Marker-Assisted Backcrossing to Improve Terminal Drought Tolerance in Pearl Millet

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Summary

Several alternative marker-assisted backcrossing (MABC) procedures are described that can be used for transferring quantitative trait loci (QTLs) from a donor to an elite recurrent parent when these two lines have been used in forming the base mapping population. We describe ICRISAT's experience to date in using these methods in pearl millet (Pennisetum glaucum (L.) R. Br.). We are attempting to improve terminal drought tolerance of elite inbred pollinator H 77/833-2 using donor PRLT 2/89-33, and elite inbred seed parent maintainer line ICMB 841 using donor 863B. The advantages and disadvantages of the alternatives are discussed.

Key Words

Backcrossing, contiguous segment substitutions, hybrid parental lines, marker-assisted selection, RFLP

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the staple food and fodder crop of millions of poor rural families in the hottest and driest dryland agricultural environments of Asia and Africa. Although grain and stover of this crop are not commercially important commodities (FAO and ICRISAT 1996), as most is consumed in the homesteads where they are produced, crop losses are economically important. These losses can be attributed to biotic stresses (principally *Striga* sp., birds, diseases, and insects) and abiotic stresses (principally nutrient deficiencies, drought, and heat). Increased yield and yield stability of pearl millet grain and stover would contribute to improving living standards and food security of poor families living in these harsh agricultural production regions.

ICRISAT, in collaboration with researchers in the UK and India, and supported by the Plant Sciences Programme of the UK's Department for International Development (DFID), has made a considerable research investment targeted to the development and application of molecular genetic tools for improving the yield and yield stability of pearl millet hybrid cultivars. Such hybrid cultivars are currently sown on >5 m ha each year by small holders in India. They have contributed to the substantial increase in pearl millet grain yields (ca. 100%) and grain production that has occurred in India over the past five decades. It is noteworthy that this increase has occurred during a time when the area sown to this crop has not only decreased, but shifted to more marginal lands thereby freeing up better land for production of higher value crops. Identification of marker-flanked quantitative trait loci (QTLs) associated with superior grain yield performance under terminal drought stress conditions has been a major part of this research activity during the past five years (see Yadav et al., this proceedings).

This paper describes several alternative procedures that can be used in pearl millet, and perhaps other crops, for marker-assisted backcross transfer (MABCT) of QTLs from a donor to an elite recurrent parent when the donor and recurrent parent have been used in forming the base mapping population. Advantages and shortcomings of each alternative are discussed.

Materials and Methods

Mapping of drought tolerance QTLs in pearl millet (Yadav et al. 1999a, b) began as a secondary target trait in a project intended to identify QTLs for seedling thermotolerance in pearl millet (Howarth et al. 1997). This first pearl millet mapping population with drought tolerance as a target trait was based on the cross of thermotolerant, drought-sensitive elite inbred pollinator line "H 77/833-2" from Haryana Agricultural University and thermosensitive, drought-tolerant breeding line "PRLT 2/89-33" from ICRISAT (Hash and Witcombe 1994). Studies of this population were followed with development and evaluation of a second pearl millet mapping population having terminal drought tolerance as its primary target trait. In this case, the drought-sensitive parent was "ICMB 841" (Singh et al. 1990) and the drought-tolerant parent was "863B." Both ICMB 841 and 863B were bred at ICRISAT-Patancheru and are elite maintainer lines of hybrid seed parents that are extensively used in India. Both PRLT 2/89-33 and 863B are derived from the Iniadi landrace of pearl millet (Andrews and Anand Kumar 1996). Mapping population development was as described by Hash and Witcombe (1994), with RFLP skeleton mapping, trait phenotyping, and QTL mapping as described by Yadav et al. (1999a, b). The parental lines, skeleton maps, and skeleton-mapped progenies from these two mapping populations have been used by us as starting points in a series of marker-assisted backcrossing (MABC) programs, initiated before or after completion of QTL mapping of the target trait (terminal drought tolerance, and its components). These MABC programs are described in detail below.

Conventional MABC Programs

Conventionally, MABC programs begin only after QTL mapping has identified the map position and closely linked flanking markers for donor parent gene blocks that contribute substantially to target trait phenotypic variation in the mapping population. At that point the breeder selects one or more genotyped (and preferably phenotyped) progenies from the mapping population that combine(s), as a minimum, heterozygosity for donor parent markers in the vicinity of the target QTL with homozygosity for the recurrent parent marker genotype in most of the remainder of the mapped genome. There are then two broad avenues that can be pursued (along with many paths between these). The first of these makes extensive use of marker genotyping in nontarget regions of the genome to reduce the number of backcrosses required to recover a desirable segregant (Hospital et al. 1992, 1997). The other extreme is to marker genotype only at points immediately flanking (and inside) the target region, and use serial backcrossing to more rapidly recover the recurrent parent genotype in nontarget regions of the genome. Choice between these two extremes, and/or some intermediate path, will largely be determined by the type of molecular markers available and length of the vegetative phase of the crop life cycle. For species with a long juvenile phase in which microsatellite markers (SSRs) are available, extensive use of marker genotyping would make a lot of sense; however, for pearl millet this is not the case.

- ***Advantages*:** It is less likely that any MABC program that is started will have to be abandoned, since the marker polymorphism of the donor and recurrent parents is already characterized and the markers identified appear to be linked to substantial differences in phenotypic performance (i.e., significant QTLs of large effect have purportedly been found).
- ***Disadvantages:*** A long time is required before the MABC program can start. Further, this program is, of course, restricted to using as its starting point the best marker genotype segregant(s) present in the original mapping population (which is largely a function of genotyped mapping population size).
- ***ICRISAT experience:*** In pearl millet we have a crop with a short life cycle, and short juvenile phase that can be reduced further by artificially reducing day length to induce early flowering. Combined with RFLP markers as the only co-dominant marker system currently available, this has lead us to initiate a program of MABC based on two mapping progenies from the cross H 77/833-2 × PRLT 2/89-33 (Fig. 1). Both selections were homozygous

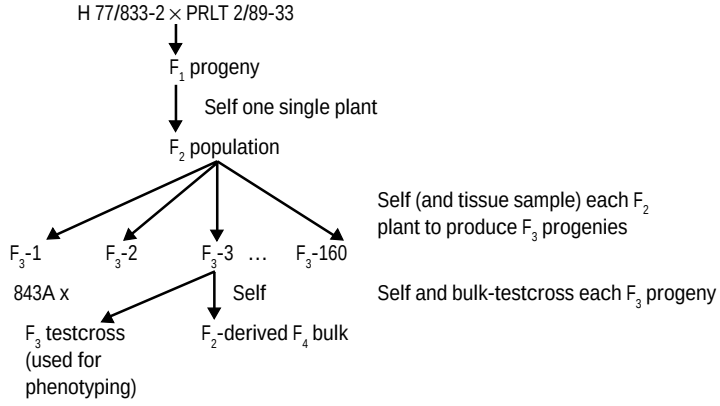
for two drought tolerance QTLs from linkage group 2 (LG2) and LG4 of PRLT 2/89-33, and at least heterozygous for the drought tolerance QTL on LG6 of H 77/833-2. These have been backcrossed to H 77/833-2, and the resulting BC_1F_1 progenies will be backcrossed again, yielding BC_2F_1 progenies segregating 1:1:1:1 for the two QTLs from PRLT 2/89-33. Individual plants from these progenies will then be genotyped at three markers flanking and centered over each of the three target drought tolerance QTLs.

Jump-started Marker-assisted Backcrossing

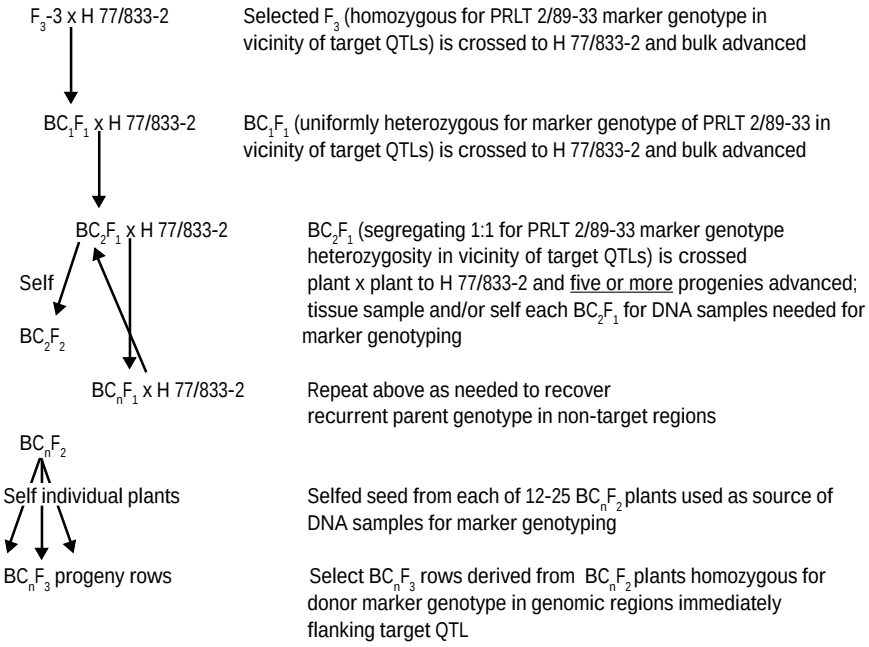
The procedure in this case begins during mapping population development itself, and perhaps even before marker polymorphism of the two parents has been fully characterized. The individual F_1 plant from which the mapping population will be derived (and itself the product of a cross between the trait donor and recurrent parent) is backcrossed to the parent weakest for the target trait. Alternatively, but less reliably, selfed progeny from the individual plant of the donor parent used in creating the mapping population can be used as the trait donor in the backcrossing program. This procedure uses probability theory (Sedcole 1977) to ensure that every possible QTL for the target trait is carried forward as rapidly as possible through the backcrossing generations. This continues until such time as markers become available, when a minimum of two markers per chromosome or linkage group arm can be used to identify segregants in which individual donor chromosome arms have been transferred into the recurrent parent genetic background. Once QTL mapping has succeeded in identifying flanking markers for QTLs of large effect, these can be used to rapidly bring the MABC program to its logical conclusion—one or more derivatives of the recurrent parent, each carrying a small segment of the donor genome consisting of a QTL for improved drought tolerance (or one of its components) and two flanking markers.

- ***Advantages:*** The major advantage of this procedure is early and rapid recovery of the recurrent parent genotype in nontarget regions. This is made possible by the early onset of the backcrossing program—even before QTL mapping, skeleton mapping, or in extreme cases even determination of parental line marker-polymorphism, have been completed.

Figure 1. Schematic for conventional marker-assisted backcross improvement of terminal drought tolerance in pearl millet inbred line H 77/833-2 based on quantitative trait loci from donor parent PRLT 2/89-33.



After F_2 skeleton mapping, F_3 testcross phenotyping, and QTL mapping, then use marker genotypes to select one or more F_2-derived F_4 bulks (or their F_2-derived F_3 progenitors) for use as drought tolerance donor for marker-assisted improvement of H 77/833-2, and proceed as below:



- ***Disadvantages:*** The down side of this procedure is that if the F_1 used as nonrecurrent parent does not have a marker and QTL genotype identical to that mapped, all of the efforts may go waste.
- ***ICRISAT experience:*** We have used this procedure to transfer the drought tolerance QTL identified on LG2 of PRLT 2/89-33 to H 77/833-2, advancing to generation BC_4F_1, where we have identified progenies likely to segregate for the target QTL and its flanking markers based on marker genotypes of the nonrecurrent parents used to produce them (Table 1). The nonrecurrent parent plants were visually very similar to the H 77/833-2 recurrent parent, so we have high hopes of quickly completing marker-assisted improvement of terminal drought tolerance of this elite male parent of several popular hybrid cultivars.

Contiguous Segmental Substitution Line Sets

A logical extension of the two procedures outlined above is the development of a contiguous segment substitution line ("contig line") set (Fig. 2).

- ***Advantages:*** This procedure will also permit detection of QTLs associated with smaller portions of the phenotypic variability for the target trait than can be detected by phenotyping modest-sized mapping populations. Further, it results in a small set (say 25-35) near isogenic homozygous lines that differ from each other by pairs of introgressed segments. For QTL mapping it will be much less expensive, and probably even more effective, to phenotype this small set of near isogenic substitution lines than a conventional mapping population. Finally, it will be possible to use the substitution line set to map QTLs for many traits that individually would not be worth the effort. An example of this is fertility restoration for the A_1 cytoplasmic-genetic male-sterility system in pearl millet, which we have mapped to LG3 while developing a contig line set of "ICMP 85410" substitutions in the background of elite maintainer line "843B" (Hash, Witcombe, and Kolesnikova-Allen, unpublished).

Figure 2. Graphical genotypes of linkage group 2 substitution line and three derived contiguous segment substitution lines (produced by backcrossing to recurrent parent and selfing out segmental substitution homozygotes).

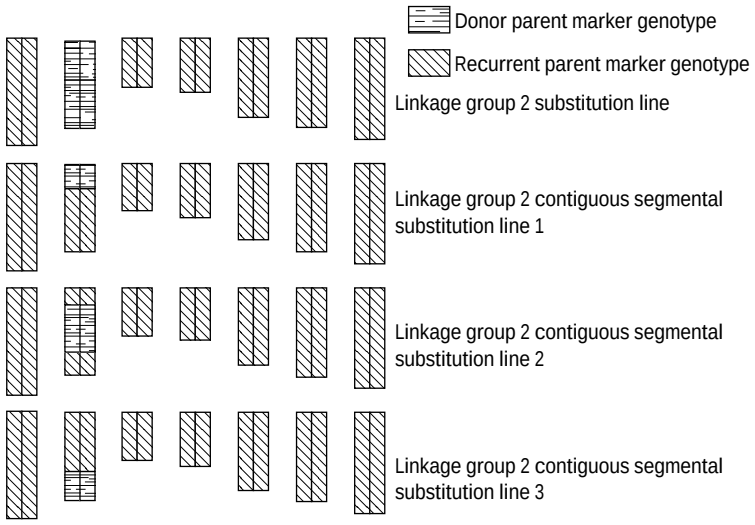


Table 1. Marker genotypes (A = donor allele homozygote; H = heterozygote; B = recurrent parent allele homozygote; - = missing data) of 25 seed parents of most recent generation of jump-started marker-assisted backcrossing program targeting transfer of improved downy mildew resistance (linkage groups 1 and 4) and terminal drought tolerance from PRLT 2/89-33 to elite pearl millet pollinator H 77/833-2. Plant numbers not "bolded" have marker genotypes indicative of crossing failure in the previous generation.

Linkage group	Probe	Enzyme	**BC3F1/**BC2F2 **1**	2	3	4	5	**6**	7	**8**	**9**	**10**	**11**	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
1	PSM757	*Eco*RV	H	A	A	B	H	H	A	H	B	B	H	A	B	B	B	B	B	B	H	H	H	B	B	H	H
	PSM565	*Hind*III	H	A	H	B	H	H	A	H	B	B	H	A	B	B	B	B	B	B	H	H	H	B	B	H	H
	PSM386	*Eco*RI	H	A	A	B	H	H	A	H	H	H	H	A	H	H	H	B	H	H	H	H	H	B	B	H	B
2	PSM322	*Eco*RI	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	PSM214	*Dra*I	B	H	A/H	H	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	PSM321	*Eco*RV	H	H	A/H	H	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
4	PSM716	*Hind*III	H	B	H	H	H	H	H	H?	H	H	H	B	B	B	B	B	B	B	H	H	H	H	H	H	B
	PSM416	*Hind*III/U	H	H	H	B	B	H	B	H	B	B	-	B	H	B	H	B	B	B	-	-	-	-	-	-	-
		*Hind*III/L	H	H	H	A	H	B	B	H	B	B	-	B	B	B	B	B	H	B	-	-	-	-	-	-	-
	PSM612	*Dra*I	H	H	H	-	H?	H	H	H	H	H	B	A	B	B	B	B	B	B	H	B	B	H	B	B	B

- ***Disadvantages:*** These substitution line sets are rather expensive (in terms of both human and operational resources) and time-consuming to produce. Therefore, they are probably not worthwhile unless several of the derived lines are expected to prove economically useful. This in turn will generally require multiple target traits and extremely diverse parents, at least one of which is extremely elite.
- ***ICRISAT experience:*** We have just initiated development of a (reciprocal) contiguous segment substitution line set based on the cross ICMB 841 × 863B (Table 2), and plan to use it for mapping drought tolerance QTLs of small effect.

Recommendation

In pearl millet, for most cost-effective MABCT of a small number of QTLs of large effect, we recommend advancing to BC_2F_2 and BC_3F_1 by advancing five random plants in each of five BC_2F_1 progenies (each derived from a single BC_1F_1 plant having a 50% probability of carrying any given marker or QTL). DNA restriction digests of the 25 advanced generation segregants (BC_2F_2/BC_3F_1 pairs), the donor and recurrent parent, and the "Tift 23DB" standard genotype will fit on a 30-well filter along with molecular weight markers on each end. This gives >90% probability of having advanced any target QTL, located anywhere in the donor parent genome, to BC_3F_1 in the recurrent parent genetic background before spending any resources on marker-genotyping the backcross progenies. Further, once the appropriate BC_3F_1 progeny has been identified for advancement, five plants from it can be randomly advanced to BC_4F_1, and five plants from each of these randomly advanced to BC_4F_2 and BC_5F_1 before the next round of marker genotyping is necessary. This should be followed by two generations of selfing and one more cycle of marker genotyping to produce the desired homozygous substitution lines. If target QTLs have been identified by the time the BC_3F_1 selection must be done, it is possible to get by with just 25 BC_4F_2/BC_5F_1 pairs (and 25 BC_5F_2 plants) per target QTL. If target QTLs have not yet been identified, then the amount of marker genotyping required in later generations will be much larger, and probably not economical except for high value traits of low heritability (Hospital et al. 1997) despite the potential time savings, unless development of a full or partial contiguous segment substitution line set is intended.

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Table 2. List of 19 F_3 progenies, from the (ICMB 841 × 863B)-derived pearl millet mapping population of 160 F_2 individuals, selected as possible starting points for development of a reciprocal set of contiguous segment substitution lines in pearl millet. Selection was based on marker genotype homozygosity for alleles of a given parent across the length of the indicated linkage groups. Terminal drought tolerance differences are expected among linkage group 2 substitution lines (Bold) .

	Target linkage groups													
F_3 progeny	841-1	841-2	841-3	841-4	841-5	841-6	841-7	863-1	863-2	863-3	863-4	863-5	863-6	863-7
F_3-4							X	X						
F_3-12										X	X			
F_3-13				X		X								
F_3-22		**X**						X		X	X	X		
F_3-34	X		X		X	X								
F_3-35			X		X									
F_3-47												X		X
F_3-57		**X**	X					X						
F_3-77					X		X			X				
F_3-85	X								**X**					
F_3-96		**X**				X								
F_3-97				X		X	X							
F_3-101	X											X		
F_3-107			X					X						
F_3-112			X					X						
F_3-122													X	X
F_3-127		**X**		X	X									
F_3-128						X			**X**			X		
F_3-148			X										X	
	Backcross these lines to 863B							Backcross these lines to ICMB 841						

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References

Andrews, D.J., and K. Anand Kumar. 1996. Use of the West African pearl millet landrace Iniadi in cultivar development. *Plant Genet. Resour. Newsl*. 105: 15–22.

FAO and ICRISAT. 1996. *The World Sorghum and Millet Economies: Facts, Trends and Outlook.* Rome, Italy: FAO and Patancheru 502 324, Andhra Pradesh, India.: ICRISAT.

Hash, C.T., and J.R. Witcombe. 1994. Pearl millet mapping populations at ICRISAT. In J.R. Witcombe and R.R. Duncan (eds.) *Use of Molecular Markers in Sorghum and Pearl Millet Breeding for Developing Countries*. London: Overseas Development Administration.Pp. 69–75.

Hospital, F., C. Chevalet, and P. Mulsant. 1992. Using markers in gene introgression breeding programs. *Genetics* 132:1199–1210.

Hospital, F., L. Moreau, F. Lacoudre, A. Charcosset, and A. Gallais. 1997. More on the efficiency of marker-assisted selection. *Theor. Appl. Genet*. 95:1181–89.

Howarth, C., G. Cavan, K. Skøt, R. Yadav, E. Weltzien R., and T. Hash. 1997. Mapping 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*, Sep 22-27 1996, Lubbock, Texas. INTSORMIL Publication No. 97-5. Lincoln, Nebraska: USAID Title XII Collaborative Research Support Program on Sorghum and Pearl Millet (INTSORMIL). Pp. 650–51.

Sedcole, J.R. 1977. Number of plants necessary to recover a trait. *Crop Sci*. 17: 667–68.

Singh, S.D., P. Singh, K.N. Rai, and D.J. Andrews. 1990. Registration of ICMA 841 and ICMB 841 pearl millet parental lines with A1 cytoplasmic-genic male-sterility system. *Crop Sci*. 30: 1378.

Yadav, R.S., C.T. Hash, F.R. Bidinger, and C.J. Howarth. 1999. QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. In *Genetic Improvement of Rice for Water-Limited Environments*. Manila, Philippines: IRRI. (In press)