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

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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 is crucial for plant breeders to adopt the capacity of SNP marker development and marker assisted selection. However, cost of utilizing high throughput SNP detection system is possibly the most important factor that limits the implementation of 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 and validation of SNP based quantitative trait loci (QTL). A mapping population at F6 and F7 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 F2-3 stage from this mapping population, with *IR29* (♀) and *Horkuch* (♂). KASP markers were designed and genotyping assay was done with F6 sample DNA where polymorphism in seven out of eight SNP markers were detected. Based on physiological analysis for the presence of desired QTLs, a subset of plants were chosen and advanced to F7 generation and SNP based QTLs were validated applying KASP assay. Hence, in the overall study KASP genotyping method was found more suitable as a marker validation system than other methods due to its high accuracy, low cost, flexibility in assay design and fluorescence based detection method. Therefore, was used further for potential donor (tolerant) plants selection that can be used in marker assisted breeding program.

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