Contiguous segment substitution lines: New tool for elite pearl millet hybrid parental lines enhancement

Final Technical Report
Project Number: R7382

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June 2002

Acronyms

AICPMIP	All-India Coordinated Pearl Millet Improvement Project
ARS	Agricultural Research Station
CAZRI	Central Arid Zone Research Institute
CAZS	Centre for Arid Zone Studies
CCSHAU	Chaudhary Chauhan Singh Haryana Agricultural University
CMS	Cytoplasmic male sterility
DM	Downy mildew
DMI	Downy mildew incidence
DMR	Downy mildew resistance
HHB	Haryana Hybrid *Bajra*
IARI	Indian Agricultural Research Institute
ICMA	pearl millet male-sterile line (A-line) bred at ICRISAT-Patancheru
ICMB	pearl millet male-sterile maintainer line (B-line) bred at ICRISAT-Patancheru
ICMP	pearl millet pollinator line (R-line) bred at ICRISAT-Patancheru
ICMR	pearl millet male-fertility restorer line (R-line) bred at ICRISAT-Patancheru
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LG	Linkage group
MABC	Marker-Assisted Backcrossing
MAS	Marker-Assisted Selection
QTL	Quantitative Trait Locus
RAU	Rajasthan Agricultural University
RR	Rust resistance
RRS	Regional Research Station
TNAU	Tamil Nadu Agricultural University

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Executive Summary: This project bred HHB 67-like hybrid combinations of improved versions of the parental lines of popular elite pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrid HHB 67 that not only have superior host-plant resistance to pearl millet downy mildew disease (the trait originally targeted in the marker-assisted breeding program implemented in this project) caused by the oomycetic plant pathogen *Sclerospora graminicola* (Sacc.) J. Schröt., but that also have improved resistance to rust caused by the unrelated basidiomycetic plant pathogen *Puccinia substriata* Ell. & Barth. var. *indica* Ramachar & Cumm., and 25-30% higher mean grain yield than the original hybrid. This has been achieved without altering hybrid growth duration by more than a day or so. Thus these new versions of hybrid HHB 67 still well match the demands of farmers' producing a rainy season pearl millet grain and stover crop in dryland double-cropping systems on over 400,000 ha of marginal semi-arid lands in Rajasthan and Haryana. This surprisingly positive achievement is equivalent to the grain yield gains expected from a conventional hybrid breeding program of circa 10 years, and was accomplished despite unanticipated difficulties in generating the planned number of linkage group and segmental substitution lines having genomic material from ICMP 85410 introgressed into the genetic background of elite dwarf seed parent maintainer line 843B, and genomic material from 863B introgressed into the genetic background of elite tall seed parent maintainer line 841B. These difficulties occurred while trying to convert the marker-assisted breeding program in this project from more costly, slow, and tedious, hybridization-based, ^{32}P-labelled restriction fragment length polymorphism (RFLP) markers to less expensive, more rapid, polymerase chain reaction (PCR)-based simple sequence repeat (SSR) and sequence-tagged site (STS) markers that can be visualized by silver staining. Unfortunately, the levels of genome coverage for and marker polymorphism between the donor parents (ICMP 85410 and 863B) and recurrent parents (843B and 841B), was markedly less for the PCR-based markers than for the RFLP markers that we had been using previously. Hence we had to continue making use of the slower, more costly RFLP markers and this reduced the total number of plants that could be genotyped and backcrossing generations that could be completed during the course of this project. Despite these difficulties, we were able to confirm (by marker-assisted backcross transfer followed by disease reaction assessment of the substitution lines produced) the occurrence of previously mapped downy mildew resistance QTLs on pearl millet linkage group 1 (LG1) and LG4 of ICMP 451-P6; LG1, LG4, LG6, and LG7 of ICMP 85410, and LG1 of P7-3. We also identified the presence of a previously unmapped downy mildew resistance QTL on LG5 of 843B.

Two hybrids initially tested in this project during the rainy season of 2001 have been selected by our collaborators at CCS Haryana Agricultural University for entry into national trials during the rainy season of 2002. Results from evaluations in these national trials, combined with those from more extensive on-station and on-farm trials in Haryana that will be conducted simultaneously, are expected to be used to support a state-release proposal for one or more of the improved versions of HHB 67 early in 2003. With this substantial quantities of Certified Seed of the new versions of HHB 67 should reach farmers by the rainy season of 2004, in time to replace the original HHB 67 before it succumbs to a downy mildew epidemic. If successful, as it should be, this will be the first time that a farmer-accepted replacement for a widely popular pearl millet hybrid has been adopted before its predecessor succumbed to an epidemic. Direct losses to pearl millet-producing farmers in India due to a major downy mildew epidemic on this popular hybrid, which can be avoided if

the improved versions of HHB 67 reach farmers in time, would be in the range of £3,300,000[1].

1 Background

Pearl millet [*Pennisetum glaucum* (L.) R. Brown] is the staple food and fodder crop of millions of poor rural families living in the hottest, driest dry-land agricultural environments of Asia and Africa. Though grain and stover of this crop are not commercially important commodities, as most are consumed in the homesteads where they are produced, crop losses due to biotic and abiotic stresses are economically important, and increased yield and yield stability of pearl millet would contribute to improving standards of living for millions of poor people in these harsh production regions. For example, a 10% gain or loss in pearl millet production in India in one year represents about £20,000,000[2].

Although pearl millet in India is a crop of the rural poor in the harshest agricultural environments in that country, F_1 hybrid seed is used to sow nearly 70% of the 10 m ha on which this crop is grown because the potential yield improvements obtainable from such hybrids more than pay for seed costs and other risks associated with hybrid cultivation—and the amount of money required to purchase the seed to sow an average holding is small (e.g., £1 to £5 for seed to sow 1 ha of the cheapest to the most expensive hybrids commercially available). Unfortunately, although pearl millet hybrids often give better grain yields than local open-pollinated cultivars, the genetically uniform single-cross hybrids currently available are more vulnerable to epidemics of pearl millet downy mildew, caused by *Sclerospora graminicola* (Sacc.) J. Schröt. Such epidemics constitute the major risk to cultivation of well-adapted pearl millet hybrids. These risks can be reduced by effective crop improvement research. Losses in individual fields can reach nearly 100%, and are estimated to average 14% across 10 m ha in India. Further, because of the narrow range of closely related parental lines that have historically been used in breeding pearl millet hybrids in India (Hash, 1997), when disease resistance of one hybrid is overcome by rapidly evolving pathogen populations, that of a range of other hybrids—generally those having a common parental line—soon follow. This results in rapid cultivar turnover—most of which is driven by disease pressure rather than yield or quality improvements. Pearl millet consumers, producers, and all those involved in the seed trade, lose out.

This project aimed to improve the efficiency and effectiveness of breeding programmes targeting improvement of hybrid pearl millet grain and stover yield and yield stability across dry-land production environments in India, by increasing the probability that seed parents evaluated are genetically distinct yet produce hybrids with good local adaptation. This project sought to reduce the risk of downy mildew epidemics on pearl millet hybrids in India and thereby contribute to improved stability of grain and stover production in the arid and semi-arid tracts of that country where millions of the world's poorest farm families make their living from dry-land agriculture. Further, this project aimed to develop the genetic materials necessary to extend these findings to African pearl millet production regions, where

[1] Average pearl millet grain yield in India of 700 kg/ha; conservative estimate of grain market price is Rs2/kg; circa 400,000 ha of HHB 67 were grown in Haryana in 2001, with an additional circa 150,000 ha in Rajasthan; downy mildew yield losses typically reach 30-40% in an epidemic; therefore 30% × 700 kg/ha × Rs2/kg = Rs420/ha = £6/ha; 0.55 m ha of pearl millet in India × £6/ha = £3.3 m.

[2] 10% of average pearl millet grain yield in India of 700 kg/ha = 70 kg/ha; conservative estimate of grain market price is Rs2/kg; therefore, 70 kg/ha × Rs2/kg = Rs140/ha = £2/ha; 10 m ha of pearl millet in India × £2/ha = £20 m.

limited soil phosphorus availability is often a major abiotic constraint, in a subsequent project.

How demand for the project for the project was identified: Following downy mildew epidemics on it in Maharashtra in 1994 and 1995, a widely-cultivated pearl millet hybrid (MLBH 104), thought to be produced on male-sterile line 843A[3], was withdrawn from the market by the private company that developed, multiplied and marketed it. A similar early-maturing hybrid (HHB 67, a public-sector release produced on 843A) has become very popular throughout northwestern India and is now also vulnerable to such an epidemic. Such disease epidemics had occurred time and again on pearl millet hybrids in India over the past 30 years, and constitute the major risk associated with cultivation of well-adapted pearl millets that can be reduced by effective crop improvement research. The effect of hybrid cultivar withdrawals due to disease susceptibility—which in each case have occurred before an acceptable, agronomically superior cultivar of similar maturity was available to farmers—is large economic losses to pearl millet consumers, pearl millet producers, and the seed industry in the affected region.

Medium-term planning at ICRISAT (Kelley et al., 1995) identified downy mildew as a major researchable constraint to pearl millet productivity, second in priority only to drought. ICRISAT placed great importance on this project and entered into explicit collaborative research arrangements with public sector components of the Indian national pearl millet improvement programme that will facilitate achieving early impact of this research in farmers' fields.

2 Project Objectives

The objectives of this project were:

- to test line and hybrid performance of segmental substitution lines bred in the genetic backgrounds of elite pearl millet hybrid parental lines by conventional and marker-assisted backcrossing,
- to produce additional segmental substitution line sets in the genetic backgrounds of recurrent parents 843B and 841B
- introduce promising hybrid parental lines from African pearl millet breeding programmes, initiate backcrossing programmes to these with current mapping population parental lines as trait donors, and initiate a mapping population based on a cross between pearl millet seed parent maintainer lines of interest to African pearl millet breeding programmes
- generate marker finger-printing data for pearl millet seed parent maintainer lines from African and Indian pearl millet breeding programmes, and
- evaluate the introduced African seed parent lines, all mapping population parental lines, and 843B-background segmental substitution lines in for phosphorus uptake ability.

[3] Private-sector companies do not disclose the parentage of their proprietary hybrids, but characteristics of 843A were readily identifiable in the seed parent in hybrid seed multiplication plots of MLBH 104.

3 Research Activities

3.1 Test substitution lines

The inbred substitution lines developed in this project, and their recurrent parents, were evaluated for agronomic performance in a replicated field trial conducted at Patancheru during the rainy season of 2001. In addition, these lines were evaluated for downy mildew reaction in the downy mildew nursery at Patancheru as well as in three time-replicated greenhouse downy mildew screens against the most virulent pathogen populations of Indian origin that are currently maintained by ICRISAT plant pathologists.

3.2 Complete contiguous segment substitution and linkage group substitution line sets (ICMP 85410 segments in 843B background)

RFLP-based marker-assisted selection was conducted in selfed and backcrossed progenies based on recurrent parent 843B and donor parent ICMP 85410 in an attempt to develop a full set of linkage group and contiguous segment substitution lines based on these two elite d_2 dwarf hybrid parental lines. A parallel program to develop A_1-cytoplasm CMS line counterparts for each of the substitution lines by repeated backcrossing used 843A, the male-sterile counterpart of recurrent parent 843B, as the source of male-sterile cytoplasm. Unfortunately, attempts to completely move the marker-data generation for this extensive marker-assisted backcrossing program from the slow and tedious hybridization-based RFLP markers to faster PCR-based STS and SSR markers were not successful. This was because of the reduction in polymorphism for the STS markers relative to the RFLP markers from which they were derived, and the limited number and restricted genome coverage of SSR markers for pearl millet that were developed at JIC in the course of a parallel project. The SSR markers were hence less readily useable for this purpose than was anticipated during the design phase of this project and it has taken longer than originally planned to produce the required marker data for each generation of the marker-assisted backcrossing. Thus fewer generations have been completed, and a reasonably complete set of linkage group or contiguous segment substitution lines for pearl millet is still being developed.

3.3 Test performance of hybrids produced with substitution lines

Hybrids of the inbred substitution lines developed in this project, and their recurrent parents, were evaluated for agronomic performance in replicated field trials conducted in ten environments at Patancheru and in northwestern India during the rainy season of 2001. In addition, these hybrids were evaluated for downy mildew reaction in the downy mildew nursery at Patancheru as well as in three time-replicated greenhouse downy mildew screens against the most virulent pathogen populations of Indian origin that are currently maintained by ICRISAT plant pathologists.

The experiments were a line × tester study conducted in the genetic background of elite hybrid HHB 67 and based on 22 versions of elite seed parent 843A each crossed with three versions of elite pollinator H 77/833-2 (Table 1). At each site, experimental units were sown in 3-4 replications of 2-row plots of 4-m length arranged in alpha-lattice designs appropriate for the large number of trial entries. Agronomic performance data sets were received from seven of the ten environments in which field trials were conducted (the remaining three trials failed due to poor seedling emergence or severe mid-season drought stress), and six of these data sets proved useful (rain at flowering had badly affected seed set in the seventh data set).

The downy mildew screens of these testcross hybrids broadly supported the findings from screens of their parental lines.

Table 1. Hybrid parental lines evaluated in a line × tester study conducted in the genetic background of elite hybrid HHB 67, rainy season 2001.

Number of lines	**Names of parental lines**	**Origin of male-fertile lines**
1	843A	Recurrent parent 843B, which was reselected at ICRISAT from material originally developed at Kansas State University; seed maintainer parent of HHB 67
1	ICMA 97444	DM-resistant pedigree-bred derivative of 843B from ICRISAT
3	ICMA 01027 ICMA 01029 ICMA 01030	Marker-assisted backcrossing at ICRISAT of DM resistance from P7-3 into 843B background
3	ICMA 01026 ICMA 01031 ICMA 01032	Marker-assisted backcrossing at ICRISAT of DM resistance and random segments from ICMP 85410 into 843B background
14	ICMA 98004 ICMA 99011 ICMA 99012 ICMA 99013 ICMA 99014 ICMA 99015 ICMA 99016 ICMA 99017 ICMA 99018 ICMA 99019 ICMA 99020 ICMA 99021 ICMA 99022 ICMA 99023	Conventional backcrossing at ICRISAT of DM resistance from ICML 22 into 843B background
1	H 77/833-2	CCSHAU-bred pollinator line; male parent of HHB 67
2	ICMR 01004 ICMR 01007	Marker-assisted backcrossing at ICRISAT of DM resistance from ICMP 451-P6 into H 77/833-2 background

Additional sets of hybrids based on substitution lines developed in the course of this project were evaluated for agronomic performance in the summer drought nursery at Patancheru during the summer seasons of 2001 and 2002. These trials consisted of 3-5 replications of 2-row plots of 4-m length sown in each of three moisture regimes: a fully irrigated control, a late-onset (grain-filling) terminal drought stress treatment, and an early-onset (flowering) terminal drought stress treatment.

3.4 Produce substitution lines (863B segments in 841B background)

RFLP-based marker-assisted selection was conducted in selfed and backcrossed progenies based on recurrent parent 841B and donor parent 863B in an attempt to develop a full set of linkage group and contiguous segment substitution lines based on these two elite D_2 tall hybrid seed parent maintainer lines. Unfortunately, attempts to completely move the marker-data generation for this extensive marker-assisted backcrossing program from the slow and tedious hybridization-based RFLP markers to faster PCR-based STS and SSR markers were not successful for the same reasons given in 3.2 above. Hence, fewer generations have been completed than originally planned and a reasonably complete set of linkage group or contiguous segment substitution lines derived from this program is still several years from completion.

3.5 Identify promising hybrid parental lines for Africa, introducing these to India, make initial crosses and advancing generations without MAS

Ten elite seed parent maintainer lines were introduced to Patancheru from each of ICRISAT's pearl millet breeding programmes in Niger and Zimbabwe. After passing through Post-Entry Quarantine Isolation Area grow-outs in the post-rainy season of 1999/2000, these 20 lines were grown in rainy season breeding nurseries at Patancheru in 2000 and 2001, where they were first crossed to several sources of downy mildew resistance QTLs effective against pathogen populations of West African origin, and the resulting F_1 hybrids then backcrossed to their respective elite parents. In addition, several crosses were made between pairs of phenotypically diverse introduced seed parent maintainer lines, and these were advanced to F_2 seed for possible future use in developing mapping populations involving agronomically elite lines of importance to African pearl millet hybrid breeding programmes. One of these mapping populations (actually two populations of 250 segregants each, based on a pair of plant × plant crosses between ICMB 89111 and ICMB 90111 = ICMP 423) has been advanced to F_4 self bulks, DNA samples prepared, and SSR marker genotyping initiated.

3.6 Fingerprinting of elite set of pearl millet seed parent maintainer lines from Africa and India

Nearly 200 inbred lines, including parental lines of all available pearl millet mapping populations, the 20 introduced seed parent maintainer lines referred to above, all named maintainers of male-sterile lines bred in the pearl millet breeding program at ICRISAT-Patancheru between 1981 and 2001, all finished products of conventional and marker-assisted backcrossing to improve downy mildew resistance of elite maintainer line 843B, and all finished products of marker-assisted backcrossing to improve downy mildew resistance, stover yield potential, and/or terminal drought tolerance of elite pollinator line H 77/833-2, are being genotyped at 28 polymorphic SSR loci that have mapped across the seven pearl millet linkage groups. Cluster analysis of the resulting data set and comparisons of graphical genotypes of these lines, will provide information on the relationships between this large set of elite hybrid parental lines, permitting a more rational choice of parents of crosses to be made for hybrid parental line improvement. In addition, the marker data set will provide information on possible introgression of donor segments in linkage groups that were not targeted in the marker-assisted backcrossing programmes for H 77/833-2, and on segments introgressed into the background of 843B in ICRISAT's conventional pedigree and backcrossing programmes. Finally, the marker polymorphism data will provide information necessary for choosing which of several available BC_2F_1 progeny sets based on mapping population donor parents and

introduced seed parent maintainer lines of African origin are most suitable for advancement in a marker-assisted backcrossing activity planned for R7379.

3.7 Evaluate the African seed parent lines, all mapping population parental lines, and 843B-background segmental substitution lines in for phosphorus uptake ability

This activity has proven more difficult than to implement expected because of staffing changes within ICRISAT (soil chemist J Adu-Gyamfi was transferred from Patancheru, India to Bamako, Mali in 2000, and then to Ibadan, Nigeria in 2001; cereals physiologist FR Bidinger retired with effect from June 2000 and his position has been merged with that of departing legume physiologist C Johansen, and which has in turn been filled by R Serraj), and difficulties in establishing a repeatable greenhouse screen to assess differences in phosphorus uptake ability between the inbred pearl millet lines originally proposed in this study. As a consequence, we have produced testcross hybrid seed of the available mapping population parental lines and other breeding materials to be screened for this trait, and are making arrangements for these to be screened under phosphorus-deficient field conditions in Nigeria for the coming rainy season of 2002.

4 Outputs

4.1 Field verification of DM resistant versions of HHB 67

The downy mildew resistant versions of HHB 67 (=843A × H 77/833-2) that are bred by crossing the A-lines corresponding to versions of 843B with genomic segments introgressed from ICML 22, ICMP 85410, or P7-3 with improved pollinators bred by introgressing genomic segments from ICMP 451-P6 have performed remarkably well. First trials of hybrids produced with the improved pollinators were conducted in the rainy season of 2000 at Patancheru and are reported in the PhD thesis of Arun Sharma (Sharma, 2001). These were followed up by a 66-entry line × tester study that was conducted successfully in six environments during the rainy season of 2001. Results from this multi-locational field trial are summarized in Annex 1 and Annex 2.

Briefly, the new versions of HHB 67 are remarkably similar in plant architecture to the original, with essentially similar phenology and yield potential, but with markedly improved downy mildew resistance where previously detected downy mildew resistance QTLs have been introgressed into either or both of the parents of these new versions of the hybrid. Surprisingly, nearly all of the new versions of the hybrid had mean grain yield performances across the six reporting locations that were as good as or significantly better than that of the original HHB 67 (see Annex 1). In some cases the grain yield superiority of the new versions over the original HHB 67 averaged 30% across test locations, and were accompanied by markedly improved downy mildew resistance, improved plant height (likely to be associated with improved stover yield), and agronomically trivial changes in flowering time and effective tiller number.

None of the new versions of HHB 67 field tested in 2001 were sufficiently similar to the original HHB 67 to allow a “simple” swapping of nucleus and breeder seedlots used in the hybrid seed multiplication chain. However, several hybrid combinations very similar to HHB 67 will be field tested in 2002. Two of the new versions of HHB 67 that were evaluated multi-locationally in 2001 will be entered in national trials in 2002, along with several more in state trials, with the intent of generating data to support the 2003 state release in Haryana of one or more of these new versions as a replacement for the original HHB 67 that appears finally to be succumbing to downy mildew.

4.2 Incomplete sets of pearl millet contiguous segment substitution lines and linkage group substitution lines for 843B, and partial sets for 842B and H 77/833-2.

A list of "finished" backcross-derived substitution lines based on donor parents ICML 22 = 7042DMR, ICMP 85410 and P7-3 and recurrent parent 843B, donor parent ICMP 85410 and recurrent parent 842B, and, donor parents ICMP 451-P6 and PRLT 2/89-33 and recurrent parent H 77/833-2, that have been developed in the course of this project, its immediate predecessor, and an associated pearl millet drought tolerance project, are listed in Annex 3. These sets of substitution lines are smaller, and the set of ICMP 85410 substitutions in 843B background is considerably less complete, than originally targeted in this project, due to difficulties in making the transition from hybridization-based RFLP markers to PCR-based STS and SSR markers due to a combination of inadequate polymorphism detectable by the STS markers and inadequate genome coverage for polymorphic SSR markers, as described in 3.2 above.

4.3 Field and greenhouse testing of the finished substitution lines under biotic and abiotic stress conditions

Annex 4 provides a summary of three greenhouse downy mildew screens of finished pearl millet substitution lines, their parents, and standard controls that were conducted at ICRISAT-Patancheru against highly virulent *Sclerospora graminicola* populations of Indian origin during 2001. In addition to these three highly heritable greenhouse screens, these substitution lines and the line × tester set described in 4.1 above were evaluated for disease reaction in the field downy mildew nursery at ICRISAT-Patancheru during the 2001 rainy season. The results of the disease screens (field and greenhouse) of the line × tester set, and the field screen of the substitution lines themselves, were largely in agreement with those of the greenhouse screens for which results are presented: downy mildew resistance reaction was improved relative to the recurrent parent whenever a previously mapped downy mildew resistance QTL was introgressed into the recurrent parent, but in at least one case backcross-derived substitution lines with poorer resistance than their recurrent parent were identified. In this case (ICML 98007 and ICML 98009), it appears that the introgressed segment(s) replaced a previously unmapped downy mildew resistance gene on LG5 of recurrent parent 843B with a less effective allele from donor ICMP 85410. Thus we have identified a genomic region on LG5 of recurrent parent 843B that is better left undisturbed during step-wise improvement of this line.

Greenhouse screens of the full set of introgression lines (donors ICMP 451-P6 and PRLT 2/89-33) in the genetic background of H 77/833-2 are currently underway at ICRISAT-Patancheru, and will be completed before the end of the project.

Field screening drought sensitivity of two sets of substitution line sets in hybrid form was initiated in the summer drought nursery at ICRISAT-Patancheru in 2001. Only small differences in sensitivity to terminal drought stress were detected between the various substitution lines in the genetic background of 843B in these preliminary trials, and these differences could be fully attributed to flowering time differences of the lines and their hybrids. However, a remarkably large difference in terminal drought tolerance of hybrids produced with testers H 77/833-2 (more tolerant) and RIB 335/74 (more sensitive) was noted. This means that we have identified an agronomically elite drought-sensitive pollinator (RIB 335/74) that could be used as a mapping population parent in any future QTL mapping of pearl millet terminal drought tolerance.

A small number of hybrid combinations of the 843B-like and H 77/833-2-like substitution lines were evaluated for drought tolerance differences in a rainout shelter

experiment conducted at ICRISAT-Patancheru during the rainy season of 2001. Again, no substantial differences in drought tolerance of the hybrids were noted that could not be attributed to flowering time differences. Additional field trials assessing differences in drought sensitivity of a wider range of substitution lines in the backgrounds of the parental lines of popular hybrid HHB 67 (i.e., 843B and H 77/833-2) were conducted in the summer drought nursery at ICRISAT-Patancheru in 2002. Initial analyses of results from these trials will not be available until after the end of this project.

4.4 Progress towards a complete contiguous segment substitution lines set for QTL mapping in 841B × 863B

Selected progenies (with target QTLs from 863B for downy resistance, terminal drought tolerance, and/or improved leaf blade digestibility) have been advanced to BC_4F_1. For the remainder of 863B segments being introgressed into the background of 841B, genotyping of the BC_2F_1 generation (to identify BC_3F_1 progenies to be advanced) is underway. Progress has been slower than expected, as described in section 3.2 above, but in most cases it should still be possible to complete development of finished substitution lines within 2-3 generations, which can be expected to be completed within 2 years of the end of this project (as originally planned) assuming continued funding of this activity.

4.5 Identification and initial crosses of hybrid parental materials for southern and western Africa

A mapping population (actually two populations of 250 segregants each, based on a pair of plant × plant crosses between ICMB 89111 and ICMB 90111 = ICMP 423), based on the cross of two seed parent maintainer lines bred at ICRISAT-Patancheru and identified as having potential for use in breeding hybrids for western Africa, has been advanced to F_4 self bulks, DNA samples prepared, and SSR marker genotyping initiated. In addition, several additional hybrid parental lines from southern and western Africa have been crossed to sources of downy mildew resistance QTLs effective against pathogen populations of West African origin, and the resulting F_1 hybrids backcrossed to their elite parent. Decisions on which of the resulting BC_1F_1 families to advance are awaiting completion (by the end of this project) of an SSR-based genetic diversity assessment of the lines available for use as donor and recurrent parents.

5 Contribution of Outputs

The outputs of this project will contribute directly to the achievement of DFID development goals. Marker-assisted breeding of segmental substitution lines in the genetic backgrounds of the elite parental lines of popular early-maturing pearl millet hybrid HHB 67 has succeeded in generating an array of elite hybrid parental lines suitable for breeding the next generation of downy mildew resistant hybrids having higher grain and stover yields while maintaining the early maturity of HHB 67 that is critical to the current widespread acceptance of this hybrid in the pearl millet production zone of northwestern India.

a) What further market studies need to be done?

Few if any additional market studies need to be conducted prior to initial commercialization of products of this research project as the seed industry in India is now well aware of the breeding lines that have been produced in this project, and private-sector breeding programs have been active in requesting samples of them. Further, public sector breeding programs at

the state (in Haryana) and national level have initiated testing of hybrids that is required prior to their official release. However, on-farm trials of HHB 67-like hybrids produced from crosses of the improved versions of 843A and H 77/833-2 bred in this project will be essential to identify the combinations that best meet farmers' needs.

b) How will the outputs be made available to intended users?

The intended users of the outputs of this project are largely- and private-sector pearl millet breeding programs in India. Substitution lines bred in this project are made available to these breeding programs under the terms and conditions of ICRISAT's standard Material Transfer Agreement for materials of ICRISAT origin (Annex 5).

c) What further stages will be needed to develop, test and establish the manufacture of products?

None of the new versions of HHB 67 field tested in 2001 were sufficiently similar to the original HHB 67 to allow a "simple" swapping of nucleus and breeder seedlots used in the hybrid seed multiplication chain. However, several such combinations will be field tested in 2002. Up to three of the new versions of HHB 67 that were evaluated multi-locationally in 2001 will be entered in national trials in 2002, along with several more in state trials, with the intent of generating data to support the 2003 state release in Haryana of one or more of these new versions as a replacement for the original HHB 67 that appears to finally be succumbing to downy mildew.

d) How and by whom, will the further stages be carried out and paid for?

The state and national trials to generate data to support the 2003 state release in Haryana of one or more of the new versions of HHB 67 as a replacement for the original will be conducted by, and paid for by, the Indian national pearl millet improvement programme.

6 Acknowledgements

This project was conducted with core support provided by a multiplicity of donors from the Consultative Group on International Agricultural Research (CGIAR) to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and with special project support from the Plant Sciences Research Programme of the UK's Department for International Development (DFID) [CAZS-DFID Project Number R7382, File Number PB98.12 — Contiguous segment substitution lines: New tool for elite pearl millet hybrid parental lines enhancement], which is gratefully acknowledged.

In addition, this project has received non-financial support and research collaboration from scientists and support staff of many public-sector research institutions including the International Livestock Research Center (ILRI) through its staff posted at ICRISAT-Patancheru; the John Innes Centre (JIC) in Norwich, England; the Institute for Grassland and Environmental Research (IGER), in Aberystwyth, Wales; the Centre for Arid Zone Studies (CAZS) at the University of Wales, Bangor; the Russian Academy of Sciences, in Moscow, Russia; La Trobe University, in Bundoora, Australia; the Institute for Genomic Diversity at Cornell University in Ithaca, New York, USA; the maize and sorghum research center of the Brazilian national agricultural research program (CNPMS-Embrapa), in Sete Lagoas, Minas Gerais, Brazil; the USDA-ARS Coastal Plain Experiment Station in Tifton, Georgia, USA; the All-India Coordinated Pearl Millet Improvement Project (AICPMIP), coordinated from Rajasthan Agricultural University's Agricultural Research Station Mandor, in Jodhpur, Rajasthan, India; the Indian Agricultural Research Institute (IARI), in New Delhi, India; the Central Arid Zone Research Institute (CAZRI), at Jodhpur, Rajasthan, India; the Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), in Hisar, Haryana, India and its Regional Research Station at Bawal, Haryana,India; the Tamil Nadu Agricultural University (TNAU) campuses at Madurai and Coimbatore in Tamil Nadu, India; the Rajasthan Agricultural University (RAU) Agricultural Research Station Durgapura at Jaipur, Rajasthan, India and Regional Research Station Nagaur, at Nagaur, Rajasthan, India; and the JNKVV College of Agriculture at Gwalior, Madhya Pradesh, India. Without the cooperation of these many institutions, especially those under which several PhD thesis research programs were conducted in the course of this project, we would not have been able to make anywhere near the amount of progress that we have in fact achieved in the course of the past three years.

Finally, this project has received un-stinting support from ICRISAT research managers, including Dr Paula J Bramel, Dr Rodomiro Ortiz, Dr Jonathan H Crouch, and Dr Jillian Lenne, as well as tremendous support from technical and administrative staff reporting to the ICRISAT scientists involved in it.

Thank you one and all!

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9 Annexes

Annex 1. Summary of performance for agronomic characters of hybrids of substitution lines in the genetic backgrounds of parental lines of elite pearl millet hybrid HHB 67, compared to control entry HHB 67; results from replicated field trials conducted in six environments (fewer reporting environments for those entries shaded blue) across India during the rainy season of 2001. Entries highlighted in yellow were selected for joint submission by CCS Haryana Agricultural University and ICRISAT to national trials in India in 2002.

Entry name	Seed parent	Pollinator	Across-location mean grain yield (kg ha^{-1})	Across-location mean percentage of location-mean grain yield	Rank for across-location mean percentage of location-mean grain yield	Across-location mean time to 50% flowering (d)	Across-location mean plant height (cm)	Across-location mean effective tiller number
HHB 67	**843A**	**H 77/833-2**	**1787**	**85**	**63**	**41**	**150**	**3.02**
ICMH 01081	ICMA 97444	H 77/833-2	2272	98	43	----	-----	-----
ICMH 01082	ICMA 98004	H 77/833-2	2163	101	32	40	165	2.68
ICMH 01083	ICMA 01029	H 77/833-2	2308	103	27	42	163	2.63
ICMH 01084	ICMA 01030	H 77/833-2	2120	92	53	39	162	2.64
ICMH 01085	ICMA 01027	H 77/833-2	2014	88	60	40	157	2.55
ICMH 01086	ICMA 01031	H 77/833-2	2025	87	62	41	154	2.70
ICMH 01087	ICMA 01032	H 77/833-2	2012	92	52	40	158	2.79
ICMH 01088	ICMA 01026	H 77/833-2	1788	82	65	----	-----	-----
ICMH 01089	ICMA 99011	H 77/833-2	2336	108	14	39	158	2.94
ICMH 01090	ICMA 99012	H 77/833-2	2152	96	46	38	156	3.09
ICMH 01091	ICMA 99013	H 77/833-2	2396	110	8	39	158	2.78
ICMH 01092	ICMA 99014	H 77/833-2	2025	87	61	42	158	2.52
ICMH 01093	ICMA 99015	H 77/833-2	2300	98	42	41	157	2.69
ICMH 01094	ICMA 99016	H 77/833-2	2276	95	48	40	157	2.97
ICMH 01095	ICMA 99017	H 77/833-2	1781	85	63	----	-----	-----
ICMH 01096	ICMA 99018	H 77/833-2	2378	103	30	40	158	2.62
ICMH 01097	ICMA 99019	H 77/833-2	2280	97	44	42	159	2.69
ICMH 01098	ICMA 99020	H 77/833-2	2222	99	39	41	163	2.96
ICMH 01099	ICMA 99021	H 77/833-2	2016	88	58	40	156	2.67
ICMH 01100	ICMA 99022	H 77/833-2	2426	103	28	41	155	2.85
ICMH 01101	ICMA 99023	H 77/833-2	2403	107	17	40	161	2.73
ICMH 01102	843A	ICMR 01007	2199	91	55	39	154	2.46
ICMH 01103	ICMA 97444	ICMR 01007	2585	111	6	----	-----	-----
ICMH 01104	ICMA 98004	ICMR 01007	2121	93	51	39	163	2.44
ICMH 01105	ICMA 01029	ICMR 01007	2264	94	49	40	157	2.34
ICMH 01106	ICMA 01030	ICMR 01007	2132	90	56	39	161	2.30
ICMH 01107	ICMA 01027	ICMR 01007	2520	108	15	39	161	2.55
ICMH 01108	ICMA 01031	ICMR 01007	2361	100	36	39	157	2.56
ICMH 01109	ICMA 01032	ICMR 01007	2340	105	24	39	160	2.51
ICMH 01110	ICMA 01026	ICMR 01007	1783	88	59	----	-----	-----
ICMH 01111	ICMA 99011	ICMR 01007	2326	105	23	38	158	2.32
ICMH 01112	ICMA 99012	ICMR 01007	2564	118	2	39	155	2.55

ICMH 01113	ICMA 99013	ICMR 01007	2138	93	50	39	159	2.28
ICMH 01114	ICMA 99014	ICMR 01007	2500	107	19	38	162	2.40
ICMH 01115	ICMA 99015	ICMR 01007	2459	107	18	39	162	2.62
ICMH 01116	ICMA 99016	ICMR 01007	2205	92	54	39	157	2.28
ICMH 01117	ICMA 99017	ICMR 01007	1997	99	41	----	-----	-----
ICMH 01118	ICMA 99018	ICMR 01007	2579	110	10	39	159	2.77
ICMH 01119	**ICMA 99019**	**ICMR 01007**	**2484**	**108**	**16**	**39**	**158**	**2.42**
ICMH 01120	ICMA 99020	ICMR 01007	2467	106	21	40	159	2.36
ICMH 01121	ICMA 99021	ICMR 01007	2320	100	35	40	157	2.40
ICMH 01122	**ICMA 99022**	**ICMR 01007**	**2692**	**119**	**1**	**39**	**164**	**2.49**
ICMH 01123	ICMA 99023	ICMR 01007	2548	110	11	39	163	2.41
ICMH 01124	843A	ICMR 01004	2342	103	26	40	166	2.84
ICMH 01125	ICMA 97444	ICMR 01004	2929	108	12	----	-----	-----
ICMH 01126	ICMA 98004	ICMR 01004	2600	101	31	----	-----	-----
ICMH 01127	ICMA 01029	ICMR 01004	2720	106	22	----	-----	-----
ICMH 01128	ICMA 01030	ICMR 01004	2568	116	3	40	163	2.94
ICMH 01129	ICMA 01027	ICMR 01004	2232	95	47	40	162	2.88
ICMH 01130	ICMA 01031	ICMR 01004	2260	103	29	39	166	2.83
ICMH 01131	ICMA 01032	ICMR 01004	2324	100	34	40	172	2.76
ICMH 01132	ICMA 01026	ICMR 01004	--	--	--	----	-----	-----
ICMH 01133	ICMA 99011	ICMR 01004	2404	111	7	39	164	2.82
ICMH 01134	ICMA 99012	ICMR 01004	2280	97	45	40	161	2.76
ICMH 01135	ICMA 99013	ICMR 01004	2257	101	33	39	158	2.58
ICMH 01136	ICMA 99014	ICMR 01004	2288	100	38	41	166	2.83
ICMH 01137	ICMA 99015	ICMR 01004	2381	104	25	41	166	2.82
ICMH 01138	ICMA 99016	ICMR 01004	2134	89	57	40	163	2.90
ICMH 01139	ICMA 99017	ICMR 01004	2627	115	4	----	-----	-----
ICMH 01140	ICMA 99018	ICMR 01004	2294	100	37	42	167	2.78
ICMH 01141	**ICMA 99019**	**ICMR 01004**	**2379**	**106**	**20**	**42**	**170**	**2.74**
ICMH 01142	ICMA 99020	ICMR 01004	2335	110	9	40	165	3.01
ICMH 01143	ICMA 99021	ICMR 01004	2320	99	40	40	167	3.03
ICMH 01144	**ICMA 99022**	**ICMR 01004**	**2602**	**112**	**5**	**42**	**163**	**3.09**
ICMH 01145	ICMA 99023	ICMR 01004	2489	108	13	41	167	2.62
		SE_d (±)	389			1.3	6.7	0.47
		SE_m (±)	275			0.9	4.8	0.33
		Across locations means	**2350**			**40.0**	**160.7**	**2.70**
		CV (%)	20.26			3.87	5.13	21.09
		LSD	762			2.5	13.2	0.91

Annex 2. Summary of general combining abilities (GCAs) for agronomic characters of selected substitution lines in the genetic backgrounds of parental lines of elite pearl millet hybrid HHB 67, results from trials conducted in six environments across India during the rainy season of 2001. Parents of hybrids selected for submission to national trials in 2002 are highlighted in yellow, while their recurrent parents (which are the parental lines of the original HHB 67) are highlighted in green.

	Grain yield (kg ha^{-1})	Panicle yield (kg ha^{-1})	Time to 50% flowering (d)	Plant height (cm)	Panicle length (cm)	Plant population density ('000/ha)	Panicle number ('000/ha)	Effective tillers per plant
Pollinator GCA estimates								
H 77/833-2	**-133**	**-140**	**0.4**	**-3.1**	**-1.2**	**-0.6**	**6.6**	**0.09**
ICMR 01007	**90**	**64**	**-0.9**	**-1.4**	**-1.2**	**-0.8**	**-17.0**	**-0.24**
ICMR 01004	**43**	**76**	**0.5**	**4.5**	**2.4**	**1.4**	**10.4**	**0.15**
Seed parent GCA estimates								
843A	**-112**	**-234**	**-0.2**	**-4.9**	**-1.3**	**-4.1**	**-18.1**	**-0.06**
ICMA 01030	-26	-14	-0.5	1.4	0.7	-0.7	-4.4	-0.06
ICMA 01027	-15	-50	-0.2	-0.6	0.4	-2.7	-5.7	-0.03
ICMA 01031	-119	-182	-0.2	-1.6	0.4	-7.1	-9.1	0.01
ICMA 01032	-121	-163	-0.2	2.8	0.4	-2.1	-9.1	0.00
ICMA 99011	54	49	-1.2	-0.6	-0.9	0.3	6.6	0.00
ICMA 99012	0	-15	-0.9	-3.2	0.7	-0.4	8.9	0.11
ICMA 99013	-58	-70	-0.9	-2.2	-0.6	-1.4	-7.4	-0.14
ICMA 99014	-51	-90	0.5	1.4	0.7	4.6	-4.7	-0.11
ICMA 99015	89	197	0.5	1.1	0.4	0.3	3.6	0.02
ICMA 99016	-120	-161	-0.2	-1.6	-0.3	-1.7	-2.1	0.03
ICMA 99018	87	197	0.5	0.8	-0.6	3.3	13.6	0.03
ICMA 99019	**109**	**145**	**1.1**	**1.8**	**0.1**	**2.3**	**1.3**	**-0.07**
ICMA 99020	54	93	0.5	1.8	0.1	0.6	-0.4	0.09
ICMA 99021	-79	-126	0.1	-0.6	0.1	-0.7	-0.1	0.01
ICMA 99022	**252**	**335**	**0.8**	**0.1**	**-0.6**	**4.6**	**13.6**	**0.12**
ICMA 99023	107	90	0.1	3.1	-0.6	4.9	6.3	-0.10

Annex 3. Summary of pearl millet substitution lines developed during the course of this project (R7382), its predecessor (R6667), and an associated drought tolerance project (R7375).

Line name	Recurrent parent	Donor parent	Introgressed segment(s)
ICML 98005	842B	ICMP 85410	LG7: M269 ... M526 + bits of LG1 & LG2
ICML 98006	842B	ICMP 85410	LG1: M413 … M386 + bits of LG2
ICMB 98004	843B	ICML 22	Two DMR genes and intermediate height
ICMB 99011	843B	ICML 22	LG4: one DMR gene and D_2 tall height
ICMB 99012	843B	ICML 22	LG4: one-two DMR genes and D_2 tall height
ICMB 99013	843B	ICML 22	LG4: one DMR gene and D_2 tall height
ICMB 99014	843B	ICML 22	LG4: two DMR genes and D_2 tall height
ICML 99015	843B	ICML 22	Two DMR genes
ICML 99016	843B	ICML 22	Two-three DMR genes
ICMB 99017	843B	ICML 22	Two-three DMR genes
ICMB 99018	843B	ICML 22	Two-three DMR genes
ICMB 99019	843B	ICML 22	Two-three DMR genes
ICMB 99020	843B	ICML 22	Two-three DMR genes
ICMB 99021	843B	ICML 22	Two DMR genes
ICMB 99022	843B	ICML 22	Two DMR genes
ICMB 99023	843B	ICML 22	Two DMR genes
ICMR 98001	843B	ICMP 85410	LG3U: M37/EI…M451.1/ EV, including long panicles & fertility restoration for the A_1 CMS system
ICML 89002	843B	ICMP 85410	LG7L: M330.2/EI ... M526/EI +LG6L: M613/EV…M737/DI
ICML 98003	843B	ICMP 85410	LG7L: M330.2/EI ...M526/EI +segregating LG6L: M575/H …M737/DI
ICML 98007	843B	ICMP 85410	LG5: M815/EV…M735.1/DI
ICML 98008	843B	ICMP 85410	LG5: M815/EV…M735.1/DI
ICML 98009	843B	ICMP 85410	LG5L: M749/DI…M735.1/DI
ICML 98010	843B	ICMP 85410	LG5L: M749/DI…M735.1/DI

Line name	Recurrent parent	Donor parent	Introgressed segment(s)
ICMB 01025	843B	ICMP 85410	LG1Q+ & LG2C
ICMB 01026	843B	ICMP 85410	LG1Q & LG4Q; shorter panicles & reduced height
ICMB 01031	843B	ICMP 85410	LG5
ICMB 01032	843B	ICMP 85410	LG5
ICMB 01027	843B	P 7-3	LG1Q
ICMB 01028	843B	P 7-3	LG1Q
ICMB 01029	843B	P 7-3	LG1Q
ICMB 01030	843B	P 7-3	LG1Q
ICMR 01001	H 77/833-2	ICMP 451-P6	*Br* & height on LG1
ICMR 01002	H 77/833-2	ICMP 451-P6	*Br* & height on LG1
ICMR 01003	H 77/833-2	ICMP 451-P6	DMR on LG1
ICMR 01004	H 77/833-2	ICMP 451-P6	DMR on LG4
ICMR 01005	H 77/833-2	ICMP 451-P6	DMR on LG4
ICMR 01006	H 77/833-2	ICMP 451-P6	DMR on LG4
ICMR 01007	H 77/833-2	ICMP 451-P6	DMR & RR on LG1
ICMR 01008	H 77/833-2	ICMP 451-P6	DMR & RR on LG1
ICMR 01010	H 77/833-2	ICMP 451-P6	DMR on LG1 & LG4
ICMR 01011	H 77/833-2	ICMP 451-P6	DMR on LG1 & LG4
ICMR 01028	H 77/833-2	PRLT 2/89-33	LG2: drought tolerance (& DMR?)
ICMR 01029	H 77/833-2	PRLT 2/89-33	As above
ICMR 01030	H 77/833-2	PRLT 2/89-33	As above
ICMR 01031	H 77/833-2	PRLT 2/89-33	As above
ICMR 01032	H 77/833-2	PRLT 2/89-33	As above
ICMR 01033	H 77/833-2	PRLT 2/89-33	As above
ICMR 01034	H 77/833-2	PRLT 2/89-33	As above
ICMR 01035	H 77/833-2	PRLT 2/89-33	As above
ICMR 01036	H 77/833-2	PRLT 2/89-33	As above
ICMR 01037	H 77/833-2	PRLT 2/89-33	As above
ICMR 01038	H 77/833-2	PRLT 2/89-33	As above
ICMR 01039	H 77/833-2	PRLT 2/89-33	As above
ICMR 01040	H 77/833-2	PRLT 2/89-33	As above
ICMR 01046	H 77/833-2	PRLT 2/89-33	As above
ICMR 02041	H 77/833-2	PRLT 2/89-33	As above
ICMR 02042	H 77/833-2	PRLT 2/89-33	As above
ICMR 02043	H 77/833-2	PRLT 2/89-33	As above
ICMR 02044	H 77/833-2	PRLT 2/89-33	As above
ICMR 02045	H 77/833-2	PRLT 2/89-33	As above
ICMR 02047	H 77/833-2	PRLT 2/89-33	As above

Annex 4. Summary of performance for pearl millet substitution lines in the genetic backgrounds of the parental lines of elite hybrid HHB 67 in greenhouse downy mildew screens against three pathogen populations (Sg298 from IARI, New Delhi; Sg153, from ICRISAT, Patancheru; and Sg139 from CAZRI, Jodhpur); results from time-replicated (3×) screens conducted at ICRISAT-Patancheru during the rainy season of 2001.

Substitution line name	Mean greenhouse downy mildew incidence (DMI, %)			Mean arcsin-transformed greenhouse DMI (radians)			Comments
	Sg298 (New Delhi)	Sg153 (Patan-cheru)	Sg139 (Jodh-pur)	Sg298 (New Delhi)	Sg153 (Patan-cheru)	Sg139 (Jodh-pur)	
ICML 98001	97.66%	97.59%	95.43%	1.395	1.354	1.271	Highly susceptible
ICML 98002	91.17%	86.72%	95.86%	1.228	1.062	1.356	Highly susceptible
ICML 98003	82.35%	70.85%	68.96%	1.055	0.804	0.781	Improved resistance
ICMB 98004	15.40%	11.63%	11.30%	0.155	0.117	0.113	Moderately resistant
ICML 98005	69.64%	42.45%	63.76%	0.851	0.442	0.856	Improved resistance
ICML 98006	67.01%	54.69%	55.61%	0.887	0.580	0.607	Improved resistance
ICML 98007	100.00%	99.50%	100.00%	1.571	1.513	1.571	Highly susceptible
ICML 98008	98.57%	98.72%	92.22%	1.473	1.478	1.338	Highly susceptible
ICML 98009	100.00%	100.00%	97.16%	1.571	1.571	1.432	Highly susceptible
ICML 98010	98.45%	99.28%	96.53%	1.469	1.501	1.417	Highly susceptible
ICMB 99011	9.96%	8.95%	9.91%	0.100	0.090	0.099	Moderately resistant
ICMB 99012	0.49%	0.00%	1.00%	0.005	0.000	0.010	Highly resistant
ICMB 99013	3.27%	1.04%	0.00%	0.033	0.010	0.000	Highly resistant
ICMB 99014	8.71%	3.31%	1.60%	0.087	0.033	0.016	Resistant
ICMB 99015	0.46%	0.00%	1.89%	0.005	0.000	0.019	Highly resistant
ICMB 99016	0.00%	0.00%	2.56%	0.000	0.000	0.026	Highly resistant
ICMB 99017	0.48%	2.71%	2.88%	0.005	0.027	0.029	Highly resistant
ICMB 99018	0.00%	0.00%	1.43%	0.000	0.000	0.014	Highly resistant
ICMB 99019	0.00%	0.00%	0.00%	0.000	0.000	0.000	Fully resistant
ICMB 99020	2.70%	0.44%	2.01%	0.027	0.004	0.020	Highly resistant
ICMB 99021	0.00%	0.89%	0.65%	0.000	0.009	0.007	Highly resistant
ICMB 99022	0.00%	0.00%	0.00%	0.000	0.000	0.000	Fully resistant
ICMB 99023	1.47%	0.56%	1.59%	0.015	0.006	0.016	Highly resistant
143 self	72.88%	40.99%	62.54%	0.830	0.440	0.691	Improved resistance
ICMB 01025	61.61%	44.62%	43.74%	0.684	0.464	0.456	Improved resistance
ICMB 01026	65.80%	52.49%	76.42%	0.742	0.577	0.930	Improved resistance

ICMB 01027	16.30%	55.61%	37.99%	0.164	0.593	0.401	Improved resistance
ICMB 01028	17.34%	67.58%	53.34%	0.175	0.747	0.595	Improved resistance
ICMB 01029	52.13%	77.03%	68.97%	0.566	0.889	0.901	Improved resistance
ICMB 01030	35.06%	53.38%	45.17%	0.362	0.581	0.472	Improved resistance
ICMB 01031	94.33%	97.74%	98.17%	1.309	1.397	1.414	Highly susceptible
ICMB 01032	97.35%	97.39%	94.43%	1.391	1.385	1.323	Highly susceptible
ICMR 01007	3.51%	50.12%	49.88%	0.035	0.528	0.526	Improved resistance
ICMR 01004	9.64%	39.25%	32.58%	0.097	0.404	0.332	Improved resistance
ICMR 01001	92.32%	96.54%	95.93%	1.180	1.372	1.343	Highly susceptible
H 77/833-2	94.18%	92.73%	99.17%	1.264	1.190	1.496	Elite susceptible line, and recurrent parent
842B	58.96%	91.30%	80.89%	0.639	1.159	0.942	Elite susceptible line, and recurrent parent
843B	95.18%	89.44%	95.19%	1.389	1.135	1.274	Elite susceptible line, and recurrent parent
81B	78.74%	65.05%	68.32%	0.956	0.711	0.780	Elite susceptible line
ICMP 85410	86.11%	83.84%	83.04%	1.255	1.228	1.218	Resistance donor for 842B and 843B
ICMP 451-P6	24.67%	82.42%	41.72%	0.250	1.017	0.435	Resistance donor for H 77/833-2
ICMP 451-P8	4.09%	49.99%	45.83%	0.041	0.533	0.491	Elite moderately resistant line
7042(S)	82.54%	74.27%	87.43%	0.993	0.843	1.068	Susceptible control
HB 3	96.55%	96.86%	94.85%	1.373	1.371	1.334	Susceptible control
ICML 22 = 7042(DMR)	12.90%	19.42%	18.05%	0.130	0.196	0.183	Resistance donor for 843B
700651	18.17%	14.66%	4.41%	0.184	0.147	0.044	Resistant control
P 7-3	0.48%	25.74%	4.75%	0.005	0.264	0.048	Resistance donor for 843B
Grand mean	45.08%	49.74%	48.62%	0.595	0.633	0.632	
Standard error (±)	6.09%	6.06%	7.23%	0.105	0.092	0.125	
CV (%)	23.39%	21.11%	25.77%	30.70%	25.26%	34.34%	
F-ratio	44.34	39.78	28.47	30.25	34.56	19.42	All highly significant
$h^{2,1}$	0.935	0.928	0.902	0.907	0.918	0.860	Plot basis heritability
$h^{2,2}$	0.977	0.975	0.965	0.967	0.971	0.949	Mean basis heritability

Annex 5.

Standard Order Form (Material Transfer Agreement) for Genetic Material Developed at ICRISAT

I/We order the following genetic material in the form of seed/vegetative propagules/tissue samples/DNA:

S.No	Pedigree	Source	Quantity	Remarks
1				
2				
3				
4				
5				

In so far as this genetic material has been developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) with public funds provided through the Consultative Group on International Agricultural Research (CGIAR) by donors from around the world. Hence, I/we agree the material contained herein is furnished by ICRISAT under the following conditions:

1 ICRISAT is making the material indicated above or in the attached list available as part of its policy of maximizing the utilization of genetic material for research. The material developed by ICRISAT is made freely available for any agricultural research or breeding purposes.
2 Recipients are free to commercialise ICRISAT research products in the form they are provided with due notification to ICRISAT. Prior to the application of any form of intellectual property rights (IPR) on this germplasm and related information, written permission must be obtained from ICRISAT. Moreover, while ICRISAT recognizes the validity of IPR, it reserves the right to distribute all material in accordance with paragraph (1) above.
3 The recipient agrees that any subsequent person or institution to whom they provide samples of this material is bound by these same provisions.
4 Although the material and associated information being supplied by ICRISAT were developed following careful and comprehensive research, ICRISAT makes no warranties as to the safety or title of the material nor as to the accuracy of correctness of any passport or other data provided with the material. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the material being furnished. The phytosanitary condition of the material is warranted only as described in the attached phytosanitary certificate. The recipient assumes full responsibility for complying with the recipient nation's quarantine or biosafety regulations and rules as to import or release of genetic material.
5 The recipients agree to furnish ICRISAT performance data collected during evaluations. Recipients should give due acknowledgement to ICRISAT in their reports for having provided the source material used for their research or to derive a process or product.

Place and date:
Indentor's signature:
Name and institutional affiliation of the person requesting the genetic material:
Address (and Shipping address):