

RNRRS PROJECT COMPLETION SUMMARY SHEET

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TITLE OF PROJECT: Genetic Improvement of pearl millet seedling thermotolerance and terminal drought tolerance
R NUMBER: R6451
RNRRS PROGRAMME: Plant Sciences
PROGRAMME MANAGER (INST): J. R. Witcombe, CAZS, Bangor
SUB-CONTRACTOR: IGER, Aberystwyth
RNRRS PROGRAMME PURPOSE: Production of target crops on impoverished soils in semi-arid conditions improved by selection and genetic enhancement
RNRRS PRODUCTION SYSTEM: Semi-Arid
COMMODITY BASE: Pearl millet
BENEFICIARIES: Pearl millet farmers and breeders
TARGET INSTITUTIONS: ICRISAT and pearl millet breeding programmes in India
GEOGRAPHIC FOCUS: India

1. Project Purpose:

Plant genes coding for high temperature tolerance and terminal drought tolerance transferred into adapted genetic background of pearl millet.

Specific objectives for this project were:

- 1.1 Develop an additional mapping family (841B x 863B), genotype and produce a genetic map.
- 1.2 Produce testcrosses of 2 pearl millet mapping families, screen for response to terminal drought and identify QTLs for tolerance.
- 1.3 Assess screening systems for thermotolerance, characterise and evaluate products of bi-directional selection for thermotolerance.
- 1.4 Map and analyse QTLs for seedling thermotolerance and other traits as appropriate.
- 1.5 Initiate marker assisted selection.
- 1.6 Assess importance of various putative components of both seedling thermotolerance and terminal drought tolerance using QTL analysis.
- 1.7 Write a series of scientific publications describing this work.

2. Outputs:

- 2.1 The mapping family developed from cross 3 (841B-P3 × 863B-P2) has been advanced to F₄. 147 F₂s have been genotyped with a total of 59 markers giving a total map length of 535 cM. The markers used include genomic RFLP probes from pearl millet, cDNAs encoding genes putatively involved in the response to drought, AFLPs and microsatellites.
- 2.2 The genetic map of cross H 77/833-2 × PRLT 2/89-33 has been expanded to 52 markers with an average spacing of approximately 7 cM and a total map length of 352cM.
- 2.3 Test-crosses have been produced on the F₄s of mapping family H 77/833-2 × PRLT 2/89-33 using 843A as the tester and produced on the F₃ progeny of 841B × 863B using two testers; H 77/833-2 and PPMI 301.
- 2.4 The test-crosses derived from mapping family H 77/833-2 × PRLT 2/89-33 have been screened in 7 different terminal drought stress environments (with a range of stress conditions encountered) and 5 control environments at ICRISAT along with 6 trials in the target environment in N.W. India.
- 2.5 QTL analysis for grain yield and its component traits under both control and terminal drought conditions has been completed. Amongst the QTLs identified, 2 (on linkage

groups 2 and 6) were associated with improved grain filling under stress; 2 (on linkage groups 1 and 2) with increased panicle grain number under stress; and 2 (on linkage groups 1 and 2) with increased grain yield under stress. In particular, a QTL for grain filling under terminal drought stress conditions, from linkage group 2 of parent PRLT 2/89-33, appears to have considerable potential for improving parent H 77/833-2. Across drought stress environments, this QTL was consistently observed to be linked with 100- seed weight, with a LOD score of up to 4.7

- 2.6 A dissection of the roles of yield potential, flowering time and drought tolerance on performance under terminal drought stress has been conducted and QTLs associated directly with drought tolerance have been identified.
- 2.7 The test-crosses from 841B × 863B have been screened once (1998) for yield and component traits under control and drought stress conditions, for the physiological response to drought and for the nutritional quality of the stover.
- 2.8 Screening for seedling thermotolerance of the F₄ progeny from cross H 77/833-2 × PRLT 2/89-33 and QTL analysis of the results has been completed. QTLs have been identified in 11 regions of the genome that provide enhanced thermotolerance; interestingly for many of the QTLs obtained, the parental allele that increased seedling thermotolerance was found to come from PRLT 2/89-33 rather than H77/833-2. QTLs for growth at control temperature and emergence also came from the PRLT 2/89-33 parent and co-mapped with QTLs for survival in the sand bed screening tank. It is possible that the increased vigour provided by the PRLT 2/89-33 parent resulted in a faster rate of development thus altering the response to temperature. The H 77/833-2 parent however did provide QTLs for enhanced growth at high temperature.
- 2.9 The test-crosses derived from mapping family H 77/833-2 × PRLT 2/89-33 have also been screened in the sand bed screening tank, for membrane thermostability, for seedling vigour, for growth after exposure to high temperature and in the field in Rajasthan. The 2 field trials were conducted in 1998 under extreme temperature conditions. Again, QTLs were identified from both parents for enhanced thermotolerance.
- 2.10 The F₄ progeny from cross ICMP 451 × H 77/833-2 have been screened twice in the field for their response to downy mildew, yield and morphological traits. In addition, 5 glasshouse downy mildew screens have been conducted with isolates from Patancheru, Mysore, Nigeria, Niger and Mali. A total of 12 QTLs for the response to downy mildew were identified and back cross improvement of elite parent H 77/ 833-2 with donor ICMP 451 has commenced at ICRISAT.
- 2.11 The markers themselves, the skeleton-mapped mapping populations and the back cross progeny containing the individual markers are also outputs from this project. These will be used both in plant breeding programmes for the improvement of other economically important traits, including pearl millet downy mildew resistance and ruminant nutritional quality, as well as in further studies elucidating the physiological and molecular mechanisms of the response to drought, downy mildew and other constraints.
- 2.12 The outputs from project R5418cb have also been extensively evaluated in the field and laboratory. These included the bi-directional selection products for seedling thermotolerance selected by three different techniques, their test-crosses, parents and other controls and the C0 bulk. 9 separate field screens have been conducted in Rajasthan over the 3 years; in 6 of these little stress was encountered and in the remaining 3, stress was extremely severe and affected seedling emergence as well as seedling survival. This confirms the need for laboratory based screening techniques. In all trials however, parent IP 3201 and thermotolerant control HHB 67 consistently out-performed the thermosensitive controls. Laboratory screening confirmed that selections made from screening in the sand-bed screening tank had increased tolerance to high temperature.

2.13 Papers, posters and oral presentations indicated in sections 4-6 are indicators of the success of this project.

3. Contribution of Outputs to Project Goal.

- 3.1 Close genetic linkage established between QTLs for component traits of terminal drought tolerance and molecular markers
- 3.2 Correlation between thermotolerance screening techniques for thermotolerance established
- 3.3 Effect of selection for thermotolerance based on different screening techniques determined
- 3.4 Appropriate markers for both downy mildew and terminal drought tolerance developed for use in marker-assisted breeding programmes
- 3.5 Marker assisted selection commenced for both response to terminal drought and to downy mildew
- 3.6 Relationships between different components of a trait established

4. Publications:

CAVAN, G. P., SKØT, K. P. STEVENS, M. J. HOWARTH, C. J. (1996). HSP17.9 from pearl millet. *Plant Molecular Biology* **30**, 1075.

CAVAN, G. P., SKØT, K. P. STEVENS, M. J. HOWARTH, C. J. (1996). HSP17.0 from pearl millet. *Plant Molecular Biology* **30**, 1076.

CAVAN, G. P., SKØT, K. P., STEVENS, M. J., HOWARTH, C. J. (1996). HSP16.9 from pearl millet. *Plant Molecular Biology* **30**, 1076.

HOWARTH, C. J., POLLOCK, C. J., PEACOCK, J.P. (1997). Development of laboratory - based methods for assessing seedling thermotolerance in pearl millet. *New Phytologist* **137**:129-139.

HOWARTH, C. J., WELTZIEN, E., BIDINGER, F.R., HARRIS, D. (1997). Seedling survival of abiotic stress: sorghum and pearl millet. In: *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*. 22-27 September 1996. Lubbock, TX. INTSORMIL University of Nebraska, Publ. 97-5. pp379-399.

HOWARTH, C. J., CAVAN, G. P., SKØT, K. P. YADAV, R. S. WELTZIEN, E., HASH, C. T. (1997). Mapping of quantitative trait loci for seedling thermotolerance and other traits in pearl millet. In: *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*. 22-27 September 1996. Lubbock, TX. INTSORMIL University of Nebraska, Publ. 97-5. pp650-651.

HOWARTH, C. J., SKØT, K. P. WELTZIEN, E., BIDINGER, F.R. (1997). Development and assessment of methods for evaluating seedling thermotolerance in pearl millet In: *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*. 22-27 September 1996. Lubbock, TX. INTSORMIL University of Nebraska, Publ. 97-5. pp651-652.

THOMAS, H, HUMPHREYS, M.W., BETTANY, A., HOWARTH, C .J. (1997) Plants for all seasons. IGER Innovations 1997 pp 6- 9 A.J. Gordon (ed).

HOWARTH,C.J. (1998) Growth and Survival at Extreme Temperatures: Implications for Crop Improvement. In: *Crop Productivity and Sustainability: Shaping the Future. Proceedings of the 2nd International Crop Science Congress*, R.B.Singh et al Chopra et al (eds). Oxford & IBH Publishing Co.Pvt.Ltd. and Science Publishers, Inc., USA. pp 467-484.

YADAV, R. S., BIDINGER, F.R., HASH, C.T., DEVOS, K.M., HOWARTH, C.J. (1998). Mapping quantitative trait loci associated with the response to drought in pearl millet

(*Pennisetum glaucum* L.). Plant & Animal Genome VI Conference.
<http://probe.nalusda.gov:8300/pag/6/review/229.html>

HOWARTH, C. J., CAVAN, G. P., SKOT, K. P., YADAV, R. S., WELTZIEN, E., HASH, C.T. (1998). Mapping of quantitative trait loci associated with seedling thermotolerance in pearl millet. *J. Exp. Bot.* **49**, P 10.26

YADAV, R. S., SKOT, K.P., HOWARTH, C.J. DEVOS, K.M., BIDINGER, F.R., HASH, C.T. (1998). Mapping quantitative trait loci associated with the drought response in pearl millet. *J. Exp. Bot.* **49**, P 10.27

YADAV, R. S., HOWARTH, C. J., CAVAN, G. P., SKØT, K. P., BIDINGER, F.R., MAHALAKSHMI, V., HASH, C.T. Drought tolerance mapping in pearl millet. *International Sorghum and Millets Newsletter* (in press)

YADAV, R. S., HASH, C. T., BIDINGER, F. R., HOWARTH, C. J. QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet In: *Genetic improvement of rice for water- limited environments*, IRRI, The Philippines, December 1998 (in press)

5. Internal Reports:

Annual report to DFID 1997 and 1998

6. Other Dissemination of Results:

Posters presented:

HOWARTH, C. J., CAVAN, G. P., SKØT, K. P., HASH, C.T., WELTZIEN, E., BIDINGER, F. R. Molecular physiology of plant acclimation to stress. *Gatersleben Research Conference*, Germany, June 1996.

HOWARTH, C.J., CAVAN, G.P. , SKØT, K. P., YADAV, R., WELTZIEN, E. , HASH, C.T. (1996). Mapping of quantitative trait loci for seedling thermotolerance and other traits in pearl millet. *2nd International Crop Science Congress*, Delhi, November 1996

HOWARTH, C.J., SKØT, K.P., WELTZIEN, E., BIDINGER, F.R. (1996). Development and assessment of methods for evaluating seedling thermotolerance in pearl millet. *2nd International Crop Science Congress*, Delhi, November 1996

Oral presentations:

C. J. Howarth 'Genetic mapping of seedling thermotolerance in pearl millet', ICRISAT, India, April 1996

C. J. Howarth 'Recent advances in genetic mapping of seedling thermotolerance, the response to downy mildew and the response to terminal drought stress' Pearl millet strategy meeting, Llangollen July 1996

C. J. Howarth was invited to speak at the ICRISAT/ INTSORMIL conference on '*Genetic Improvement of Sorghum and Pearl Millet*' in Lubbock, U.S.A. in September 1996 and presented a paper entitled 'Seedling survival of abiotic stress: sorghum and pearl millet.'

C. J. Howarth was invited to speak at the *2nd International Crop Science Congress*, Delhi, November 1996 and presented a paper entitled 'Growth and Survival at Extreme Temperatures: Implications for Crop Improvement'.

C. J. Howarth 'The use of genetic mapping to understand the response to high temperature and drought in tropical cereals and temperate grasses'. *BBSRC/ British Council meeting, Lisbon, Portugal*, March 1997

C. J. Howarth was invited to speak at the *2nd Tansley conference on 'Putting Plant Physiology on the Map'* at Bangor University in April 1997 and presented a paper entitled

'The use of genetic mapping to understand the response to drought and high temperature in pearl millet (*Pennisetum glaucum*)'

R. S. Yadav presented a paper entitled 'Drought tolerance mapping in pearl millet' at the DFID PSP-ICRISAT sponsored meeting on '*the use of molecular markers for pearl millet improvement in developing countries*' in November 1997, ICRISAT, India.

R. S. Yadav presented a paper entitled 'Use of molecular marker tools in drought tolerance breeding' at Texas A&M University, Amarillo, U.S.A. in September 1998.

R. S. Yadav was invited to speak at the Rockefeller Foundation sponsored workshop on '*Genetic improvement of rice for water-limited environments*' at IRRI, The Philippines in December 1998 and presented a paper entitled 'QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet'

R. S. Yadav presented a paper at the Aberystwyth Cell Genetics Group conference entitled 'Mapping and marker-assisted breeding for increased drought tolerance in pearl millet', January 1999.

7. Follow-up indicated / planned:

- 7.1 Develop new version of elite parent H 77/833-2 using marker assisted backcrossing of QTLs associated with terminal drought tolerance.
- 7.2 Develop a new pollinator population derived from selected F₃s from cross H 77/933-2 x PRLT 2/89-33, containing the drought tolerance QTLs, which can be improved either as a topcross pollinator, or used to derived new drought tolerant inbred pollinators.
- 7.3 Develop near isogenic lines with differential tolerance to terminal drought in H77/833-2 background. These will provide invaluable genetic tools for field experiments for not only evaluating the biological and economic advantages of incorporating these QTLs but provide material for fine mapping the response to terminal drought stress.
- 7.4 Continue marker assisted back-crossing of tolerance to downy mildew from ICMP 451 to H 77/833-2.
- 7.5 Identify QTLs for response to downy mildew in progeny of mapping families H 77/933-2 x PRLT 2/89-33 and 841B x 863B and initiate marker assisted backcrossing.
- 7.6 Identify QTLs for stover yield and quality in progeny of mapping family 841B x 863B and use for marker assisted selection.
- 7.7 Analyse results from control data obtained during screening for terminal drought tolerance of mapping families H 77/933-2 x PRLT 2/89-33 and 841B x 863B and use for marker assisted selection.
- 7.8 Develop water budgets for drought trials so far conducted and for future trials to assist in analysis of results obtained.
- 7.9 Verify QTLs for response to terminal drought stress, identify additional QTLs using mapping family from 841B x 863B and assess stability and robustness of QTLs obtained.
- 7.10 Initiate marker assisted selection for the improvement of elite parents 841B and 863B for response to terminal drought stress
- 7.11 Identify QTLs for physiological traits thought to confer terminal drought tolerance. This will allow the quantification of the value of these traits (and their components) under drought stress, and lead to the identification of the mechanisms and genes involved in adaptation to drought conditions.
- 7.12 Screen test-crosses of parents of other mapping families for response to drought and other traits to determine their suitability for future QTL analysis.