RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2007.33.207

*ScienceAsia* 33 (2007): **207-214**

# Developing KDML105 Backcross Introgression Lines Using Marker-Assisted Selection for QTLs Associated with Drought Tolerance in Rice

**Jonaliza L. Siangliw[a], Boonrat Jongdee[b], Grienggrai Pantuwan[c] and Theerayut Toojinda[a*]**

[a]Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, Kasetsart University, Kamphangsaen, Nakhon Pathom 73140, Thailand.
[b]Ubon Rice Research Center, Muang, Ubon Ratchatani 34000, Thailand.
[c]Rice Research Institute, Chatuchak, Bangkok 10900 Thailand.

* Corresponding author, E-mail: theerayut@dna.kps.ku.ac.th

*Received 9 Feb 2006*
*Accepted 28 Aug 2006*

**ABSTRACT:** Marker-assisted selection (MAS) has been employed to improve the efficiency and speed up breeding programs, specifically in selection. A major use of MAS is in assisting backcrossing of genes/QTLs to elite cultivars. Markers aid in the selection of target alleles and in the assessment of a recipient's parent genome. Introgression and selection of QTLs using molecular markers in foreground selection may have additional problems, since the exact position of the target is often not known. In such cases, QTL detection must be estimated with the expectation that its most likely position is within a confidence interval limit. The backcrossing process with target selection resulted in 103 KMDL105 introgression lines carrying 1, 2 or 3 target combinations, where 79, 20 and 4 lines were derived from KDML105 x IR68586-$F_2$-CA-143 (DH212) (cross 1), KDML105 x IR68586-$F_2$-CA-31 (DH103) (cross 2) and KDML105 x IR68586-$F_2$-CA-54 (DH126) (cross 3) crosses, respectively. Genome scanning revealed that carrier chromosomes in all crosses showed a low percentage of the recipient parent genome. Also, non-carrier chromosomes, especially in crosses 1 and 2, were found to carry segments of the donor, which was reflected in the low percentage of the recipient genome of KDML105 in all crosses. The results proved that MAS aids in the transfer of target segments and may improve the recovery of the recipient genome if background selection is employed.

*Abbreviations:* MAS, marker-assisted selection; QTL, quantitative trait loci; KDML105, Khao Dawk Mali 105; DTS, drought tolerance segment

**KEYWORDS:** backcrossing, marker-assisted selection (MAS), QTL, introgression, drought tolerance

## INTRODUCTION

Drought is a devastating stress, since its effect on the crop may range from a minor reduction of 15% in yield to a loss of more than 50%, which may lead to food scarcity. Rice plants have developed several mechanisms to withstand the effects of drought. These include having short life cycles, the ability of roots to extract water from deeper soil layers and hardpans, producing osmoprotectants and ion scavenging agents, the ability to recover from stress and high desiccation tolerance[1]. Crop improvement program involves the identification of the appropriate plant traits for environmental conditions and farmers' and consumers' preferences[2]. In the rainfed lowland environment, as well as in other types of rice ecosystems, the primary objective of plant breeders is to produce varieties with superior yields. Superior yield is accompanied by superior agronomic traits that make the plant suitable for a particular environment. In a drought or poorly irrigated environments, one of the essential characteristics to attain superior yield is moderately high yield potential[3]. This character is coupled with several agronomic traits like intermediate height, sturdy culms, moderately long and erect leaves, moderate tillering, large panicles with many grains and complete panicle exsertion[2]. There are also shoot-related adaptive traits that help in the maintenance of leaf water potential, like stomatal closure, leaf rolling, a thicker epicuticular wax layer, osmotic adjustment and accumulation of amino acids and growth regulators[4,5,6]. Likewise, root related traits that would increase water uptake, as well as ability to withstand a restricted water reservoir, are also important to the plant under drought stress[5,7]. These root-related traits include a deeper or denser root system, increased root penetration of hardpan and root osmotic adjustment.

Backcrossing is a way by which these genetically

inherent rice characteristics against drought stress can be transferred to an elite line. Backcrossing aided simultaneous discovery and transfer of valuable QTLs from unadapted germplasm to an elite breeding line was demonstrated by Tanksley and Nelson[8]. The same study demonstrated the success of using molecular markers in gene transfer. Huang et al.[9], and Sanchez et al[10] used this method coupled with molecular techniques to select lines carrying resistance genes for bacterial leaf blight and also in pyramiding different loci for this bacterial leaf blight resistance. The use of various molecular markers in maintaining the target genes, fast recovery of the recurrent genome and reducing linkage drag to improve the efficiency of gene transfer with backcross breeding proved the success of using MAS in breeding programs[11]. The use of marker-assisted selection (MAS) has been demonstrated in transferring root traits for drought tolerance[12], selecting low glutelin content in rice[13] and a lot more.

In drought tolerance improvement, the identification and characterization of QTLs controlling the adaptive traits for drought tolerance are necessary to understand the control and expression of these traits. Drought traits like yield, yield components and morpho-physiological characters evaluated in a doubled haploid population from CT9993-5-10-1-M (CT9993) crossed with IR62266-42-6-2 (IR62266) were used to identify QTLs controlling these traits. The QTL information then served as background information in transferring target QTLs to a traditional variety that has high farmer's acceptability and adaptation to the environment.

Khao Dawk Mali 105 (KDML105) is a famous Thai cultivar which has good cooking and eating qualities but is moderately susceptible to drought. Incorporating drought tolerance traits into KDML105 will minimize yield loss under drought conditions in rainfed lowland rice growing areas, particularly in northeast Thailand. Incorporating the drought tolerance traits via introgression or hybridization of KDML105 with doubled haploid lines from a cross between a rice genotype with a good rooting system (CT9993) and a rice genotype with high osmotic adjustment (IR62266), followed by backcrossing to KDML105, will allow the development of a drought tolerant KDML105.

Introgressing the traits of interest can be followed using molecular markers that are mapped flanking or tightly linked with the traits being incorporated. The use of MAS facilitates a faster introgression since plants can be sampled and genotypes with target traits can be identified even at the early stage of development.

The main objective of this study has to develop KDML105 backcross introgression lines with QTLs for drought tolerance. This objective can be achieved by the following approaches: 1) identification of drought tolerance QTL donors 2) introgression of QTLs associated with drought tolerance by means of backcrossing and MAS and 3) evaluation of the introgressed regions by genome scanning.

## Materials and Methods

### Experimental Sites

Planting and backcrossing experiments were done in the Ubon Rice Research Center and Rice Gene Discovery Unit (RGDU), Kasetsart University starting in the year 2001 up to 2004. Marker-assisted selection (MAS) was conducted at RGDU for every generation of backcrossing. Upon reaching $BC_3F_1$ generation, selected lines were allowed to self to identify homozygous individuals ($BC_3F_2$) carrying the target genes using MAS. The $BC_3F_2$ were again allowed to self to produce $BC_3F_3$ introgression lines, which were subjected to genome scanning that was done at RGDU.

### Rice Genotypes Used in the Experiment

Three doubled haploid lines derived from a cross between CT9993 and IR62266 were used as donors of QTLs associated with drought tolerance. The selection was based on good phenotypic performance of the lines in terms of agronomic and physiological traits that were evaluated under line source sprinklers at the Ubon Rice Research Center in 2000. Lanceras et al.[14] described the experimental design, growth conditions and evaluations and measurements of traits. In the study conducted by Lanceras et al.[14], flowering time of test lines was synchronized by staggered planting and the lines were subjected to varying degrees of water created by line source sprinklers. The plants were irrigated from transplanting until late vegetative stage. Water was drained out of the field at the start of panicle initiation to impose stress at the reproductive stage. Leaf water potential, leaf rolling and drought score were measured and scored starting from water drainage until the plants experienced stress. Flowering date was also recorded from the emergence of the first panicle up to 100% flowering. At mature stage, grain yield and yield components were measured[14]. Initial genome analysis revealed that these doubled haploid lines carry the alleles of the parents contributing to QTLs for drought tolerance or good agronomic performance under drought. These doubled haploids included 1) IR68586-$F_2$-CA-31 (DH103) 2) IR68586-$F_2$-CA-54 (DH126) and 3) IR68586-$F_2$-CA-143 (DH212) that served as donors and were crossed to KDML105 as recipient of QTLs for drought tolerance. One hundred and three $BC_3F_3$ backcross introgression lines of KDML105 were generated by backcrossing and marker-assisted selection.

**Table 1.** Agronomic and physiological performance of selected donor lines under irrigated and drought stress conditions in the 2000 wet season.

| Trait | CT9993 | IR62266 | KDML 105 | Mean of Population | IR68586 -F2-CA-31 | IR68586 -F2-CA-54 | IR68586 -F2-CA-143 |
|---|---|---|---|---|---|---|---|
| Grain yield-W0 (t/ha) | 1.57 | 3.34 | 1.867 | 2.01 | 1.68 | 2.23 | 2.46 |
| Grain yield-W4 (t/ha) | 0.52 | 0.20 | 0.902 | 0.61 | 0.58 | 0.70 | 1.12 |
| Biological yield -W0 (t/ha) | 4.28 | 8.16 | 7.08 | 5.47 | 4.54 | 5.66 | 6.48 |
| Biological yield -W4 (t/ha) | 3.03 | 4.74 | 5.12 | 3.92 | 4.78 | 3.73 | 3.80 |
| Harvest index-W0 | 0.36 | 0.42 | 0.27 | 0.37 | 0.37 | 0.39 | 0.38 |
| Harvest index-W4 | 0.15 | 0.03 | 0.18 | 0.14 | 0.12 | 0.19 | 0.30 |
| Days to flowering *-W0 | 26 | 30 | 20 | 24 | 33 | 25 | 23 |
| Days to flowering *-W4 | 42 | 55 | 24 | 38 | 32 | 28 | 23 |
| Total number of spikelet-W0 | 127 | 94 | 120 | 105 | 104 | 86 | 92 |
| Total number of spikelet-W4 | 122 | 86 | 100 | 106 | 85 | 84 | 96 |
| Spikelet sterility -W0 (%) | 33.7 | 28.3 | 47.8 | 36.7 | 26.0 | 37.2 | 34.1 |
| Spikelet sterility -W4 (%) | 49.5 | 62.4 | 59.8 | 58.6 | 40.8 | 47.5 | 45.0 |
| Panicle numberW4 (-hill) | 5 | 11 | 5.8 | 9 | 8 | 10 | 8 |
| Panicle numberW4 (-hill) | 5 | 8 | 6.1 | 8 | 6 | 9 | 9 |
| Leaf water potential -early stress (MPa) | -1.68 | -1.92 | -2.03 | -1.83 | -1.72 | -1.47 | -1.55 |
| Leaf water potential -late stress (MPa) | -2.04 | -2.40 | -2.30 | -2.16 | -1.88 | -1.70 | -2.15 |
| Drought response index-W1 | -0.14 | -0.07 | -1.6412 | 0.00 | 0.51 | -0.42 | -0.19 |
| Drought response index-W4 | 0.44 | -1.45 | 0.4048 | -0.02 | 0.59 | 0.54 | 0.91 |

* days after the initiation of water gradient.
W0 – control condition[14].
W4 – severe stress[14].

**Table 2.** Allelic composition of donor lines in peak markers associated with drought-related traits in the selected drought tolerance segments.

| Chromosome | Trait[a] | Marker[b] | Allele | IR68586 -F2-CA-31 | IR68586 -F2-CA-54 | IR68586 -F2-CA-143 |
|---|---|---|---|---|---|---|
| 1 | PN | CDO345 | IR[c] | CT | CT | CT |
| (49 cM)* | PN | CDO345 | IR | CT | CT | CT |
| | PN | CDO345 | IR | CT | CT | CT |
| | PH | RG109 | CT[d] | CT | CT | CT |
| | PH | RG109 | CT | CT | CT | CT |
| | PH | RG109 | CT | CT | CT | CT |
| | CT | RM5794 | CT | CT | CT | CT |
| | DS | RM165 | CT | CT | CT | CT |
| | DS | RM165 | CT | CT | CT | CT |
| | DS | RM165 | CT | CT | CT | CT |
| | LWP | RG109 | CT | CT | CT | CT |
| 3 | GY | EM11_9 | CT | CT | CT | CT |
| (14.8 cM)* | HI | RM81 | CT | CT | CT | CT |
| | DFAIG | EM11_9 | CT | CT | CT | CT |
| | DFAIG | EM11_9 | CT | CT | CT | CT |
| | DFAIG | EM11_9 | CT | CT | CT | CT |
| | DFAIG | RM1026 | CT | CT | - | CT |
| 4 | GY | RZ565 | IR | CT | CT | IR |
| (53 cM)* | GY | RZ565 | IR | CT | CT | IR |
| | GY | RZ565 | IR | CT | CT | IR |
| | TSN | RG476 | CT | CT | CT | CT |
| | TSN | RG476 | CT | CT | CT | CT |
| | PSS | C335 | IR | CT | CT | IR |
| | PN | RG476 | IR | CT | CT | CT |
| | PN | RG476 | IR | CT | CT | CT |
| | PN | RG476 | IR | CT | CT | CT |
| 8 | BY | ME2_11 | IR | IR | IR | IR |
| (60 cM)* | PSS | G2132 | IR | CT | IR | IR |
| | PSS | G2132 | IR | CT | IR | IR |
| | PN | EM14_1 | IR | CT | IR | CT |
| | PN | EM14_1 | IR | CT | IR | CT |
| | PN | EM14_1 | IR | CT | IR | CT |
| | PH | RZ997 | CT | CT | IR | CT |
| | PH | RZ997 | CT | CT | IR | CT |
| 9 | BY | ME9-3 | IR | IR | IR | CT |
| (30 cM)* | HI | RM1026 | CT | CT | - | CT |
| | DS | RG667 | CT | CT | CT | CT |

* refers to size of drought tolerance segment.
[a] traits clustering in the drought tolerance segment:
PN – panicle number per hill; PH – plant height (cm); CT – canopy temperature (°C); DS – rought score; LWP – leaf water potential (MPa); GY – grain yield (t/ha); HI – harvest index; DF AIG – days to flowering after iniation of water radient (d); TSN – total spikelet numb er; PSS – percent spikelet sterility (%); BY – biological yield (t/ha).
[b] peak marker associated with the trait.
[c] IR allele at a particular marker associated with the trait.
[d] CT allele at a particular marker associated with the trait.

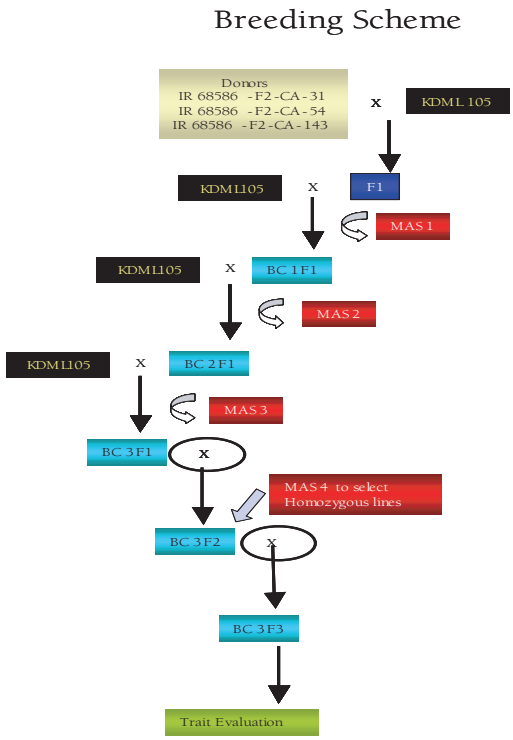


**Fig 1.** Breeding scheme used in the development of KDML105 backcross introgression lines using marker-assisted selection.

## Identification of Donor Lines and Generation of Introgression Lines by Backcrossing and MAS Methods

Three doubled haploid lines were selected as donors of QTLs associated with drought tolerance. The selection was based on phenotypic performance of doubled haploid lines and the alleles they carry in the drought tolerance segments of chromosomes 1, 3, 4, 8 and 9 based on QTL analysis. The phenotypic scores and allelic composition of doubled haploid lines are presented in Tables 1 and 2. The doubled haploid lines and KDML105 were crossed to get $F_1$ and the generation of subsequent backcrosses is presented in Figure 1. In each backcross generation, selected markers in the

regions were used to hasten the selection of lines carrying the QTLs of interest. The markers that were used in MAS are as follows:

Target 1: RM104, RM102 and P-3 (a marker developed by masking repeats in BAC clones) for the drought QTLs on chromosome 1

Target 2: RM81 and RM231 for drought QTLs on chromosome 3

Target 3: RM252, RM317 and RM470 for drought QTLs on chromosome 4

Target 4: RM515, RM80 and RM447 for drought QTLs on chromosome 8

Target 5: RM215, RM201 and RM242 for drought QTLs on chromosome 9

Target 6: waxy and RM204 flanking waxy gene

Target 7: GT11 and RM121 for QTL conferring gelatinization temperature

Target 8: 10L03FW, E03.92, RM223 and BADH for aroma QTL

At the $BC_3F_2$ generation, individual $F_2$ plants from each family were genotyped to identify homozygous lines carrying the desired QTL. The number of lines for each drought tolerance segment is listed in Table 3. There were 103 $BC_3F_2$ homozygous lines that were selected and allowed to self again to generate $BC_3F_3$ that was used in the genome scan analysis.

### Genome Scan Analysis by Genotyping $BC_3F_3$ Generation

The markers used in each chromosome were selected every 30 cM distance. The number of markers for each chromosome is listed in Table 4. The genotype at each marker such as homozygous donor, homozygous KDML105 and heterozygous, was identified in the 103 KDML105 backcross introgression lines. Counting the number of a particular allele type, dividing it with the total allele number and multiplying the value with 100% resulted in percentage of allele for every chromosome.

**Table 3.** Types of drought tolerance segments and number of KDML105 backcross introgression lines developed.

| No. of DTS * | DTS[a] | No. of lines |
|---|---|---|
| 1 | 1 | 28 |
| 1 | 3 | 13 |
| 1 | 4 | 15[b] |
| 1 | 8 | 10 |
| 1 | 9 | 7 |
| 2 | 13 | 7[b] |
| 2 | 14 | 8 |
| 2 | 18 | 1 |
| 2 | 19 | 2 |
| 2 | 34 | 10 |
| 2 | 39 | 1 |
| 3 | 134 | 2 |
| 3 | 139 | 1 |
| | TOTAL | 105 |

* number of combinations of drought tolerance segments

[a] represents the chromosome segments in single or in combination: 1–chromosome 1; 3-chromosome 3; 4–chromosome 4; 8–chromosome 8; and 9–chromosome 9.

[b] one line each from DTS 4 and 13 did not germinate making the total 103 for genome scan.

## Results and Discussion

### Identification of Donor Lines

In Thailand, the development of KDML105 introgression lines particularly for drought tolerance has been given great attention since drought affected regions in Thailand, especially in the northeast, are quite large. Breeding for drought tolerance has long been employed in improving varieties like KDML105. The variable conditions of rainfed lowland impede the development of suitable lines for this environment. Developing lines tolerant to drought by conventional method can be hastened by using marker-assisted selection or MAS. It is known that MAS is effective for genes or QTLs with large variations in phenotype[15]. However, many and small QTLs control the expression of drought-related traits[1,14,16]. Likewise, environment has a large influence on these traits[17,18]. Introgression lines for KDML105 have been developed using MAS to quickly transfer QTLs for drought tolerance traits. Also, the effectiveness of MAS was assessed for QTLs explaining small variations in the phenotype like drought-related traits.

The choice of donor is important in the development of improved lines. As mentioned earlier, drought tolerance traits are highly affected by the environment. To choose the donor, it is important that phenotypic evaluation be done several times before selecting the tolerant line that can eventually be a candidate donor. In doing so, the repeatability of expression of tolerance may be attributed to genetic effects, thus the probability of transferring QTLs for drought tolerance is higher. A doubled haploid (DH) population generated from a cross between CT9993 and IR62266 was evaluated for phenotypic performance under drought conditions. This population has been examined for several years and at several locations[1,14,16]. Table 1 shows the performance of DH lines under irrigated and stress

**Table 4.** Number of molecular markers used in genome scanning of KDML introgression lines

| chrom* | No. of markers |
|---|---|
| 1 | 22 |
| 2 | 10 |
| 3 | 19 |
| 4 | 19 |
| 5 | 8 |
| 6 | 7 |
| 7 | 9 |
| 8 | 13 |
| 9 | 13 |
| 10 | 11 |
| 11 | 8 |
| 12 | 11 |
| TOTAL | 150 |

* - refers to chromosome number

conditions. It can be noted that IR62266 had greater values for most agronomic traits than CT9993 under irrigated condition. But, under stress conditions, CT9993 performed better than IR62266. Three DH lines were selected and their performance under the irrigated condition was equal to or higher than CT9993 for some traits. Grain yields of IR68586-F2-CA-31 (DH103) (1.68 t/ha), IR68586-F2-CA-54 (DH126) (2.23 t/ha) and IR68586-F2-CA-143 (DH212) (2.46 t/ha) were higher than CT9993 (1.57 t/ha). Biological yield and harvest index had the same trend as grain yield. Comparing the flowering date of the selected lines, DH103 flowered late (33 d), while DH126 (25 d) and DH212 (23 d) as well as CT9993 (26 d) are early flowering types under normal conditions. Stress delayed the flowering time of the parents, although it did not affect the flowering time of the selected lines. Since early flowering is a strategy to escape drought stress, lines developed from these donors must have flowering dates nearly the same as KDML105. The numbers of effective panicles of the donor lines were comparatively higher than those of CT9993 or KDML105 in both irrigated and stress conditions. In addition, the percentage of spikelet sterility, a better grain yield indicator of DH103, DH126 and DH212 were lower than those of the parents as well as KDML105. Physiologically, leaf water potential of three selected lines (-1.88, -1.70 and -2.15 MPa for DH103, DH126 and DH212, respectively) were higher than the parents (-2.04, -2.40 and –2.30 MPa for CT9993, IR62266 and KDML105, respectively) at late stressing period, except for DH212 and CT9993 wherein CT9993 had slightly higher leaf water potential than DH212. Good leaf water potential coupled with good agronomic traits and early flowering will ensure the tolerance of a line under intermittent or late season drought[19]. Selection criteria based on phenotypes mentioned above supported the choice of the DH lines as donors in developing introgression lines with KDML105 background.

Phenotypic selection was also coupled with genotypic selection based on the alleles contributed by the selected lines after determining QTLs controlling drought responsive traits. Five genomic regions were identified as QTL-dense regions because many QTLs for several traits were coincidentally located. These regions included one on chromosome 1 with an estimated length of 49 cM flanked by the RG975 and RM14 markers towards the long arm of the chromosome. This region contained QTLs for leaf water potential, panicle number, total spikelet number, drought score, canopy temperature and plant height. It can be seen in Table 2, that most of QTLs for the traits mentioned were contributed by CT9993 except for panicle number. The selected donor lines had CT9993 alleles present in the markers that were highly associated with the traits in this region. Another region selected for introgression was that in chromosome 3 flanked by RG104 and RG409, which covers a total length of 14.8 cM. This segment contained QTLs for grain yield, harvest index and days to flowering. All of these traits were controlled by CT9993 and all selected lines had CT9993 alleles in this region. The region of chromosome 4 selected for introgression contained QTLs for grain yield, total spikelet number, percentage of sterile spikelet and panicle number. Most of the QTLs were contributed by IR62266, except for total spikelet number. Unfortunately, donors DH103 and DH126 had CT9993 alleles for the QTLs in this chromosome. DH212 had IR62266 alleles for some of the QTLs. This region was selected because QTLs for grain yield under stress were identified as contributed by CT9993 in chromosome 3 and by IR62266 in chromosome 4. Another interesting region for introgression was the segment flanked by markers ME5_5 to RG598 on chromosome 8. Biological yield, percent spikelet sterility, panicle number and plant height were located in this region. All QTLs except for plant height were contributed by IR62266. DH126 had IR62266 alleles for all of the QTLs. DH212 had QTLs for biological yield and percent spikelet sterility which were also contributed by IR62266. In another QTL mapping study for drought traits, this region was found associated with QTLs for osmotic adjustment[20]. Lastly, a region in chromosome 9 with a length of 30 cM was also selected for introgression. QTLs located in this region included biological yield, harvest index and drought score. The chromosomal locations of QTLs for the traits mentioned in chromosome 9 were found to be similar to the QTLs identified by Chandra Babu et al.[16]. Most of the QTLs in this segment of chromosome 9 were contributed by CT9993 and all selected donor lines had CT9993 alleles for the markers highly associated with the QTLs for the traits in this chromosome. Thus, CT9993 alleles contributing for QTLs in segments of chromosomes 1, 3 and 9 were chosen while IR62266 alleles were selected for chromosomes 4 and 8. The selection of the contributing alleles in each segment may be expected to be introgressed, since the selected donors mostly contained the desired alleles for the QTLs. It is also expected that other desirable traits may be lost due to the opposite directions of the alleles.

Combining phenotypic and genotypic information in the selection may strengthen the chance of introgressing the desired traits to improve an elite line.

## Generation of Introgression Lines by Backcrossing and MAS Methods

The three selected donors of drought tolerance segments were crossed with KDML105 and the resulting

$F_1$s were then crossed to KDML105 again to generate the first backcross generation. The process of generating the introgression lines is presented in Figure 1. Holland[21] suggested that markers are useful in selection with backcrossing in three ways. First, they effectively aid in the selection of target alleles with effects that are difficult to observe phenotypically. Second, they can be used to identify introgression lines with chromosomes containing target alleles with small amounts of surrounding DNA from the donor parent, therefore reducing linkage drag. Lastly, markers permit the selection of progeny with a high percent of recurrent parent.

The markers used in the selection were the flanking markers associated with the QTLs. According to Holland[21], the use of flanking markers may increase the chance of selecting lines containing the target more than using a single marker for selection. Co-dominant markers, like simple sequence repeats (SSR), were used to easily employ MAS. Also, selection among the introgression progeny involves the selection for heterozygous progeny, which can only be achieved with co-dominant markers. As mentioned earlier, the application of MAS for genes or QTLs of large effects in generating introgression lines may be more effective. In Lanceras et al.[14], most of the agronomic traits had LOD scores of 3 and above with heritability of >40%. With the LOD score values of the QTLs and heritability where most of the traits were controlled by 4 or less QTLs, MAS may be substantially more efficient than phenotypic selection[21]. But, for the other traits with LOD score lower than 3, the possibility of transferring those traits may be low. According to Hospital and Charcosset[22], in general, it is illusive to plan to manipulate more than three or four QTLs simultaneously in a MAS backcrossing program. In this study, the introgression of chromosomal segments containing QTLs for at least four traits were coincidentally located. MAS and introgression processes were continued, since QTLs for most of the traits identified in this population were also identified in other QTL studies[1,16]. These mapping studies were performed in different locations and conditions using the same population, and locating coincidentally the QTLs from different studies denoted the most likely positions of the QTLs.

Near isogenic lines for drought specifically for root traits were also developed in rice and the effect of the introgressed regions with grain yield were assessed[23]. At the end of the introgression, the numbers of lines generated for single, double or triple drought tolerance segment combinations are shown in Table 3. For double drought tolerance segments, the combinations identified were 13 (indicating a combination of segments from chromosomes 1 and 3), 14, 18, 19, 34 and 39. On the other hand, three drought tolerance segment combinations were 134 and 139. All triple drought tolerance segment combinations were derived from DH212, while most of the double combinations were also from DH212, except for the combination 18 that was derived from DH103. DH212 was the source of single drought tolerance segments for chromosomes 1, 3, 4 and 9. A four DTS combination (1349) was not identified, probably because the number of lines generated was too small to identify a recombinant line containing 4 DTS combination. It may be assumed that introgression lines developed with DTS combination lower than DH212 may be less tolerant to drought stress. Exposing the developed lines and parents to drought stress can test this assumption. DH103, on the other hand was the source for a single drought tolerance segment for chromosome 1 and the only source for chromosome 8. Single drought tolerance segment for chromosome 9 was also derived from DH126.

## Genome Scans of Introgression Lines: Background Screening

After the generation of $BC_3F_2$ lines that were homozygous for donor segments and for cooking and eating qualities of KDML105, selected lines were

**Table 5.** Percent of alleles in KDLM105 x DH212, KDML105 x DH103 and KDML105 x DH126 crosses, as revealed by genome scan.

| Cross | Percent Allele | Chrom 1 | Chrom 2 | Chrom 3 | Chrom 4 | Chrom 5 | Chrom 6 | Chrom 7 | Chrom 8 | Chrom 9 | Chrom 10 | Chrom 11 | Chrom 12 | Whole Genome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KDML 105 x DH212 | KDML 105 | **57.5*** | 95.2 | **69.4** | **57.3** | 59.7 | 93.9 | 100.0 | 99.2 | **84.3** | 100.0 | 90.3 | 93.7 | 76.7 |
| | Heterozygous | **16.7** | 0.0 | **8.5** | **8.7** | 10.7 | 2.5 | 0.0 | 0.6 | **4.8** | 0.0 | 5.7 | 1.7 | 7.0 |
| | DH212 | **18.8** | 0.0 | **16.8** | **29.1** | 26.8 | 3.5 | 0.0 | 0.2 | **7.1** | 0.0 | 3.6 | 4.6 | 12.9 |
| | missing data | **7.1** | 4.8 | **5.3** | **4.8** | 2.9 | 0.0 | 0.0 | 0.0 | **3.8** | 0.0 | 0.4 | 0.0 | 3.4 |
| No. of lines | 79[a] | | | | | | | | | | | | | |
| No. of markers used | 84 | | | | | | | | | | | | | |
| KDML 105 x DH103 | KDML 105 | **82.9** | 93.0 | 91.7 | 72.2 | 76.0 | 93.8 | 70.0 | **69.4** | 81.9 | 80.6 | 91.7 | 100.0 | 81.9 |
| | Heterozygous | **1.7** | 2.0 | 2.2 | 7.2 | 16.0 | 2.5 | 7.5 | **3.3** | 6.3 | 6.9 | 0.0 | 0.0 | 4.7 |
| | DH103 | **8.3** | 4.0 | 0.6 | 12.8 | 6.0 | 3.8 | 13.8 | **10.6** | 0.0 | 9.4 | 5.0 | 0.0 | 6.6 |
| | missing data | **7.1** | 1.0 | 5.6 | 7.8 | 2.0 | 0.0 | 8.8 | **16.7** | 11.9 | 3.1 | 3.3 | 0.0 | 6.8 |
| No. of lines | 20 | | | | | | | | | | | | | |
| No. of markers used | 79 | | | | | | | | | | | | | |
| KDML 105 x DH126 | KDML 105 | 93.2 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | **71.9** | 85.7 | 100.0 | 100.0 | 93.5 |
| | Heterozygous | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **3.1** | 10.7 | 0.0 | 0.0 | 1.4 |
| | DH126 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **15.6** | 3.6 | 0.0 | 0.0 | 3.1 |
| | missing data | 6.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **9.4** | 0.0 | 0.0 | 0.0 | 2.1 |
| No. of lines | 4 | | | | | | | | | | | | | |
| No. of markers used | 73 | | | | | | | | | | | | | |

* - italicized represents the chromosomes with targets introgressed

[a] – 79 lines were used instead of 81, because 2 lines did not grow

allowed to generate $BC_3F_3$ generation. The number of lines having combinations of drought tolerance segments is presented in Table 3. This generation was subjected to genome scanning, for which the percentage of alleles of the parents are presented in Table 5. Genome scanning using molecular markers simply estimates the recipient genome contents of the introgression lines. Scoring the genotype at a collection of markers over the genome and taking the ratio of the number of markers homozygous for the recipient allele over the total number of the markers estimates the recipient genome contents.

A genome scan was performed for each cross. Seventy-nine, twenty and four $BC_3F_3$ lines were scanned for KDML105 x DH212, KDML105 x DH103 and KDML105 x DH126 crosses, respectively. In the first cross, the segments introgressed were derived from chromosomes 1, 3, 4 and 9. It can be noted in the genome scan of 79 lines using 84 markers that segments of introgression for the chromosomes listed above showed low percentages of KDML105 alleles, as compared with other chromosomes. It is evident that in backcrossing focusing on the target where no selection is made for the background may introduce a genetic feature of backcross breeding known as linkage drag in the carrier chromosomes and additional segments of the donor in the non-carrier chromosomes. Linkage drag refers to the introduction of either deleterious or beneficial genes along with the target regions during backcrossing. The amount of wild or alien DNA introgressed into KDML105 was monitored using markers that were evenly distributed along the genome. The evidence showed that 27% of the donor segments of chromosome 5 were transferred and only 60% of the recipient parent was recovered. Chromosomes 6, 8, 11 and 12 shared small portions of the donor (3.5, 0.2, 3.6 and 4.6 percent, respectively).

The KDML105 x DH103 cross had introgressed segments on chromosomes 1 and 8. This cross includes 20 lines. As expected, chromosome 8 had the least percentage of background alleles. Only chromosome 12 had 100 percent KDML105 background. The rest of the chromosomes contained donor segments ranging from 0.6 to 13.8 percent. Likewise, the introgression lines under this cross have segments of donors in many parts of the genome other than the target. Those regions may contain genes or QTLs that may contribute to tolerance, which were not targeted during the introgression and selection processes. The effects of those regions may be seen after evaluating the introgression lines in the drought condition.

Lastly, the cross between KDML105 and DH126 contained only the chromosome 9 DTS with only 4 individual lines were included in this cross. Only chromosomes 9 and 10 contained the donor segments for this cross and the rest has 100 percent KDML105 background.

The results of the genome scan show that the segments of target had the highest portion of the donor. This may reflect that markers used in MAS may effectively transfer the targets. The results also reflect the importance of background selection using markers for non-carrier chromosomes. Hospital[24] discussed that 99% recipient genome content may be fully recovered at $BC_4$ with selection. This percentage of the recipient genome can only be achieved in $BC_6$ with no selection on markers. Since no selection of the background was employed, donor segments were still present in most non-carrier chromosomes examined by the genome scan. In order to reduce those segments, lines that have high recipient content, based on the genome scan and contain the target segment may be backcrossed again to get higher percentages of the recipient genome.

The generated backcross introgression lines of KDML105 must be evaluated for phenotypic performance under irrigated and stress conditions to fully assess the success in transferring drought tolerance segments using marker-assisted selection.

## Conclusion

The application of molecular genetics in breeding programs is bounded by the precision of the effects of associated markers as well as by the cost effectiveness of marker-assisted selection.

Marker-assisted selection was found useful in developing genotypes with combinations of favorable alleles. The process of introgression enables the study of specific genomic regions affecting agronomically important traits and also estimation of the QTL effects, which are often difficult to obtain because of the influence of environments and genetic backgrounds. Therefore, it is necessary to have an accurate evaluation of QTLS effects in varying environments before initiating an introgression program. Once the introgression population is made, it may provide an attractive tool to select candidate genes in the annotated data of the sequenced genome corresponding to the QTL.

It is also suggested in backcross introgression programs using MAS that selection should be done not only for the target segment but also for the background or in non-carrier chromosomes. Selection for the background may eliminate numerous unwanted genes coming from the donor as well as linkage drag.

## Acknowledgements

The authors are grateful to the Rockefeller Foundation for financial support extended to this project.

## REFERENCES


1. Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripath JN and Sarial AK, et al (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theor Appl Genet.* **103**, 19-29.
2. Mackill DJ, Coffman WR and Garrity DP (1996) Rainfed lowland rice improvement, pp 1-24. International Rice Research Institute, Los Banos, Philippines.
3. Fischer KS and Fukai S (2003) How rice responds to drought. In: *Breeding Rice for Drought-Prone Environments* (Edited by Fischer KS, Lafitte R, Fukai S, Atlin G and Hardy B), pp 32-6. International Rice Research Institute, Los Banos, Philippines.
4. O'Toole JC, Cruz RT and Seiber JN (1979) Epicuticular wax and cuticular resistance in rice. *Physiol Plant* **47**, 239-44.
5. Mitra J (2001) Genetics and genetic improvement of drought resistance in crop plants. *Cur Sci* **80**, 758-63.
6. Price AH, Cairns JE, Horton P, Jones HG and Griffiths H (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTLS approach: progress and new opportunities to integrate stomatal and mesophyll responses. *J Exp Bot* **53**, 989-04.
7. Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K and Jin D, et al (2006) Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics* **172**, 1213-28.
8. Tanksley SD and Nelson JC (1996) Advanced backcross QTLS analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor Appl Genet* **92**, 191–03.
9. Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G and Kumarvadivel N, et al (1997) Pyramiding of bacterial leaf blight resistance genes in rice marker assisted selection using RFLP and PCR. *Theor Appl Genet* **95**, 313-20.
10. Sanchez AC, Brar DS, Huang N, Li Z and Khush GS (2000) Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci* **40**, 792-97.
11. Hospital F (2005) Selection in backcross programmes. *Phil Trans R Soc B* **360**, 1503-12.
12. Li Z, Shen LS, Courtois B and Laffitte R (1999) Development of near isogenic introgression line (NIIL) sets for QTLs associated with drought tolerance in rice. Workshop in molecular approaches for the genetic improvement of cereals for stable production in water-limited environments. CIMMYT Headquarters, Mexico. http://www.cimmyt.org/ABC/map/research_tools_results/wsmolecular/workshopmolecular/WSdroughtDevelopmentnear.htm
13. Wang YH, Liu SJ, Ji SL, Zhang WW, Wang CM, Jiang L and Wan JM (2005) Fine mapping and marker-assisted selection (MAS) of a low glutelin content gene in rice. *Cell Res* **15** (8), 622-30.
14. Lanceras JC, Pantuwan G, Jongdee B and Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiol* **135**, 384-99.
15. Spelman RJ and van Arendonk JAM (1997) Effect of inaccurate parameter estimates on genetic response to marker-assisted selection in an outbred population. *J Dairy Sci* **80**, 3399-10.
16. Chandra Babu R, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Peyaprakash P and Ganesh SK, et al (2003) Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. *Crop Sci* **43,** 1457-69.
17. Wade LJ, McLaren CG, Quintana L, Harnpichitvitaya D, Rajatasereekul S, Sarawgi AK and Kumar A, et al. (1999) Genotype by environment interactions in diverse rainfed lowland rice environment. *Field Crops Res* **64**, 35-50.
18. Lafitte R, Blum A and Atlin G (2003) Using secondary traits to help identify drought-tolerant genotypes. In: *Breeding Rice for Drought-Prone Environments.* (Edited by Fischer KS, Lafitte R, Fukai S, Atlin G and Hardy B), pp 37-48. International Rice Research Institute, Los Banos, Philippines.
19. Jongdee B, Fukai S and Cooper M (2002) Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *Field Crops Res* **76**,153-63.
20. Zhang J, Nguyen HT and Blum A (1999) Genetic analysis of osmotic adjustment in crop plants. *J Exp Bot* **50,** 291-02.
21. Holland JB (2004) Implementation of molecular markers for quantitative traits in breeding programs- challenges and opportunities. Proceedings of the 4th International Crop Science Congress, Brisbane, Australia. http://www.cropscience.org.au/icsc2004/symposia/3/4/203_hollandjb.htm
22. Hospital F and Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. *Genetics* **147**, 1469-85.
23. Shen L, Courtois B, McNally K, Robin KL and Li Z (2001) Evaluation of near-isogenic lines of rice introgressed with QTLSs for root depth through marker-aided selection. *Theor Appl Genet* **103,** 75-83.
24. Hospital F (2001) Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. *Genetics* **158**, 1363-79.