A Promising Fluorescent Based Genotyping Method: Kompetitive Allele Specific PCR (KASP) successful in Marker assisted backcrossing

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Abstract

Efficient tracking of superior genetic variations through breeding process is the most important requirement for crop development now a days. In this regard competitive allele specific PCR (KASP) has proved as a high throughput and easier way to assist marker assisted backcrossing (MAB). Presence of SNP marker throughout genome and easy visualization of desirable alleles made KASP a very efficient fluorescent based method in MAB. In this work, SNP-based fluorescent PCR primers (KASP markers) have been designed for SNPs associated with salt tolerant traits. Then these KASP markers are validated and used to confirm introgression of these salt tolerant traits into high yielding varieties (BRRI dhan63 and BRRI dhan74). The F_1 population, derived from crossing of donor and elite lines, have been selected by these established KASP markers. Apart from the QTL-specific markers, around 110 KASP markers have been successfully designed to detect the background genetic material specific to the elite lines. Quick selection of the QTLs and elite genetic material using these established KASP markers will enhance the efficiency of breeding salt tolerant rice with multiple QTL loci into the fine-grained highly popular rice varieties.

Background

Crop Improvement

Climate change

Biotic stress Tolerance

Abiotic stress Tolerance

Breeding

Molecular Markers

Marker assisted bakcrossing

Salt tolerant traits/QTLs from landraces (Horkuch)

QTL of Root length and Filled grain number

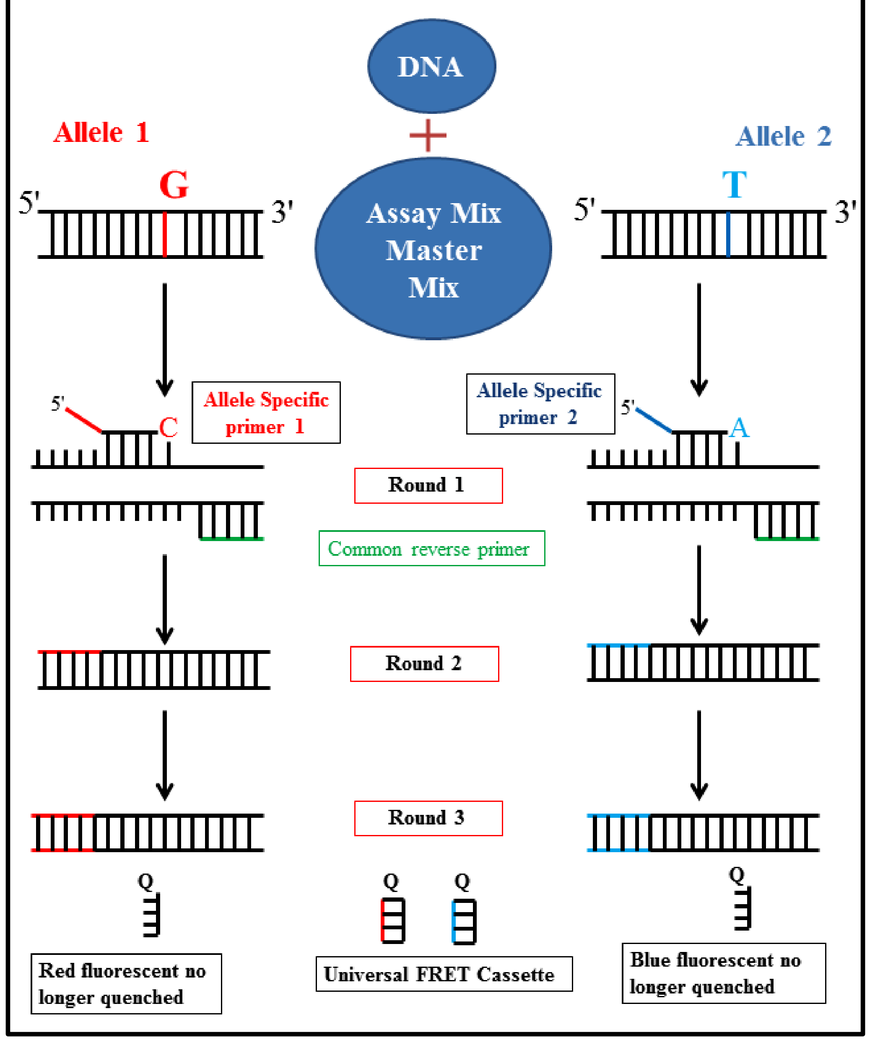
High Yielding Farmer popular rice (BRRI dhan63 and BRRI dhan74)

New Salt tolerant rice

Why KASP?

- High throughput and faster detection
- Safe as Florescent based method
- Easy to visualize than Taqman

Principle of KASP Genotyping

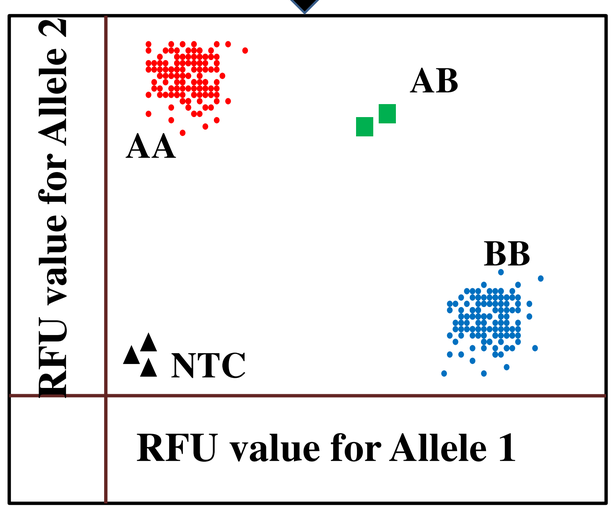


Methods

KASP Genotyping

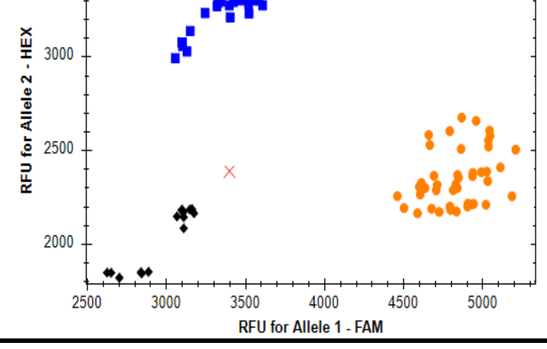
Master Mix + Plant DNA + Primer Assay Mix

KASP Reaction

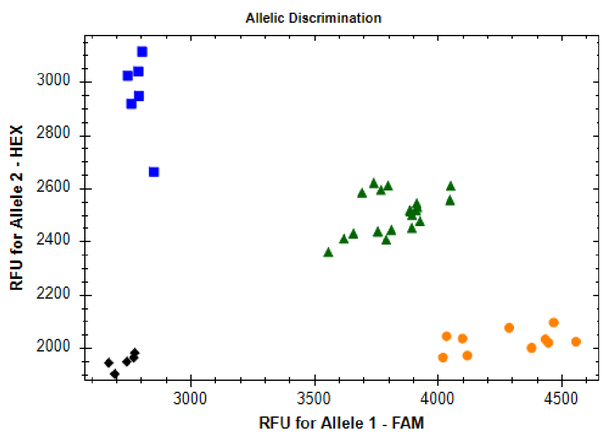


Results

Foreground Marker Chr_2_snpOS0505



Recombinant Marker Chr_2_26960862



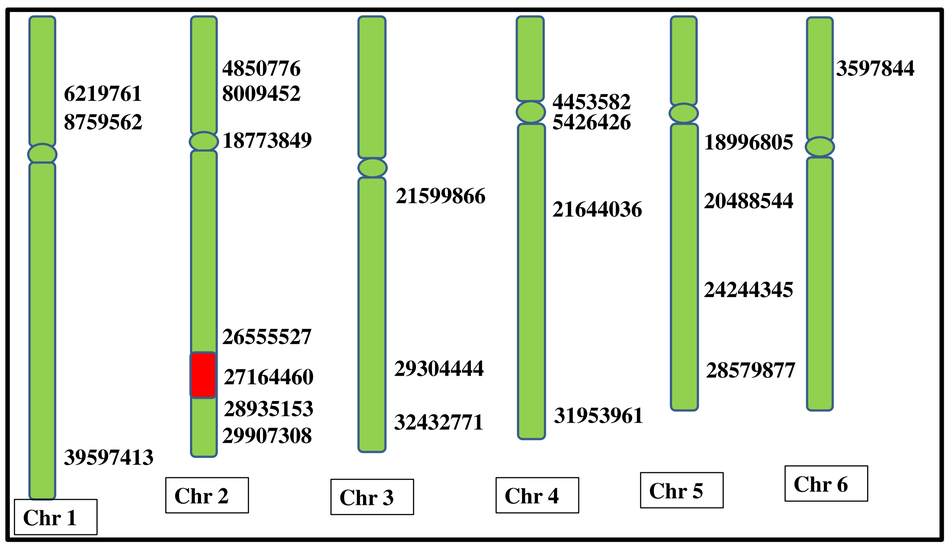
Results

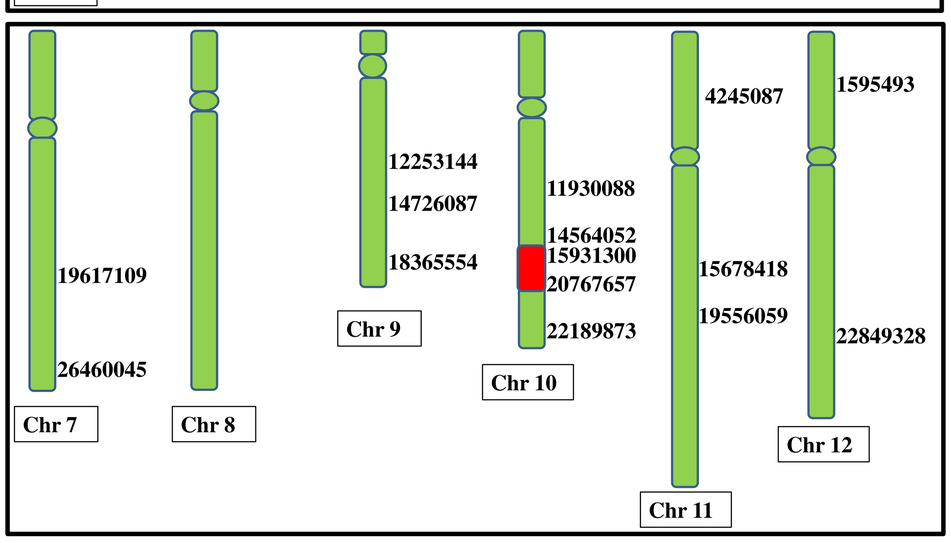
171 primers are designed and tested by KASP

AATTACCACCAAATTAT[A/T]ATCGTATTGTCGCTAGGATTAG
CGGAGTCAACGGATTGAAATCTAATCCTAGCGACAATACGATA
GACCAAGTTCATGCTGAAATCTAATCCTAGCGACAATACGATT

Donor With QTL of **RL** and **FGN** × BRRI dhan63 and BRRI dhan74

Among 400 plants **26** F1 plants having **both QTLs** were selected using **KASP**





Significance of the study

- ❑ Establishing KASP for MAB in Bangladeshi varieties
- ❑ Producing new salt tolerant varieties with high yield

Acknowledgement