# FINAL TECHNICAL RESPORT R7373

## Use of molecular markers to improve terminal drought tolerance in pearl millet.

*Institute of Grassland and Environmental Research, Aberystwyth*

In collaboration with ICRISAT, India, and JIC, Norwich

01/04/99 - 30/09/02

## Executive Summary

The purpose of the project was to identify and characterise new QTLs for terminal drought tolerance in pearl millet (by studying a mapping population from a cross between ICMB 841x 843B) and to transfer those identified in an earlier project (R 6451) into the agronomically elite pollinator H 77/833-2 using marker-assisted backcrossing. Three new QTLs for terminal drought tolerance (in addition to one identified on LG 2 by studying H 77/833-2 x PRLT 2/98-33 cross in R 6445) were identified using ICMB 841 x 843B. These QTLs were also characterised for their effects on agronomic traits such as grain and stover yield *per se* in terminal drought stress environments and for the putative physiological mechanisms associated with them. These QTLs contributed to increased grain yield in terminal drought stress environments either by their effects on increased harvest index or biomass yield or both. QTLs for physiological mechanisms underlying drought tolerance such as increased relative water content, delayed leaf rolling, osmotic adjustment and early flowering co-mapped to these QTLs indicating the role of these processes in contributing to increased grain and stover yield under terminal drought stress conditions. QTLs for morphological traits (such as plant height, panicle characteristics etc.) and for yield under non-drought conditions were also identified.

The QTL for terminal drought tolerance identified on LG 2 by studying the H 77/833-2 x PRLT 2/98-33 mapping population was transferred to elite parental line H 77/833-2 using marker assisted back crossing. NILs for individual QTL so developed were tested for their response to drought in a number of terminal drought stress environments to revalidate effect of this QTL in a range of environments. NIls were also tested in the genetic background of several different female parents to understand QTL x genetic backgrounds effects. This QTL for terminal drought tolerance was found to have a positive effect in most terminal drought stress environments without any significant effect on yield in the irrigated control environment. In some situations, significant QTL x genetic background interactions effects were observed. QTL x genotype interaction was mainly associated with plant size: high tillering backgrounds leading to large plant size were found to negate the effect of QTL. In addition to marker assisted back crossing, this QTL was also transferred into a more heterogeneous genetic background by developing top cross pollinator populations (TCPs). TCPs so developed were again tested in different water environments and genetic backgrounds and showed superior performance in terminal drought stress environments. During the project period, other QTLs for terminal drought tolerance identified by studying ICM841 x 863B mapping family were also transferred to the background of sensitive parent using marker assisted backcrossing. NILs for these individual QTLs are now ready for evaluation. Study of these NILs would lead towards detailed characterisation of

these QTLs for their usability in different water stress environments and genetic backgrounds. These QTL NILs together with those developed for other QTLs in other DFID funded projects could be utilised to accumulate useful QTLs in elite pearl millet cultivars with wider adaptability, yielding, and resistance to diseases.

## Background

Pearl millet production areas are characterised by not only low but also unpredictable rainfall, which results in extremely unstable yields. The major abiotic reason for crop failure/reduced yields in pearl millet is post-flowering drought stress. In the 1994-1998 ICRISAT Medium Term Plan, enhancement of terminal drought tolerance was given the highest priority for future pearl millet research, with an estimated annual economic value of success of over $140 million and an estimated annual yield of $630 million. The identification of molecular markers associated with superior grain and stover yield performance, and their components, under terminal drought stress will enhance both the effectiveness, and the rate of progress, of breeding of improved genotypes for these harsh environments. QTLs which provide enhanced performance under terminal drought were previously identified in DFID project R6451. In this project they were transferred into the agronomically elite pollinator, H 77/833-2, using marker assisted backcrossing, which can then be used directly to breed adapted, high-yielding, disease-resistant hybrid cultivars. The backcrossing also produced sets of near-isogenic lines which could then be used to quantify the effects and value of individual QTLs, and materials suitable for developing additional pollinators. In addition a second cross (again between elite parents) was used to verify the QTLs obtained, to identify new QTLs, to determine their stability over a range of test cross parents and environments, to assess the involvement of physiological traits in drought tolerance and to provide closely linked markers for the improvement of seed parent maintainer 841B.

## Project Purpose

New elite hybrids of pearl millet developed with improved tolerance to terminal drought

## Research Activities

**1.1.** $F_3$s with appropriate genotype identified and backcrossed with H 77/ 833-2 ($BC_1F_1$). Also develop top cross pollinator population by recombining selected $F_3$s. Testcrosses selfed to check for fertility restoration.

**1.2.** $BC_1F_1$s backcrossed with H 77/833-2 ($BC_2F_1$).

**1.3.** $BC_2F_1$ genotyped.

**1.4.** Selected $BC_2F_1$s selfed ($BC_2F_2$), and backcrossed ($BC_3F_1$).

**1.5.** $BC_3F_1$ and $BC_2F_2$ s genotyped.

**1.6.** Selected $BC_3F_1$s selfed ($BC_3F_2$) , genotyped and backcrossed ($BC_4F_1$).

**1.7.** $BC_4F_1$ selfed ($BC_4F_2$) and selected $BC_4F_2$s testcrossed to 843A and to 3 other A lines.

**1.8.** NIL testcross products and testcrosses of top cross pollinator populations screened in rain-out shelter and drought nursery at ICRISAT.

**2.1.** Testcrosses of 841B x 863B mapping population screened in drought nursery and rain out shelter at ICRISAT and in target environment.

**2.2.** Physiological response to drought of testcrosses assessed.

**2.3.** Microsatellites and AFLPs identified in regions of interest and mapped.

**2.4.** QTLs analysed.

**2.5.** QTL results compared across environments and testers.

**2.6.** QTLs and individuals identified for MAS programme to improve 841B.

**2.7.** Preparation of manuscripts.

## Outputs

**1.1-1.7** QTL for drought tolerance (DT) identified using a cross between H 77/833-2 x PRLT 2/89-33 were successfully introgressed into the genetic background of sensitive parent (H 77/833-2) of the mapping population, using marker assisted back crossing. 13 near-isogenic lines (NILs) of the restorer line, H 77/833-2, were produced each with a segment from linkage group (LG) 2 of PRLT 2/89-33. The effect of the transferred QTL into H 77/833-2 was also assessed in the genetic background of 4 different A-lines by producing testcrosses. This was to understand the effect of genetic background on expression of drought tolerance QTL on LG 2. 13 NILs produced with LG2 PRLT genomic segment in H 77/ 833-2 background were genotyped with a large number of markers on LG 2 and on other LGs of pearl millet (figure 1). This data was studied in conjunction with field performance of these lines to get closer (or fine map) definition of the region of PRLT genome on LG 2 where drought tolerance QTL on this LG seems to be reside. Genotyping of NILs with markers from other linkage groups verified the effectiveness of marker assisted back crossing programme, where background markers were not studied during the introgression of DT QTL.

**1.8** These 13 NILs along with their testcrosses were evaluated in a range of drought and irrigated control environments to quantify the effects of the transferred QTL. Initial trial results indicate that QTL significantly increased grain yield in drought environments without any penalty of yield in irrigated control environments (Table 1, Figure 2). Three top cross pollinator populations (based on QTL, field performance and random selection) were also developed and their performance assessed in number of drought and irrigated control environments and in the genetic background of 12 A-lines. The top cross pollinator population based on the presence of QTL (MAS TCP) yielded significantly better than that produced based on field based selection (Phenotype TCP) (Figures 3, 4).

**2.1, 2.2** 2 sets of 152 testcrosses of 841B x 863B mapping population (on two different testers) were screened in 24 environments (drought nursery and rain out shelter at ICRISAT and in target environment plus relevant controls). Morphological, and physiological characters were scored in addition to grain and stover yield and their components.

**2.3** An updated map of 841B x 863B was produced with 91 markers placed on the linkage map of this cross. 33 of these markers were SSRs and 2 markers were functional probes (Figure5).

**2.4- 2.5** The genetic linkage map of 841B x 863B was used to identify new QTLs for drought tolerance. (Figure 6) In addition to mapping QTLs for responses to drought tolerance, the map and mapping progenies of cross between 841B x 863B proved a good resource for mapping QTLs for downy mildew resistance, ruminant nutritional quality, morphological characters and yield under non-drought conditions.

Physiological processes associated with components of drought tolerance in pearl millet were also identified by studying 841B x 863B and H 77/833-2 x PRLT 2/89-33. The role of leaf rolling, relative water content and osmotic potential was established by co-mapping with drought tolerance QTLs indicating that these traits provided an advantage to grain yield under stress conditions. (Figure 7)

The stability of QTLs associated with terminal drought tolerance and other traits was verified by studying the two mapping population (841B x 863B and H 77/833-2 x PRLT 2/89-33) in a range of environments and genetic background.

A large number of B-lines studied for mapped SSR markers both within the drought tolerance and other economic traits QTL region as well as in rest of the genome and data generated will help in marker assisted accumulation of desired QTLs in elite pearl millet lines used in pearl millet hybrid breeding

**2.6** $F_3$ individuals were identified for MAS programme to improve 841B and marker assisted introgression commenced.

**2.7** Papers, posters and oral presentations (see section 11 below) indicate the success of the project.

## Contribution of Outputs

**1.** QTLs associated with terminal drought tolerance identified and characterized in 2 separate populations.

**2.** Value of these QTLs in terms of economic yield established.

**3.** Information on their stability and expression in genetic background and environments established which would facilitate their use in MAS improvement of these traits along with other traits of agronomic importance in this crop.

**4.** Methodology of MABC transfer of identified QTLs in desired background established

**5.** A new way of accumulating desired QTLs in heterogeneous background explored, value of the methodology established in larger perspective

**6.** Physiological mechanism associated with components of drought tolerance and yield under drought tolerance established

**7.** Maps, markers and population developed for terminal drought tolerance were shown to be useful for other traits such as downy mildew resistance, ruminant nutritional quality and other agronomic traits

**8.** MAS methodology established, MAS derived products superior in terminal drought tolerance developed, tested.

**9.** Large number of B-lines form the germplasm studied for diversity and for polymorphism in the drought and other economic traits QTL regions for accumulating identified QTLs in elite liens using MAS

**10.** New versions of elite economically important hybrid parent (pollinator H 77/833-2) developed with improved drought tolerance that is expressed in its hybrids

**11. Publications:**

**11.1.** Yadav RS, Bidinger FR, Hash CT, Yadav YP, Bhatnagar SK and Howarth CJ (2003) Mapping and characterization of QTL x E interactions for grain and stover yield determining traits in pearl millet. *Theoretical and Applied Genetics* 106: 512 -520

**11.2.** Yadav RS, Hash CT, Cavan GP, Bidinger FR, Howarth CJ (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

**11.3.** Yadav RS**,** Hash CT, Bidinger FR, Devos KM, Howarth CJ (2003) Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and testers background. *Euphytica,* Accepted, manuscript reference - EUPH6861

**11.4.** Kapila RK, Yadav RS, Rai KN, Yadav OP, Sharma A, Hash CT, Howarth CJ (2003) Diversity analysis of hybrid seed parental lines of pearl millet using SSR markers. *Molecular Breeding,* communicated, manuscript reference- MOLB752

**11.5.** Howarth CJ, Yadav, RS (2002) Successful marker assisted selection for drought tolerance and disease resistance in pearl millet. *IGER Innovations 2002,* Gordon, AJ, ed. 6: 18-21

**11.6.** Hash CT, Sharma A, Kolesnikova MA, K, Bidinger FR, Serraj R, Thakur RP, Raj AGB, Nijhawan DC, Beniwal CR, Khiarwal IS, Yadav YP, Yadav RS, Howarth CJ, Witcombe JR (2002) Marker-assisted improvement of pearl millet hybrid HHB 67. P. 16 in Souvenir & Abstracts, National Symposium on New Opportunities and Challenges for Improving Crop Productivity Through Biotechnology (Society for Plant Biochemistry and Biotechnology), Feb 13-15 2002. Department of Biotechnology & Molecular Biology, CCS Haryana Agricultural University: Hisar 125 004, Haryana, India.

**11.7.** Hash CT, Yadav RS, Sharma A, Bidinger FR, Devos KM, Howarth CJ, Chandra S, Cavan GP, Serraj R, Witcombe JR (2002) QTL mapping and marker-assisted backcrossing for components of terminal drought tolerance in pearl millet. Pp 60-64 Proceedings of Rockefeller Foundation sponsored workshop on “Progress toward developing crops for drought prone areas”, May 27-30 2002 , International Rice Research Institute (IRRI), Manila, Philippines

**11.8.** Yadav RS, Hash CT, Bidinger FR, Dhanoa, MS, Howarth CJ (2000) Identification and utilization of QTLs associated with drought tolerance in pearl millet (*Pennisetum glaucum* L.). PP

108-113 in the Proceedings of the International Strategic Planning Workshop on *Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments* (Ribaut JM and Polland D, eds). A Strategic planning workshop held at CIMMYT, EL Batan, 21-25 June 1999. Mexico D.F.: CIMMYT

**11.9.** Hash CT, Yadav RS, Cavan GP, Howarth CJ, Lui H, Qi X, Sharma A, Kolesnikova MA, Bidinger FR, Witcombe, JR (2000) Marker-assisted backcrossing to improve terminal drought tolerance in pearl millet (*Pennisetum glaucum* L.). PP 114-119 in the Proceedings of the International Strategic Planning Workshop on *Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments* (Ribaut JM and Polland D, eds). A Strategic planning workshop held at CIMMYT, EL Batan, 21-25 June 1999. Mexico D.F.: CIMMYT

**11.10.** Yadav RS, Hash CT, Sharma A, Skøt K, Qi X, Lindup S, Bidinger FR and Howarth CJ (2000) Mapping and marker-assisted selection to improve drought tolerance in the tropical cereal pearl millet. In: A. Gallais, C. Dillmann and I. Goldringer (eds*), Quantitative Genetics and Breeding Methods: the Way Ahead.* Proceedings of the XIth EUCARPIA meeting of the section Biometrics in Plant Breeding, 30th August to 1st September 2000, PARIS, France, pp295-296.

**11.11.** Yadav RS, Hash CT, Bidinger FR, Howarth CJ (1999) QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet (*Penneisetum glaucum* L.). Pp. 211-223 in *Genetic improvement of rice for water-limited environments* (Ito O, O'Toole J, Hardy B, eds). International Rice Research Institute (IRRI), Los Banos, Philippines.

**11.12.** RS Yadav, FR Bidinger, CT Hash, R Serraj and CJ Howarth (2001) Improving drought tolerance in pearl millet. Research Highlights, R7375, DFID P*lant Sciences* R*esearch* P*rogramme.* http://dfid-psp.org/highlights/2001/drought.html

**Posters presented:**

**11.13.** Hash CT, Sharma A, Kolesnikova-Allen MA, Serraj R, Thakur RP, Bidinger FR, Bhaskar Raj G, Beniwal CR, Yadav HP, Yadav YP, Srikant, Yadav RS, Howarth CJ (2002). Popular pearl millet hybrid HHB 67 has been improved in downy mildew resistance and agronomic performance by marker-assisted backcrossing of its parents. Poster presented to the annual meeting of the Crop Science Society of America, November 2002

**11.14.** Yadav RS, Bidinger FR, Hash CT, Serraj R and Howarth CJ (2002) Testing the agronomic and physiological consequences of drought tolerance QTLs in different genetic backgrounds of the tropical cereal pearl millet. P7.26 *Comparative Biochemistry and physiology*. Poster presented at SEB conference, Swansea April 2002

**11.15.** Yadav RS, Bidinger FR, Hash CT, Sharma A, Serraj R, and Howarth CJ (2002) Testing the agronomic and physiological consequences of drought tolerance QTL in different genetic backgrounds of the tropical cereal pearl millet. A poster presented at Plant, Animal & Microbe Genomes Conference X, January 12-16, 2002, http://www.intl-pag.org/pag/10/abstracts/PAGX_P444.html

**11.16.** Sharma A, Hash CT, Nijhawan DC, Yadav RS (1999) Marker-assisted backcross breeding of elite pearl millet hybrid parental line H 77/833-2. Abstracts: *Life Sciences in the Next Millennium* School of Life Sciences, University of Hyderabad, India, 11-14 December 1999, 66

**11.17.** Yadav RS, Howarth CJ, Skot KP, Devos KM, Bidinger FR, Hash CT (1998) Mapping quantitative trait loci associated with drought response in pearl millet. *J. Exp. Bot.* 49, P 10.27

**11.18.** Howarth CJ, Cavan GP, Skot KP, Yadav RS, Weltzien E, Hash CT (1998) Mapping of quantitative trait loci associated with seedling thermotolerance in pearl millet. *J. Exp. Bot*. 49, 77

**11.19.** Yadav RS, Bidinger FR, Hash CT, Devos KM, Howarth CJ (1998) Mapping quantitative trait loci associated with the response to drought in pearl millet (*Pennisetum glaucum* L.). Plant and Animal Genome VI Conference. http://probe.nalusda.gov:8300/pag/6/review/229.html

**Oral presentations:**

**11.20.** Yadav RS, Hash CT, Bidinger FR, Serraj R, Howarth CJ (2002) Genetic analysis of yield in pearl millet under irrigated and terminal drought stress conditions. Paper presented in a conference entitled Genotype – Phenotype : Narrowing the Gaps, organised by the Association of Applied Biologists, 16-18 December 2002 , Royal Agricultural College, Cirencester, UK

**11.21.** Yadav RS, Hash CT, Bidinger FR, Serraj R, Howarth CJ (2002) Genetic improvement of drought tolerance of pearl millet hybrids using marker assisted selection. Paper presented in a conference entitled Genotype – Phenotype : Narrowing the Gaps, organised by the Association of Applied Biologists, 16-18 December 2002 , Royal Agricultural College, Cirencester, UK

**11.22.** Yadav RS (2002) Marker assisted selection strategies to accumulate desirable QTLs in pearl millet. An invited paper presented in the Plant, Animal & Microbe Genomes Conference X, January, 12-16, 2002, http://www.intl-pag.org/pag/10/abstracts/PAGX_W226.html

**11.23.** Howarth CJ, Yadav RS (2001) Using genetic mapping to understand drought tolerance in tropical cereals. Seminar presented at HRI, Wellesbourne, September 2001

**11.24.** Yadav RS (2001) Using genetic maps and markers in understanding and improving drought tolerance in tropical cereal pearl millet. Seminar presented in the IGER Cell Biology Seminar series, 23 July 2001

**11.25.** Yadav RS (2001) Using genetic maps and markers in pearl millet research. An invited lecture presented in the Department of Biotechnology and Molecular Biology, Chaudhary Charan Singh Haryana Agricultural University, Hissar, India, 19 February, 2001

**11.26.** Yadav RS, Bidinger FR, Hash CT, Howarth CJ (2001) Using genetic mapping to understand and improve drought tolerance in tropical cereal pearl millet. An invited paper presented in the annual meeting of the Society for Experimental Biology (SEB) held at the University of Kent, Canterbury, UK, April 2 to 6 2001 *J. Exp. Bot.* 52, P5.30

**11.27.** Howarth CJ, Yadav RS Hash CT (2000) Using genetic mapping to understand stress tolerance in tropical cereals. Seminar presented at University of Sao Paulo / Instituto de Botanica Sao Paulo, Brazil, November 2000

**11.28.** Yadav RS, Hash CT, Sharma A, Skøt K, Qi X, Lindup S, Bidinger FR and Howarth CJ (2000) Mapping and marker-assisted selection to improve drought tolerance in the tropical cereal pearl millet. A short paper presented in the XI$^{th}$ EUCARPIA meeting of the section Biometrics in Plant Breeding entitled "Quantitative Genetics and Breeding Methods: the Way Ahead", PARIS, France, 30$^{th}$ August to 1$^{st}$ September 2000

**11.29.** Yadav RS, Hash CT, Bidinger FR, Dhanoa MS, Howarth CJ (1999) Identification and utilization of QTLs associated with drought tolerance in pearl millet (*Pennisetum glaucum* L.). An invited paper presented in the Rockefeller Foundation sponsored workshop on “Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments”, 21-25 June 1999, CIMMYT, Mexico

**11.30.** Hash CT, Yadav RS, Cavan GP, Howarth CJ, Liu H, Qi X, Sharma A, Kolesnikova-Allen M, Bidinger FR and Witcombe, JR (1999) Marker-assisted backcrossing to improve terminal drought tolerance in pearl millet (*Pennisetum glaucum* L.). An invited paper presented in the Rockefeller Foundation sponsored workshop on “Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments”, 21-25 June 1999, International Wheat and Maize Research Institute (CIMMYT), El Batan, Mexico

**11.31.** Yadav RS, Hash CT, Bidinger, FR, and Howarth CJ (1998) QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. An invited paper presented in the Rockefeller Foundation sponsored workshop on “Genetic Improvement of Rice for Water-Limited Environments”, December 1–3, 1998, International Rice Research Institute (IRRI), Manila, Philippines

**11.32.** Yadav RS (1999) Mapping and marker-assisted breeding for increased drought tolerance in pearl millet. Paper presented in the Aberystwyth Cell Genetics Group (CGG) Conference, January 4-5, 1999, Aberystwyth, Wales, UK

**11.33.** Yadav RS (1998) Use of molecular marker tools in drought tolerance breeding. Invited lecture delivered at the Texas A&M University, Amarillo (USA), November 1998

**11.34.** Yadav RS, Howarth CJ, Cavan GP, Skot KP, Bidinger FR, Hash CT (1997) Abiotic stress tolerance mapping in pearl millet. An invited paper presented in the DFID PSP-ICRISAT meeting on the "Use of Molecular Markers for Pearl Millet Improvement in Developing Countries", 18-20 November 1997, International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India

**Visitors:**

Mr Arun Sharma, ICRISAT and HAU, November 1999

Dr OP Yadav, Pearl millet Breeder, Center of Arid Zone Research Institute (CAZRI) Jodhpur, India (August to November 2002)

Dr Rakesh Kapila, Himachal Pradesh Agricultural University, Palampur, India, August 2002 to April 2003

## Follow-up indicated / planned:

- Completion of field assessment of products of MAS at ICRISAT, multi-locational trials in India and in farmers' fields
- Assessment of impact of release of products of MAS in pearl millet growing areas of India
- Further understand QTL effects in a range of drought stress environments to understand their effects and usability in drought environments (severity, timing and duration)
- Assessment of DT QTL x G and DT QTL x E interactions using hybrids based near-isogenic pairs of parents with and without DT QTL
- Evaluation of NILs to further identify physiological and morphological processes contributing to improved drought tolerance
- Marker-assisted introgression of DT QTL alleles from 863B into 841B
- To quantify the resulting benefits in terms of yield and yield stability and assess the interactions of DT QTL with environment and genetic background
- Fine mapping and map based cloning of genes associated with terminal drought tolerance
- Comparative mapping of DT QTLs within Gramineae

- Linking/ association mapping of diversity already studied in the present project (for large number of SSR markers) for available B-lines in the germplasm held at ICRISAT with the traits of economic importance in pearl millet, using disequilibrium mapping approaches and use of results obtained in marker assisted breeding.

## Authors of report

Dr Rattan S Yadav and Dr Catherine J Howarth

Appendix

Copies of papers indicated in section 11 (not supplied electronically)

**Table 1** Performance of 13 NILs transferred with drought tolerance QTL on LG 2 for grain yield and other agronomic traits evaluated during summer season of 2002 at ICRISAT Patancheru, India. CT; control, LS; Late onset stress, MS; Middle onset stress, ES; Early onset stress.

| Pollinator | Days to 50% flowering | | | | Panicle number $m^{-2}$ | | | | Stover dry weight | | | | Biomass dry weight | | | | Grain yield | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | LS | MS | ES | CT | LS | MS | ES | CT | LS | MS | ES | CT | LS | MS | ES | CT | LS | MS | ES |
| 1 | 35.9 | 36.5 | 36.8 | 37.5 | 34.2 | 31.5 | 31.6 | 23.8 | 238.9 | 193.3 | 188.6 | 168.9 | 659.1 | 488.9 | 426.0 | 329.2 | 325.6 | 202.8 | 143.6 | 95.3 |
| 2 | 35.2 | 36.4 | 36.7 | 36.5 | 32.6 | 30.5 | 26.7 | 22.6 | 226.1 | 183.6 | 169.2 | 166.2 | 623.7 | 486.6 | 409.4 | 342.0 | 305.6 | 214.9 | 151.1 | 105.4 |
| 3 | 35.0 | 35.4 | 35.7 | 36.2 | 34.4 | 30.4 | 32.8 | 26.3 | 222.4 | 168.7 | 169.7 | 167.8 | 619.3 | 459.2 | 406.3 | 333.6 | 296.6 | 203.2 | 136.5 | 95.1 |
| 4 | 34.1 | 35.1 | 34.4 | 35.1 | 32.3 | 31.2 | 31.2 | 27.0 | 224.1 | 165.6 | 152.9 | 162.9 | 625.1 | 472.3 | 382.2 | 333.5 | 309.8 | 215.2 | 145.2 | 100.8 |
| 5 | 35.1 | 35.5 | 36.1 | 35.9 | 35.3 | 32.8 | 31.3 | 28.4 | 222.8 | 171.0 | 155.7 | 158.4 | 620.6 | 460.0 | 383.5 | 319.4 | 301.2 | 200.1 | 131.7 | 91.3 |
| 6 | 33.6 | 34.5 | 34.3 | 34.8 | 32.5 | 32.8 | 31.5 | 27.2 | 179.1 | 144.8 | 141.3 | 133.5 | 528.4 | 422.7 | 354.3 | 286.2 | 262.7 | 196.4 | 135.1 | 85.0 |
| 7 | 34.4 | 35.0 | 34.8 | 35.5 | 37.2 | 32.2 | 34.2 | 28.0 | 193.4 | 170.0 | 167.1 | 158.5 | 561.2 | 462.2 | 396.8 | 322.0 | 277.2 | 206.2 | 143.4 | 95.3 |
| 8 | 34.8 | 35.6 | 35.8 | 36.0 | 32.5 | 31.9 | 30.8 | 26.1 | 204.0 | 162.5 | 146.8 | 163.0 | 582.0 | 444.6 | 357.8 | 316.5 | 291.7 | 196.6 | 133.5 | 90.0 |
| 9 | 33.8 | 33.8 | 33.9 | 34.8 | 36.4 | 29.1 | 34.1 | 26.4 | 187.3 | 146.9 | 139.3 | 134.0 | 551.4 | 439.9 | 353.5 | 281.9 | 280.3 | 215.2 | 136.4 | 88.3 |
| 10 | 35.7 | 36.1 | 36.2 | 37.2 | 37.0 | 34.6 | 33.5 | 24.9 | 222.8 | 173.6 | 175.9 | 160.8 | 623.8 | 467.2 | 397.3 | 312.3 | 298.8 | 204.1 | 126.8 | 87.2 |
| 11 | 34.6 | 35.0 | 35.6 | 36.3 | 36.5 | 32.3 | 30.1 | 25.8 | 197.2 | 162.4 | 151.4 | 143.6 | 579.1 | 458.5 | 365.3 | 294.8 | 292.9 | 206.6 | 133.2 | 85.6 |
| 12 | 36.0 | 36.4 | 36.3 | 37.2 | 34.5 | 32.1 | 32.2 | 24.3 | 224.3 | 180.6 | 161.9 | 167.4 | 624.7 | 478.2 | 387.7 | 325.5 | 308.8 | 207.3 | 135.5 | 92.2 |
| 13 | 35.3 | 35.3 | 35.8 | 36.3 | 37.9 | 32.9 | 30.7 | 27.2 | 212.7 | 164.0 | 161.0 | 151.2 | 612.2 | 472.9 | 390.6 | 308.5 | 302.0 | 215.3 | 142.8 | 88.2 |
| Recipient (PRLT 2/98-33) | 36.0 | 36.4 | 36.3 | 38.0 | 37.0 | 34.4 | 36.2 | 27.2 | 210.5 | 180.2 | 175.6 | 165.3 | 601.4 | 469.9 | 405.6 | 325.2 | 286.3 | 195.2 | 135.0 | 89.2 |
| Donor (H 77/833-2) | 36.3 | 36.6 | 37.3 | 37.4 | 20.6 | 18.3 | 18.1 | 16.2 | 196.8 | 152.6 | 146.2 | 150.7 | 600.5 | 444.1 | 366.5 | 306.2 | 323.6 | 217.7 | 144.4 | 93.4 |
| LSD | 0.34 | | | | 1.76 | | | | 10.1 | | | | 24.37 | | | | 13.46 | | | |

| Pollinator | Harvest index | | | | Average hundred seed weight | | | | Grain number per panicle | | | | Grain number $m^{-2}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | LS | MS | ES | CT | LS | MS | ES | CT | LS | MS | ES | CT | LS | MS | ES |
| 1 | 49.2 | 41.4 | 33.7 | 28.9 | 0.79 | 0.73 | 0.65 | 0.52 | 1241 | 897 | 768 | 875 | 41.5 | 27.8 | 22.1 | 18.2 |
| 2 | 48.7 | 44.0 | 36.9 | 30.8 | 0.82 | 0.78 | 0.63 | 0.56 | 1226 | 977 | 951 | 906 | 38.2 | 27.5 | 24.1 | 18.9 |
| 3 | 47.5 | 44.3 | 33.6 | 28.4 | 0.77 | 0.71 | 0.60 | 0.48 | 1122 | 971 | 720 | 858 | 38.2 | 28.9 | 22.9 | 20.0 |
| 4 | 49.6 | 45.6 | 37.9 | 29.9 | 0.87 | 0.83 | 0.70 | 0.59 | 1133 | 851 | 743 | 810 | 35.7 | 26.0 | 20.8 | 17.0 |
| 5 | 48.3 | 43.6 | 34.1 | 28.6 | 0.81 | 0.77 | 0.64 | 0.54 | 1070 | 820 | 708 | 703 | 37.1 | 26.4 | 20.6 | 17.2 |
| 6 | 49.5 | 46.5 | 38.2 | 29.6 | 0.85 | 0.81 | 0.71 | 0.59 | 980 | 757 | 674 | 660 | 30.8 | 24.3 | 19.2 | 14.4 |
| 7 | 49.2 | 44.5 | 36.2 | 29.5 | 0.85 | 0.82 | 0.73 | 0.59 | 909 | 816 | 644 | 694 | 32.9 | 25.2 | 19.9 | 16.2 |
| 8 | 49.7 | 44.4 | 37.3 | 28.6 | 0.86 | 0.82 | 0.71 | 0.59 | 1073 | 777 | 668 | 716 | 33.7 | 24.1 | 18.8 | 15.5 |
| 9 | 50.7 | 48.9 | 38.5 | 31.4 | 0.88 | 0.87 | 0.75 | 0.61 | 898 | 1546 | 606 | 674 | 32.1 | 24.8 | 18.3 | 14.5 |
| 10 | 47.9 | 43.7 | 32.0 | 28.0 | 0.81 | 0.73 | 0.60 | 0.53 | 1008 | 834 | 674 | 759 | 37.1 | 28.2 | 21.3 | 16.5 |
| 11 | 50.4 | 45.1 | 36.9 | 28.9 | 0.84 | 0.79 | 0.73 | 0.59 | 980 | 831 | 657 | 665 | 35.0 | 26.2 | 18.5 | 14.4 |
| 12 | 49.4 | 43.2 | 34.9 | 28.2 | 0.83 | 0.74 | 0.64 | 0.54 | 1116 | 899 | 687 | 843 | 37.7 | 28.1 | 21.4 | 17.4 |
| 13 | 49.2 | 45.4 | 36.5 | 28.6 | 0.82 | 0.79 | 0.70 | 0.57 | 1015 | 867 | 718 | 668 | 36.9 | 27.9 | 20.6 | 15.4 |
| Recipient (PRLT 2/98-33) | 47.6 | 41.3 | 33.2 | 27.3 | 0.69 | 0.62 | 0.51 | 0.45 | 1154 | 937 | 775 | 844 | 41.8 | 31.3 | 26.5 | 20.0 |
| Donor (H 77/833-2) | 53.8 | 48.9 | 39.5 | 30.3 | 1.00 | 0.88 | 0.71 | 0.56 | 1617 | 1378 | 1146 | 1065 | 32.6 | 24.7 | 20.3 | 16.6 |
| LSD | 1.28 | | | | 0.03 | | | | 147 | | | | 17.4 | | | |

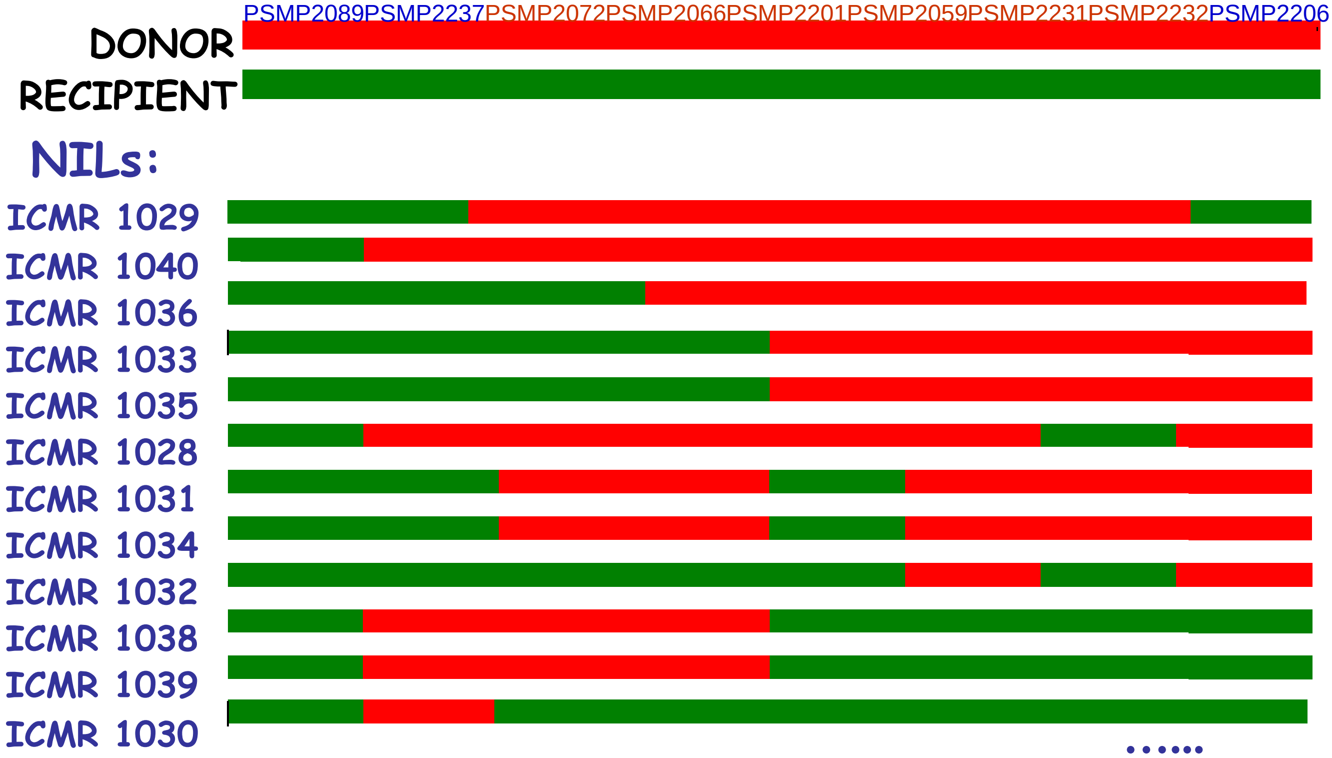



**Figure 1** Marker-assisted back cross transfer and development of NILs with homozygous drought tolerance QTL alleles in recurrent parent background; Genotype of linkage group 2 of NILs and of donor and recipient genotypes

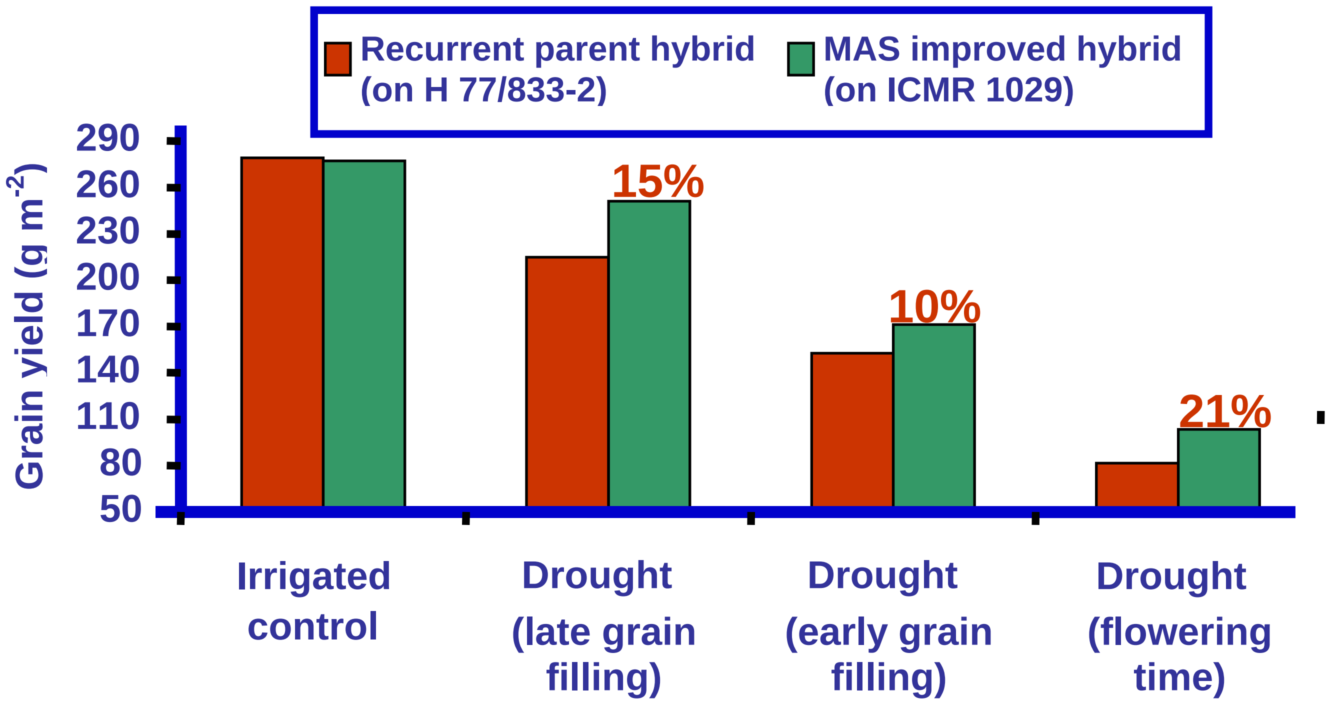
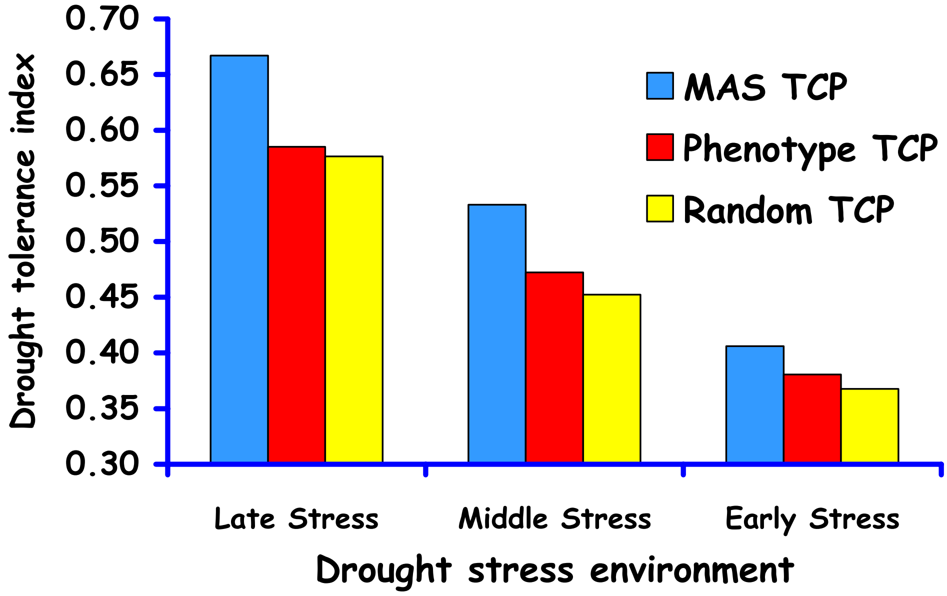



**Figure 2** Grain yield of MAS improved and recurrent parent hybrids in different water environments

**Figure 3** Mean drought tolerance indices of hybrids of three top cross pollinators evaluated in the




background of 12 A-lines in three drought stress environments.

**Figure 4** Drought tolerance indices of grain yield of hybrids of three top cross pollinators evaluated in the background of 12 A-lines under late-onset drought stress conditions.

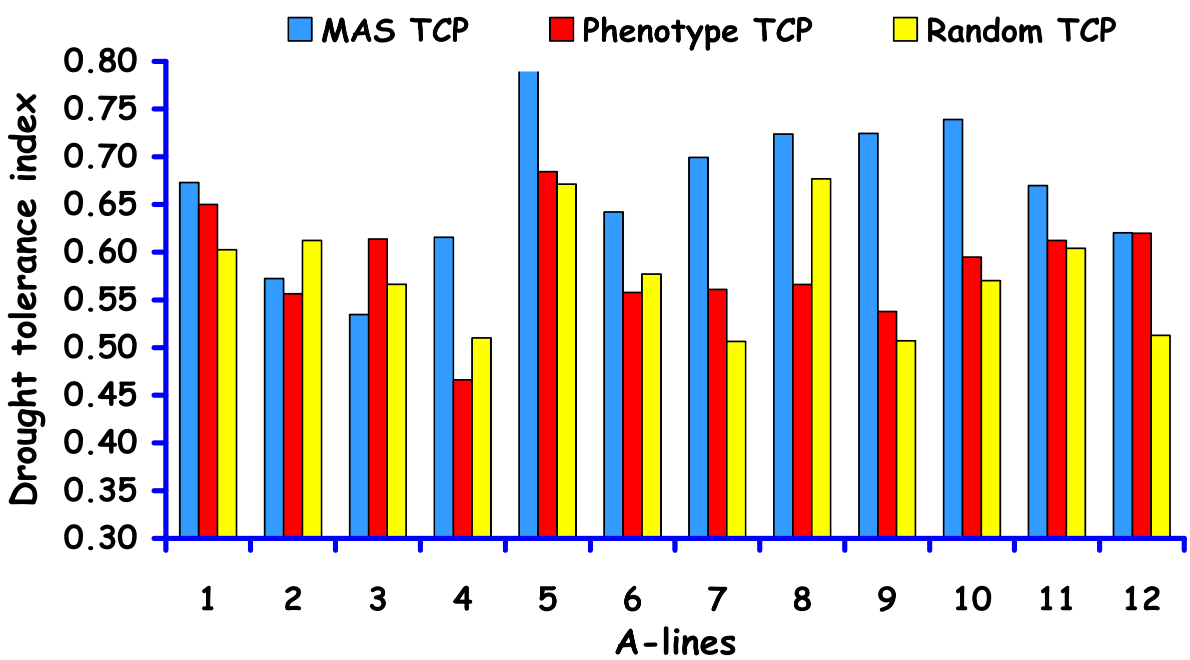


**Figure 5** Genetic linkage map of 841B x 863B

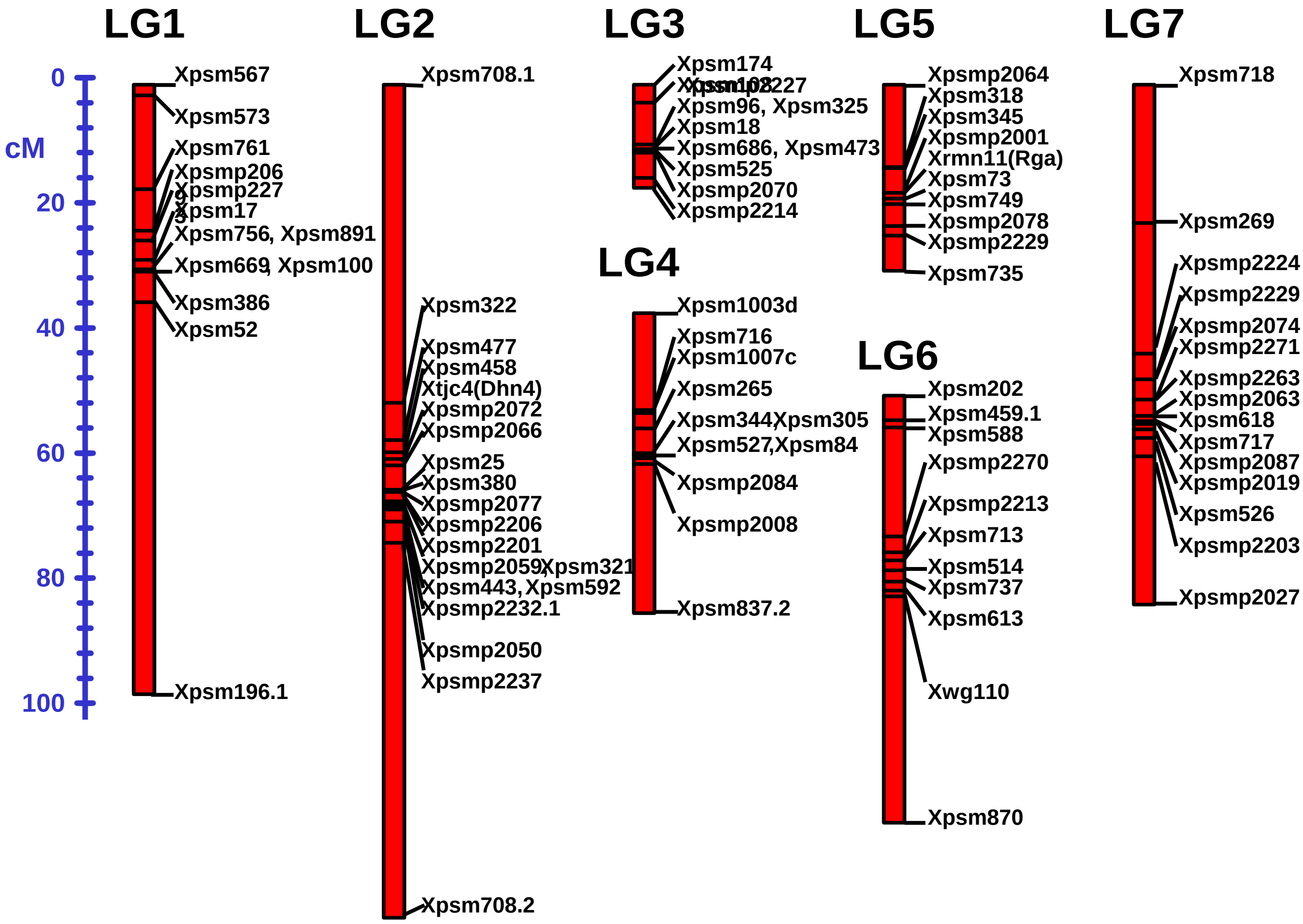
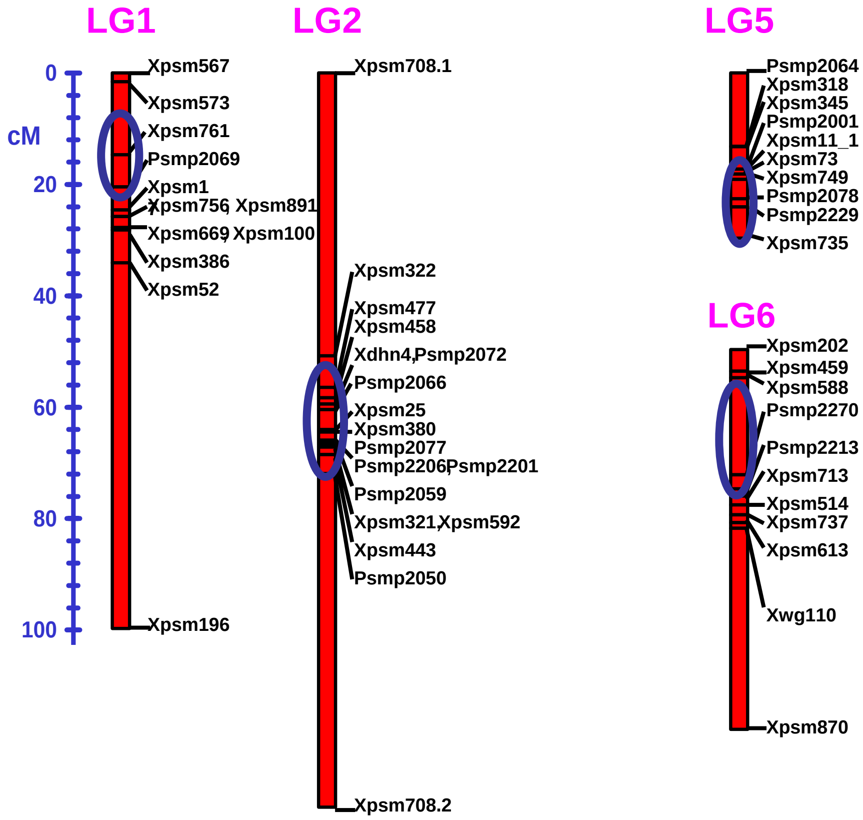


**Figure 6** Summary of location of QTL associated with grain yield, harvest index and biomass yield




during drought stress in 841B x 863B (ellipses)

**Figure 7** Summary of location of QTL associated with physiological traits during drought stress in 841B x 863B