

# Marker-assisted selection of four root QTL and aroma to improve Kalinga III: Evaluation of pyramid lines and advanced bulks from backcrossing

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## Abstract

The aim of this work was to improve early season drought tolerance in the upland rice variety Kalinga III, a variety that was not used for mapping drought-related traits. It was used as a recurrent parent in backcrossing with marker-assisted selection (MAS) with the Philippines variety Azucena as the donor parent of five carrier chromosomes for introgression. Four target segments carried a QTL for root traits and the fifth carried a recessive gene for aroma. We summarise the marker-assisted selection in three backcross (BC) generations and further pyramid crosses to stack multiple target segments from Azucena. We combined MAS with client-oriented breeding in bulk populations selected for QTLs from the BC2 generation. Phenotypic evaluation of the resultant bulks and near-isogenic lines was carried out. Screens for root traits were conducted in soil-filled pipes under field conditions in Bangalore, India. Over three years, six bulks and four advanced BC3 lines (known as PY lines) were evaluated by farmers in their own fields in eastern India. In addition replicated drought trials were carried out under upland conditions in three locations (two in eastern India and one in western India). The results demonstrate the complexities of selecting for root traits with low heritability and the effect of the evaluation environment. None-the-less, some of the products of this programme were shown to outperform Kalinga III and were acceptable to upland farmers in eastern India.

## Introduction

It is widely recognised that rice varieties with deep root systems perform best under drought. Breeding varieties for improved roots is difficult because root traits are quantitative and have low heritability. Molecular markers can be used to identify linkage to quantitative trait loci (QTL) for rooting ability and these can be selected more easily in a breeding programme than the traits themselves. It is also easier to select for markers linked to aroma than to select for the trait of aroma itself. A marker-assisted selection programme was initiated by Adam Price (University of Aberdeen, UK) and Brigitte Courtois (formerly of IRRI, Philippines) where backcrossing was made to the upland variety Kalinga III, which had not been previously used in mapping studies. We chose Kalinga III because, although it escapes end of season drought through early maturity, it is susceptible to early season drought. It is non-aromatic, and currently there are no aromatic upland varieties adapted to eastern Indian conditions. The aim was to introgress four regions containing root QTLs (on chromosomes 2, 7, 9 and 11) and a QTL for aroma (on chromosome 8). 'Pyramid' crosses were made between lines with multiple introgressions with the aim of stacking five target segments containing root QTLs. Here we describe the results of experiments conducted to test selected progeny plants containing the introgressed regions, including single and multiple root QTLs.

## Materials and Methods

### *Marker-assisted selection*

Marker assisted selection follows four main stages:

1. Linkage mapping of molecular markers in a population derived from diverse parents for the trait (s) of interest.
2. Phenotypic screens of the population to identify chromosomal regions controlling the trait(s) of interest (quantitative trait loci, or QTLs).
3. Selection of molecular markers for the donor parent at QTL in backcross progeny.
4. Testing products for expression of traits.

The first two stages for QTL mapping were carried out in a population of recombinant inbred lines from a cross between Azucena and Bala (Price et al., 1997; Price et al., 2000). The markers for selection in stage 3 were chosen so that alleles from Azucena at four targets containing root QTLs conferred genes for improved rooting ability. These were on chromosomes 2 (G39-C601), 7 (RG650-C507), 9 (G385-G1085) and 11 (RG2-C189). A fifth target at RM223 on chromosome 8 was chosen where the Azucena allele is tightly linked to aroma. Markers at some non-target loci were selected for recurrent parent genotype in backcross progeny. The use of backcrossing combined with marker-assisted selection meant that field screening for roots and aroma was avoided and that farmers or breeders could make selections for other traits in segregating bulks. Therefore, we were able to test the combination of MAS and client-oriented plant breeding (also known as participatory plant breeding).

The breeding programme was started in 1996, and by 2000, more than 20 near-isogenic lines (NILs) with 2-3 introgressed regions had been produced. Kalinga III was used as the recurrent parent for three generations of backcrossing. NILs with more combinations of introgressed segments became available after each generation. The marker systems used were RFLP (restriction fragment length polymorphisms) and SSR (simple sequence repeats or microsatellites) for introgression.

Two strategies for selection in segregating generations were compared. The first used MAS to select bulks from the BC2 generation which were fixed for individual root QTLs, but were segregating at other loci, and these were then used for collaborative and/or consultative selection in the field. Farmers were thus able to select for other desirable traits donated either by Azucena or by Kalinga III in these bulks. The second strategy used MAS alone, and individuals from the BC3 generation were crossed to 'pyramid' the targets. Advanced lines from the pyramid breeding strategy were selected or rejected in consultative selection in eastern India.

The products from this breeding programme included:

1. Six heterogeneous MAS bulks (from the BC2) with different QTLs selected in farmers' fields (Bulks 1-6, also called Richa 1-6).
2. Many BC3 lines with different QTLs (single and multiple).
3. Pyramid (PY 82, PY 83, PY 84) lines with multiple QTLs.
4. One control BC3 line with no Azucena alleles at targets (PY 81).

### *Phenotypic evaluation of products of MAS*

The selected lines and bulks were tested for phenotypic traits in three different types of experiment over 3 years since 2000. Not all lines were included in all experiments.

**1. Controlled root screens conducted in Bangalore.** Plants were grown under field conditions in 1.2 m pipes that were sunk into the ground and filled with soil taken from the same field. Plants were sown in a replicated randomised block layout with two treatments, droughted from day 45 and well-watered throughout. Sampling was conducted at either vegetative stage or at maturity and root length was recorded. There were minor modifications to the treatments used in the screens carried out in different years, and a total of seven screens were completed.

**2. "Mother and Baby" trials conducted in farmers' fields in eastern India** These trials were conducted by farmers in the GVT-project villages of three states of Eastern India. The entries for mother trials were either 6 MAS bulks or 4 Pyramid (PY) lines, and Kalinga III was always grown as a check although some farmers grew an additional local check variety. Yield data was collected from Mother trials. In Baby trials only one test entry was grown alongside the check variety and farmers' preferences were recorded. The plots were managed under standard farmers' management practices for upland conditions.

**3. Field screening of in replicated multi-location drought trials.** These trials were conducted on research stations at BAU, GVT and JNKVV under upland farmer-management with no irrigation. Measurements were taken for maturity, plant height and grain and straw yield in 2001 and 2002. The entries for testing were 6 MAS bulks, 4 PY lines, Kalinga III and Vandana.

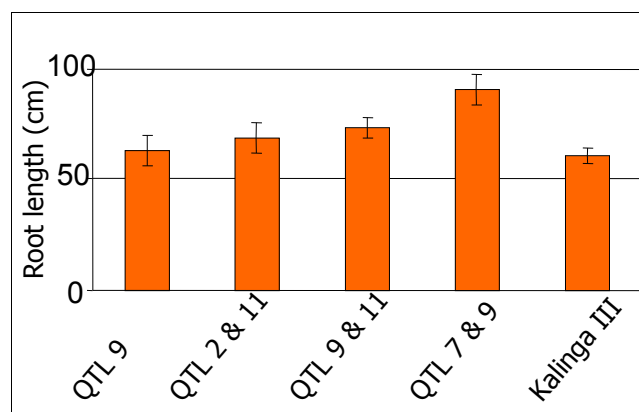
## Results of Phenotypic Evaluation

### 1. Root screens

Maximum root length was significantly different between Azucena and Kalinga III across all of the screens where the two parents were tested ( $T_{(48)} = 2.08$ ,  $P > 0.05$ ). ANOVA (GLM) was conducted on data from all the screens combined using the genotypes at 5 target regions as factors. The QTL on chromosome 9 was shown to have an effect on root length with alleles from Azucena significantly increasing root length. Drought had a significant effect on root length but there was no interaction of drought treatment with the chromosome 9 region.

Analysis with only plants sampled at maturity showed that there were significant genotype and treatment effects but no genotype by treatment interaction. Some lines containing root QTLs had roots that were approximately 21 cm longer under drought than under well-watered conditions, but the control line with no target regions did not show any difference for root length under the two water regimes.

ANOVA was also conducted on each of the screens separately, and root length was shown to be under genetic effect in three of the screens. The lines from these screens were ranked according to mean root length in the three screens to give an overall rank. Only two lines ranked lower than Kalinga III, and one of them was the control line with no introgressed QTL targets. Despite the QTLs on chromosomes 2, 7 and 11 having no significant effect on root length there was evidence that plants with multiple QTLs had better root systems than those with only one or no QTLs (Figure 1).

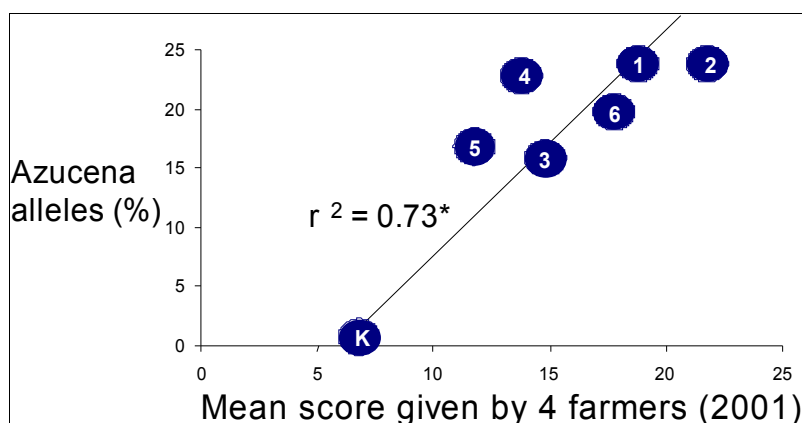


**Figure 1.** Maximum root length (75 days after sowing) of Kalinga III and four lines selected to contain root QTLs on chromosomes 2, 7, 9 and 11 (QTL targets indicated). The data were collected from one screen carried out in 2000 at UAS Bangalore, plants were grown in soil-filled pipes. Droughted and well-watered treatment means were combined because treatments were not significantly different.

## 2. Mother and Baby trials

An overall summary of the farmers' rankings for the Mother trials containing 6 MAS bulks revealed that all bulks lodged less than Kalinga III. All bulks had thicker straw than Kalinga III, and higher straw yield than either Kalinga III or Vandana. The mean scores for all traits (except flowering time) for upland conditions were higher for the bulks than for Kalinga III. Bulk 5 was the tallest, but slightly later and farmers liked its aromatic grain. The farmers' ranking correlated with the proportion of Azucena alleles in the genome of the six bulks, tested at 92 loci (figure 2).

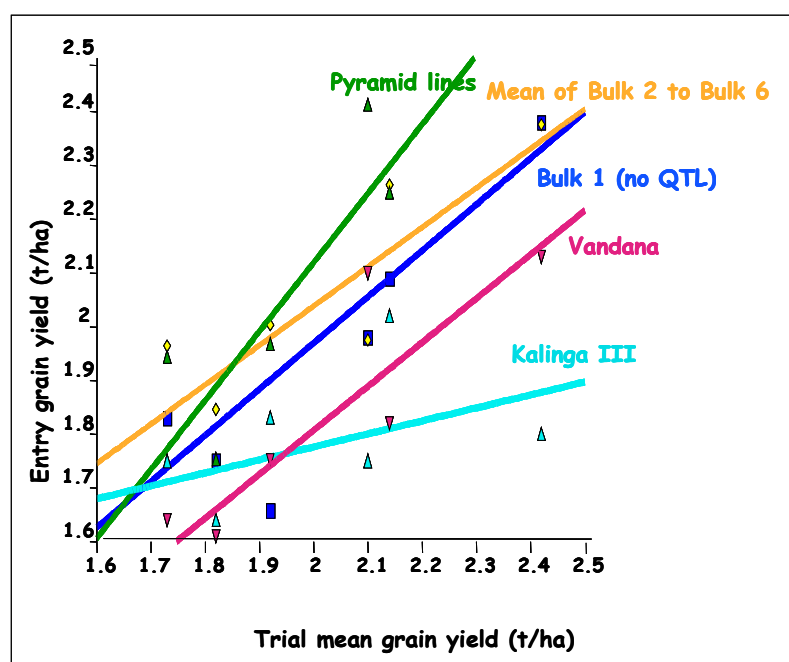
All 4 PY lines performed better than Kalinga III in farmers' fields, including PY 82 which had none of the target QTL regions.



**Figure 2.** Regression of the proportion of Azucena alleles at 92 genome-wide loci and the mean overall rank given by farmers in participatory mother trials in eastern India for six MAS bulks (1-6) and Kalinga III (K). \* $\leq 0.05$ .

## 3. Results from multi-location drought trials

For grain yield the five bulks containing QTLs yielded on average 15% more than Kalinga III. The three PY lines with multiple QTLs yielded 21% higher than Kalinga III while the PY line with no QTL yielded 18% more, although it was slightly later than Kalinga III. The MAS bulks and PY lines showed wide adaptation across different environments (figure 3).



**Figure 3.** Entry grain yield plotted against trial mean grain yield from 6 research trials conducted at 3 locations (GVT upland farm, Ranchi, BAU, Ranchi and JNKVV, Jhabua, Madhya Pradesh) in 2001 and 2002. Entries plotted are the mean of three PY (82, 83 and 84) lines containing root QTLs, the mean of Bulks 2-6 containing QTLs, Bulk 1 with no root QTL selected, Vandana and Kalinga III.

## **Summary and Conclusions**

The root QTL on chromosome 9 of Azucena significantly increased root length when introgressed into Kalinga III, and there was some evidence that the effect of multiple QTLs was greater than one alone. Their effect on both root length and yield was dependant on the evaluation environment. The Azucena genome (with up to 25% introgression including non-target regions) contributes positively in Kalinga III. Marker-assisted selection was combined with client-oriented breeding methods in segregating heterogeneous bulks and for the selection of pure lines. Kalinga III has been improved for roots, aroma, straw thickness and lodging resistance. The 'pyramid' lines with multiple QTLs showed greatest improvement over Kalinga III in terms of grain yield in the field. We expect to release the best varieties developed in this collaborative programme for the rain-fed uplands of Eastern India.

## **References**

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