

Concepts for application of marker techniques in Africa

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Abstract

Cost-effective application of molecular marker technology to agriculturally important problems in Africa cannot be done in isolation. Research workers in Africa must build, with partners elsewhere, on previous findings in order to develop integrated systems to deliver improved cultivars in the minimum possible time and with the minimum additional operational expense. As an example of what may prove to be an appropriate path to pursue in the medium term, 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. ICRISAT's experiences to date in using these methods in pearl millet (*Pennisetum glaucum* (L.) R. Br.) are described. 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 these alternatives are discussed.

Introduction

As this training seminar draws to a close, I have been asked to briefly review the costs of molecular marker techniques and discuss some concepts of how these might be appropriately applied to crop improvement here in Africa. I hope that you will agree, based on the earlier presentations that there are areas where these tools should be brought to bear on agricultural production constraints on this continent. The important questions are biological and economic — which problems should we concentrate on and how are we going to afford to use these technologies that can be so expensive, at least in the development phase? My answer to these questions — not unbiased, I might add — is that we should concentrate in four areas: assessment of genetic diversity where appropriate sets of marker are available, development of high through-put co-dominant marker systems for economically important crops in which these are not already available in the public sector, application of marker-assisted selection

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using previously identified QTLs and their flanking markers, and finally, development of multiple trait-targeted mapping populations in our crops based on parents that are adapted to African agricultural production systems.

Cost-effective application of molecular marker technology to agriculturally important problems in Africa cannot be done in isolation. Research workers in Africa must build, with partners elsewhere, on previous findings in order to develop integrated systems to deliver improved cultivars in the minimum possible time and with the minimum additional operational expense. I will not waste your time here in presenting a summary of how much each type of markers will cost you per individual data point, because these figures are changing day by day, and vary tremendously depending on the number of data points that you need to obtain. In any case, most of us cannot afford to be generating our own marker data—at least in the short term. Applied crop breeders in Africa, like their counterparts in smaller private companies and public sector breeding programs elsewhere in the world, will get much better value for money by contracting out the small jobs that most of us can afford (at least initially) to labs having high through-put capacity and considerable experience in tweeking these marker systems to get them to work. If this means contracting out AFLP marker work to KeyGene, or similar labs elsewhere in developed or developing countries, then so be it. Such service laboratories will be essential to making this technology cost-effective even into the medium term.

Where new mapping populations must be developed targeting traits or trait complexes that are specific to this region, it will be most cost effective to select as parental pairs of each mapping population complementary genotypes that differ for several traits of economic importance. Skeleton mapping of a single population and phenotyping its progeny for several traits will provide more information per unit of operational investment than will attempts to tag genes for these different trait in a larger number of populations based on bulk segregant analysis (BSA) of extreme segregants in each population. Further, these skeleton-mapped populations potentially provide a tool upon which to build future QTL mapping efforts, whereas BSA tagging is not sufficiently powerful to be used in isolation and does not provide as firm a foundation to support future efforts.

As a beginning, where such things exist, I recommend starting with marker-assisted backcrossing into African adapted cultivars of QTLs that have been detected elsewhere for traits of interest in this region. As examples of how this can be done in a cost effective manner, I will describe some of the work ICRISAT has been involved with that attempts to improve pearl millet hybrid parental lines for conditions in south Asia.

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 are 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, with the collaboration of researchers in the UK and India supported by the Plant Sciences Programme of the UK's Department for International Development (DFID), has made a considerable research investment targeting development and application of molecular genetic tools for use in 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 while area sown to this crop has not only decreased, but shifted to more marginal lands--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.

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 (Figure 1). 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 non-target 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 non-target 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 daylength to induce early flowering. Combined with RFLP markers as the only co-dominant marker system currently available, this has lead us to initiate this year a program of MABC based on two mapping progenies from the cross H 77/833-2 × PRLT 2/89-33. 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 from LG6 of H 77/833-2. F₃ plants derived from these skeleton-mapped F₂ selections have been backcrossed to H 77/833-2, and the resulting BC₁F₁ progenies will be backcrossed again, yielding BC₂F₁ 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

In this case backcrossing begins during mapping population development itself, and perhaps even before marker polymorphism of the two parents has been fully characterized. The individual F₁ 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 homozygous 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 non-target 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.
- Disadvantages: The down side of this procedure is that if the F₁ used as non-recurrent 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 (Table 1), advancing to generation BC₄F₁, where we have identified progenies likely to segregate for the target QTL and its flanking markers based on marker genotypes of the non-recurrent parents used to produce them. The non-recurrent 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 Indian 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 (Figure 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 to 35) near-isogenic homozygous lines that differ from each other by pairs of overlapping 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 A₁ 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).
- **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, and any other crop having a relatively short vegetative growth phase, for most cost-effective MABC transfer of a small number of QTLs of large effect we recommend advancing to BC₂F₂ and BC₃F₁ by advancing seven random plants in each of seven BC₂F₁ progenies (each derived from a single BC₁F₁ plant having a 50% probability of carrying any given marker or QTL). DNA restriction digests of the 49 advanced generation segregants (BC₂F₂/BC₃F₁ pairs), the donor and recurrent parent, and the Tift 23DB standard genotype will fit on two 30-well filters along with molecular weight markers on each end. This gives >98% probability of having advanced any target QTL, located anywhere in the donor parent genome, to BC₃F₁ in the recurrent parent genetic background before spending any resources

on marker-genotyping the backcross progenies. Further, once the appropriate BC₃F₁ progeny has been identified for advancement, seven plants from it can be randomly advanced to BC₄F₁, and seven plants from each of these randomly advanced to BC₄F₂ and BC₅F₁ 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 BC₃F₁ selection must be done, it is possible to get by with just 49 BC₄F₂/BC₅F₁ pairs (and 49 BC₅F₂ 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 economic 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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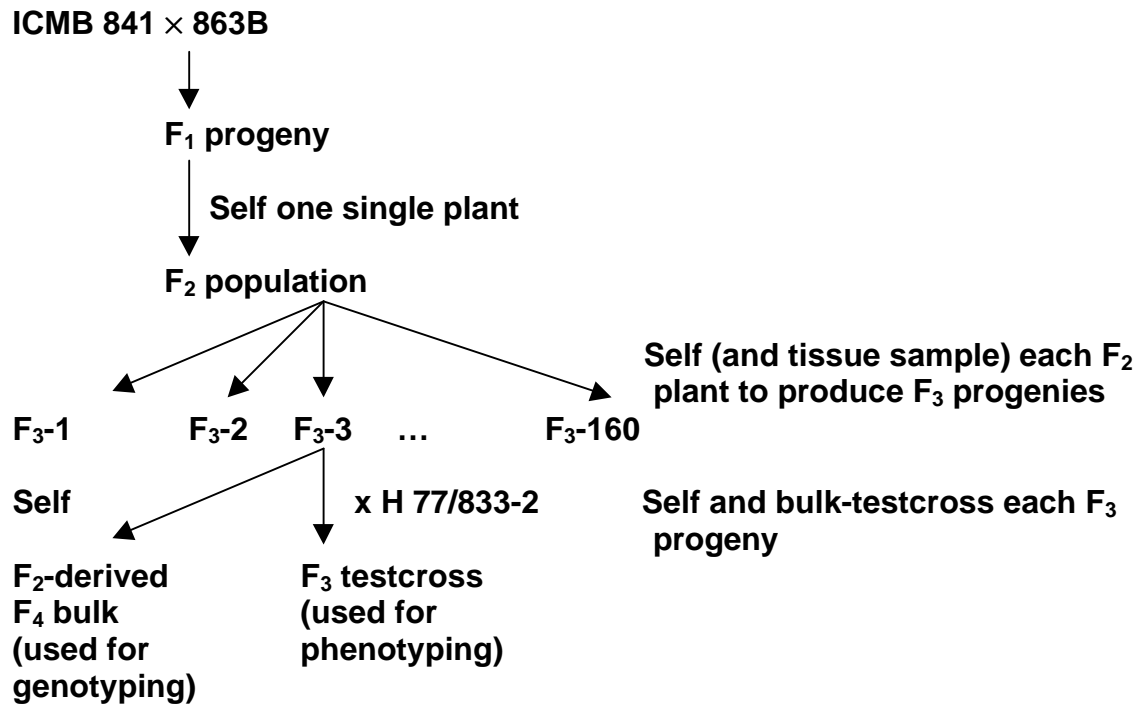


Figure 1. Schematic for conventional marker-assisted backcross improvement of terminal drought tolerance in pearl millet inbred line ICMB 841 based on quantitative trait loci from donor parent 863B.

Figure 1. continued.

After F₂ skeleton mapping, F₃ testcross phenotyping, and QTL mapping, then use marker genotypes to select one or more F₂-derived F₄ bulks (or their F₂-derived F₃ progenitors) for use as drought tolerance donor for marker-assisted improvement of ICMB 841, and proceed as below:

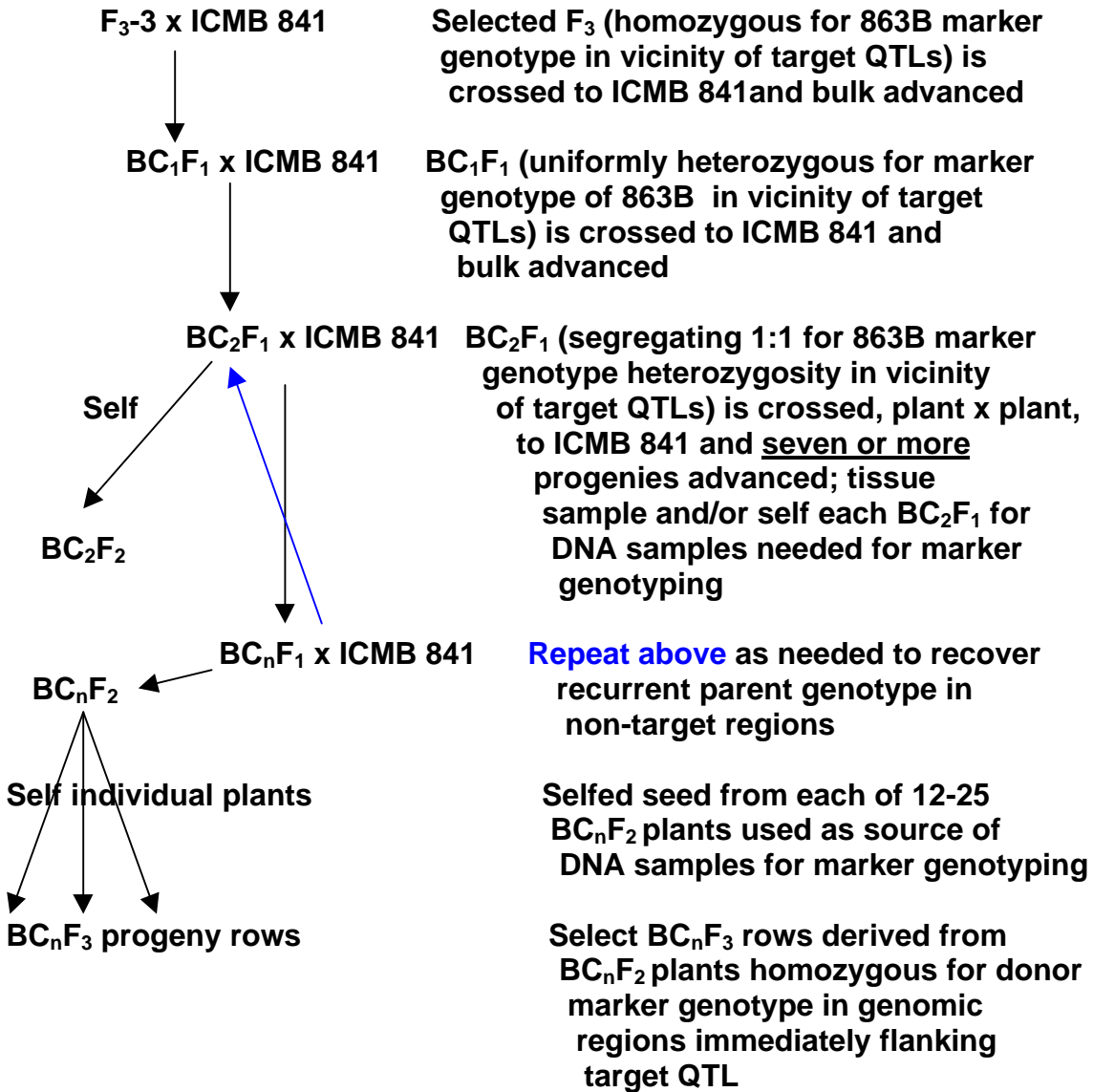


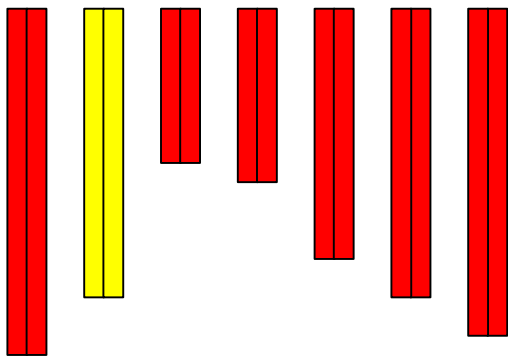
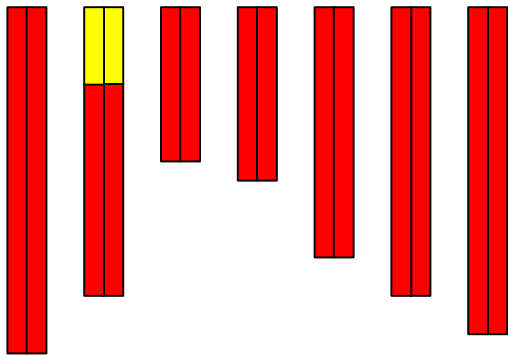


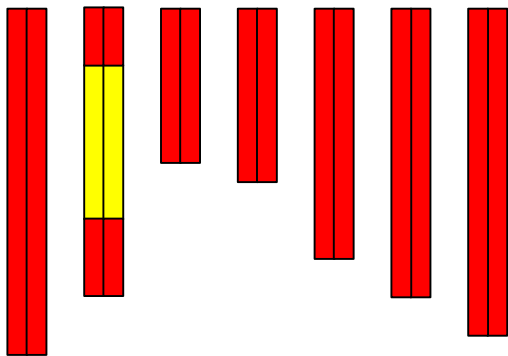
Figure 2. Graphical genotypes of linkage group 2 substitution line and three derived contiguous segment substitution lines (produced by backcrossing to recurrent parent and selfing out segmental substitution homozygotes). Donor parent marker genotype = ; recurrent parent marker genotype =  .



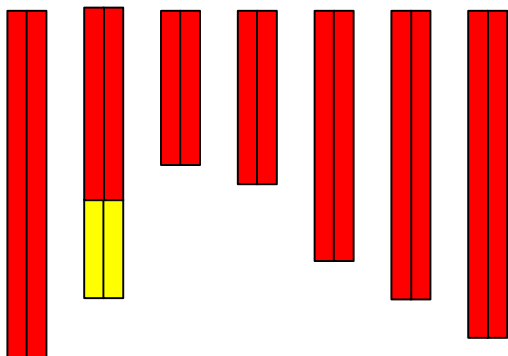
Linkage group 2 substitution line



Linkage group 2 contiguous segmental substitution line 1



Linkage group 2 contiguous segmental substitution line 2



Linkage group 2 contiguous segmental substitution line 3

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. Marker data courtesy of Mr. Arun Sharma.

Link -age group	Probe	Enzyme	BC3F1/BC2F2						BC4F1/BC3F2																		
			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	<i>EcoRV</i>	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	<i>HindIII</i>	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	<i>EcoRI</i>	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	<i>EcoRI</i>	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	<i>DraI</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	<i>EcoRV</i>	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	<i>HindIII</i>	H	B	H	H	H	H	H	H?	H	H	H	B	B	B	B	B	B	H	H	H	H	H	H	H	B
	PSM416	<i>HindIII/U</i>	H	H	H	B	B	H	B	H	B	B	-	B	H	B	H	B	B	B	-	-	-	-	-	-	-
		<i>HindIII/L</i>	H	H	H	A	H	B	B	H	B	B	-	B	B	B	B	H	B	B	-	-	-	-	-	-	-
PSM612	<i>DraI</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	

Table 2. List of 19 F₃ progenies, from the (ICMB 841 × 863B)-derived pearl millet mapping population of 160 F₂ 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 target linkage groups. Terminal drought tolerance differences are expected among substitution lines for linkage group 2 (**Bold**).

F ₃ progeny	Target linkage groups (donor parent-linkage group)													
	<u>841-1</u>	841-2	<u>841-3</u>	<u>841-4</u>	<u>841-5</u>	<u>841-6</u>	<u>841-7</u>	<u>863-1</u>	863-2	<u>863-3</u>	<u>863-4</u>	<u>863-5</u>	<u>863-6</u>	<u>863-7</u>
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