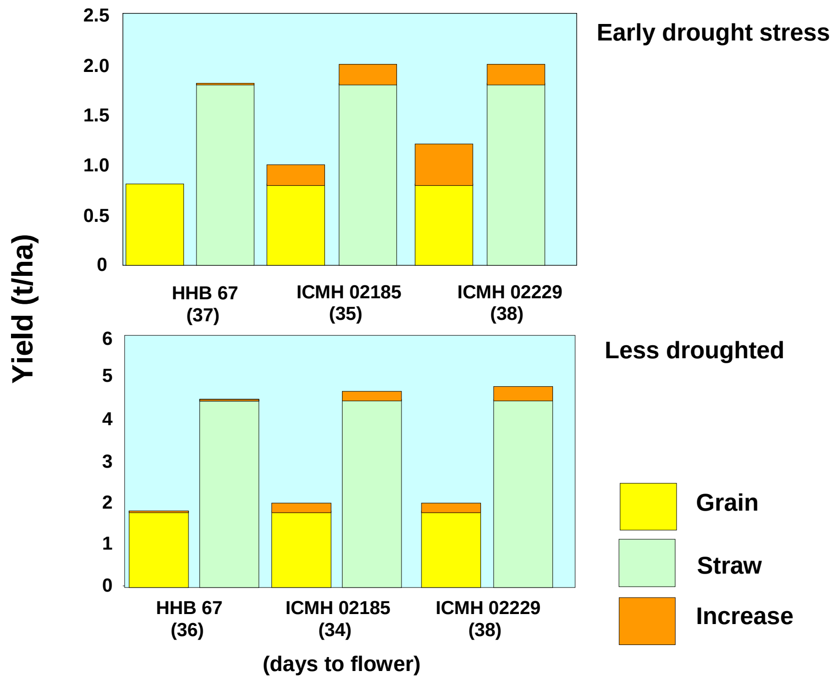

***Figure 2*: Summary of the performance of two most advanced marker-assisted hybrids (ICMH 02185 and ICMH 02229) relative to the control (HHB 67) under two moisture regimes in field trials conducted at ICRISAT, summer 2002.**

UNPREDICTED OUTPUTS OF RESEARCH

Additional improvements to the new hybrids include:

- Improved grain yield - new versions of the hybrid HHB 67 have been shown to be superior for non-target traits such as yield. Rates of genetic gain of 3.5% per annum have been found for non-target traits of grain yield.
- Improved stover yield – via increased plant height.
- Improved ruminant nutritional quality of stover – via better foliar disease resistance.

Financial Impact

HBB 67 is widely grown by resource-poor farmers in northern India, particularly in western Rajasthan and Haryana where it covers ca. half a million ha. In recent years, the original hybrid has shown signs of susceptibility to a new race of downy mildew pathogen and the usual course of events for a single cross hybrid, such as this, is that in the following year the disease is more widespread and by the next year there will be an epidemic that causes severe losses.

Impact from downy mildew resistance
Past experience suggests that in the first year of a major pearl millet downy mildew epidemic (which occurs before farmers can adopt replacement cultivars), 30% or more of the entire grain harvest on the newly susceptible hybrid is lost. For HHB 67 this would amount to a grain yield loss in the first year of the epidemic worth at least £7.8 million (assuming 30% of the grain harvest from 550,000 ha yielding 700 kg/ha and grain price of Rs 5/kg).

Impact from yield increases
About £2.6 million worth of additional grain yield could be expected annually from a 10% increase in grain yield of the best new versions of HHB 67 (the mininum expected from the new downy mildew resistant hybrid showing on-station advantages of about 30%).

Combined impact
Assuming that we should soon be able to combine the QTLs for improved downy mildew resistance and drought tolerance in a single pollinator line, the additional yield in years of drought stress would be even more valuable as both grain and straw prices are higher.