Fluorescence based Kompetitive (competitive) Allele specific PCR (KASP) for high-throughput SNP marker detection and validation

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Abstract:

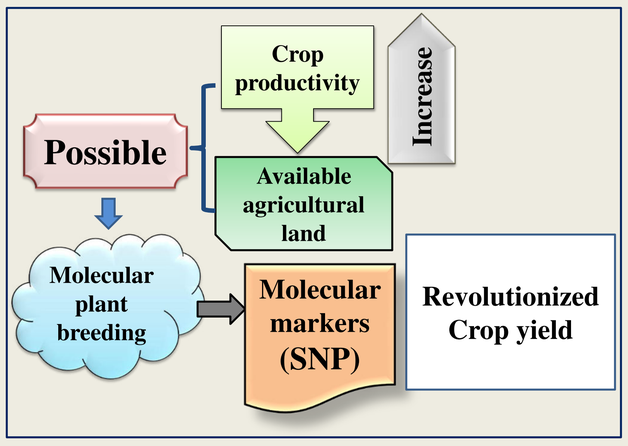
Considering the enormous potential of DNA markers in plant breeding and recent advances in single nucleotide polymorphism (SNP) genotyping for its promising role in crop improvement, it has become inevitable for the plant breeders to adopt the capacity of SNP marker development and marker assisted selection (MAS).While, gene cloning allows insertion of a single gene, MAS enables insertion of multiple loci for pyramiding of different tolerance mechanisms, leading to a higher level of tolerance. Cost of utilizing high throughput SNP detection system is possibly the major hindrance in implementing marker assisted selection (MAS). In the current work, we have attempted to establish fluorescence-based Kompetitive Allele Specific PCR (KASP) technology for easy and efficient detection of SNP alleles. KASP was applied for the validation of the identified salt tolerance quantitative trait loci (QTL) with an aim to select potential donor (tolerant) allele/plant for use in marker-assisted breeding.A mapping population at F_6 and F_7 with the salt tolerant rice landrace *Horkuch* and sensitive but high yielding *IR29*, was used to establish KASP genotyping. Specific salt tolerance SNP-based QTLs had been previously identified at the $F_{2\text{-}3}$ stage from this mapping population, with *IR29* (♀) and *Horkuch* (♂).KASP markers were designed for these loci and genotyping was done with the DNA of the F_6 and F_7 population for molecular validation. Physiological analysis was also done in both seedling and reproductive stages. Later correlation analysis of both physiological and molecular data was done to select appropriate donor plant.KASP genotyping was successfully established as fluorescence based SNP detection method by detecting seven out of eight SNPs. From the correlation between physiology and molecular analysis few F_6plants have been found as potential donors multiple traits or QTLs.In the overall study KASP genotyping method was found more suitable as a marker validation system than other methods due to its speed, high accuracy, low cost, flexibility in assay design and fluorescence based detection method. The method is now being used in a breeding program for introgression of multiple QTLs in a commercial Rice variety.

Background

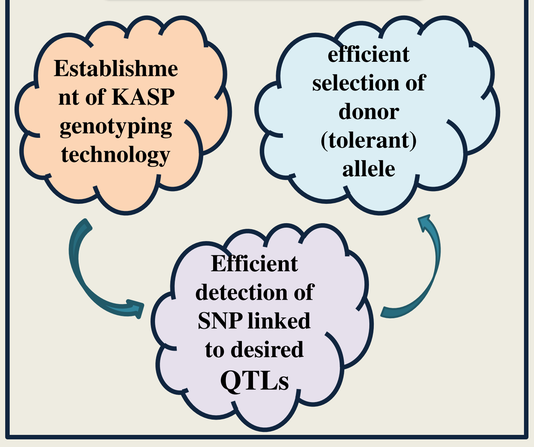
To feed the overgrowing population, No alternative of rice production in coastal lands of Bangladesh

'Rice security' is equivalent to 'food security'

Location of Bangladesh near sea level makes it particularly vulnerable to climate change-induced salinity

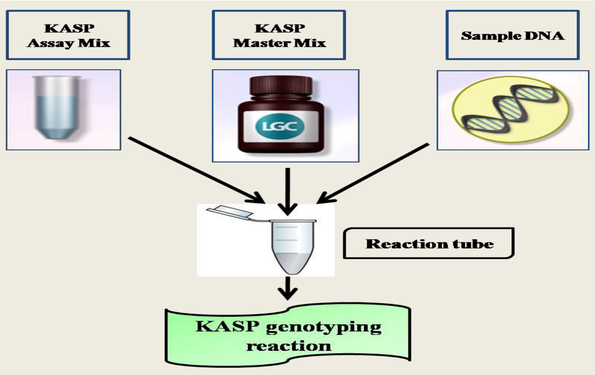


Major Purposes of this study



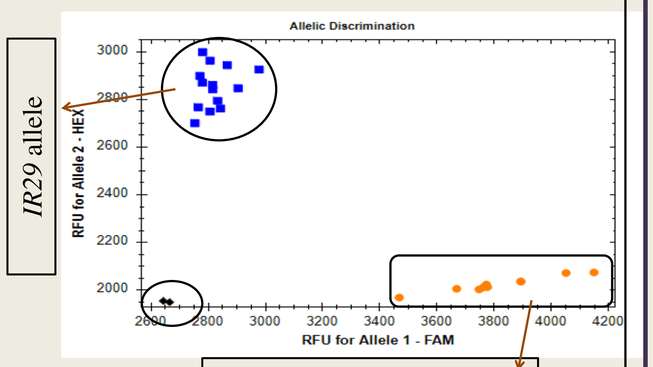
Establishment of KASP(Kompetitive Allele Specific PCR) genotyping

Materials and Methodology



KASP result on selected F_6 plants

Marker SF0140 (Donor Parent *IR29*)

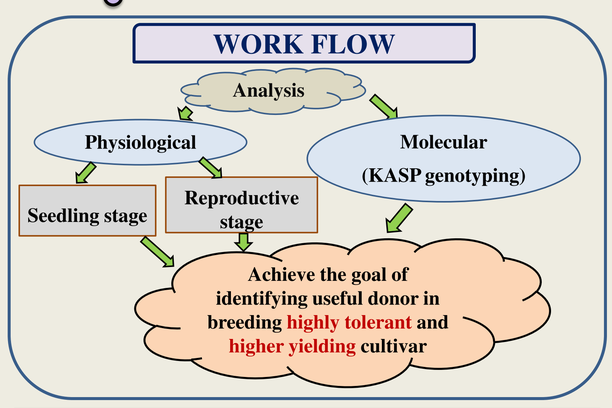


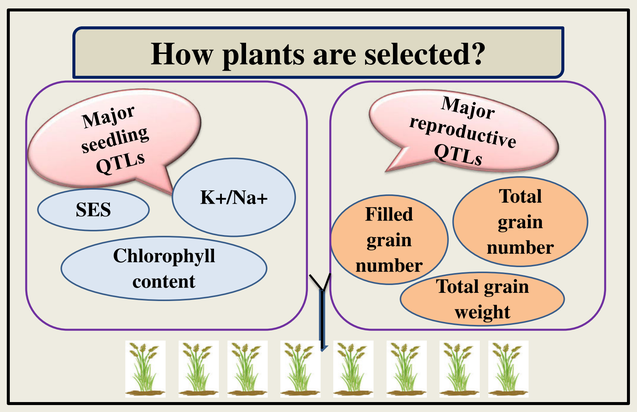
SNP markers to be detected by KASP genotyping

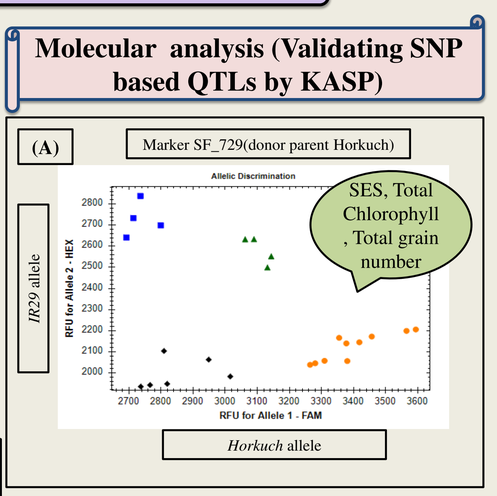
SNP markers	Chromosome	Associated QTLs	Donor Parent
SF_726	Chromosome-7	Root length	Horkuch
SF_018	Chromosome-1	Root dry weight	Horkuch
SF_085	Chromosome-8	Filled grain number	Horkuch
SF_1017	Chromosome-10	Spikelet damage	Horkuch

Acknowledgement

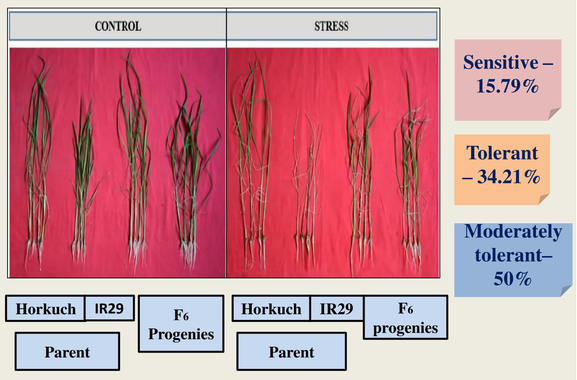
Physiology and Molecular Analysis(applying KASP) of Plants for selecting potential donor allele



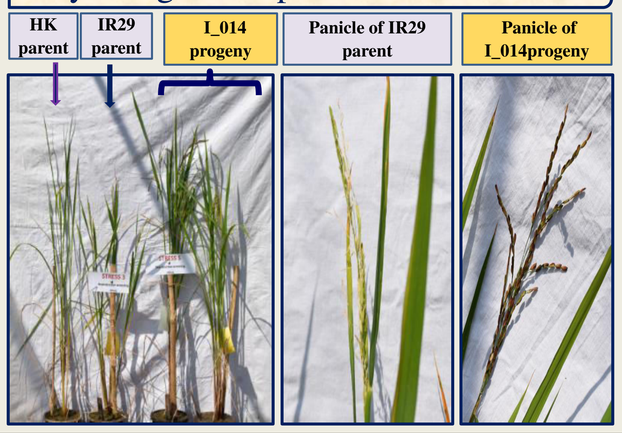




Physiological response under salt stress(seedling stage)



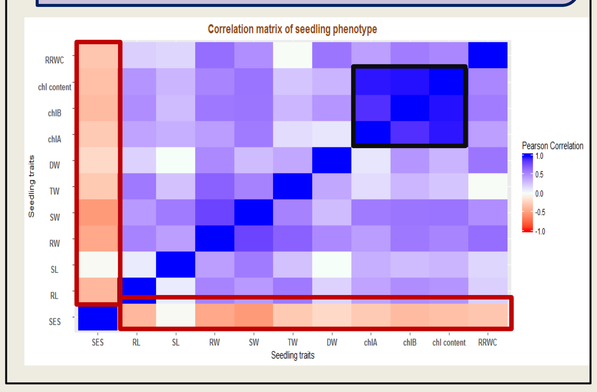
Physiological response under salt stress



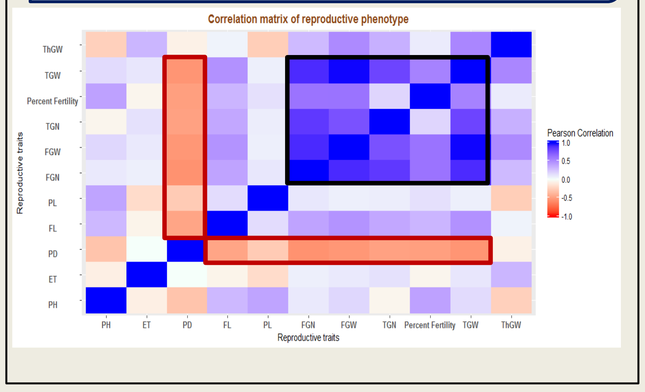
Plants confirmed to be selected as potential donor

Plants	SNP markers	Associated QTL	Phenotype		
			High	Moderate	Low
I_014	SF_0140, SF_729	SES, Shoot Length, Filled grain weight	Yield, Tch		SES
I_119	SF_0140,	Total chlorophyll, Filled grain number	Yield, Tch		SES
I_014	SF_0140, SF_71	SES, Shoot Length, Filled grain weight, Total Chlorophyll		Yield	SES

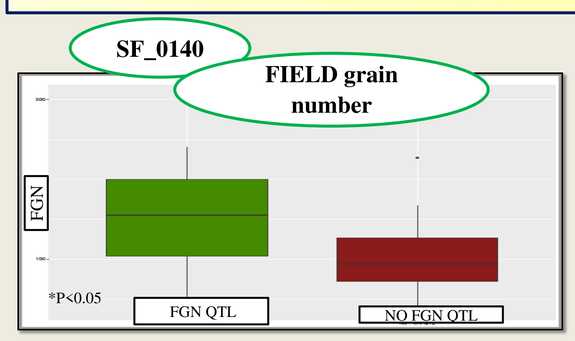
Are the seedling phenotypes correlated???

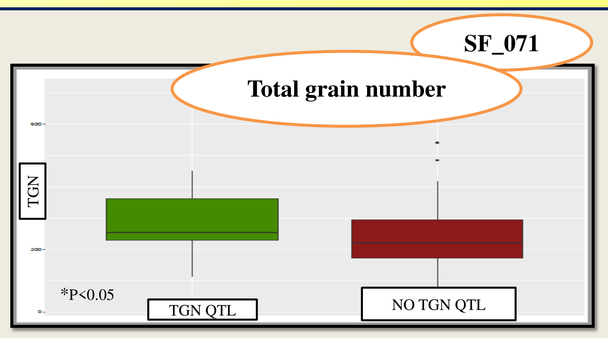


Are the Reproductive phenotypes correlated???



Do the SNP (associated with QTLs)have effect on phenotypes???





Future Prospects

KASP – a hope for molecular plant breeders

Pyramiding of more useful QTLs expected to show better tolerance under stress

Crosses can be made between progenies having multiple desirable QTLs to develop highly tolerant and higher yielding rice variety

We can't stop hoping !!!! We can't stop dreaming !!!!