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Farmers' and academia's views"

Development of specific SNP markers for identification of rice species conserved in AfricaRice genebank

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Abstract

Some of the major challenges facing genebank managers and plant genetic resource scientists relate to taxonomic classification and labeling. Misclassification and mislabeling lead to errors in genebank operations, including those related to conservation, management dissemination and use of germplasm. AfricaRice's genebank team reported that 3.1 % of 3,134 accessions from four rice species (*Oryza glaberrima*, *Oryza barthii*, *Oryza longistaminata*, *Oryza sativa* spp. *indica* and *japonica*) were either mislabeled or misclassified, leading to the effective use of germplasm in various ways. Such errors have been reported in others crop species and error rates vary from 3 to 28 %. Most instances of taxonomic misclassification and mislabeling are due to human error during planting of material, characterisation of accessions for phenotypic traits, and misreading of the germplasm names. These types of errors can best be avoided by implementing routine genotyping quality control (QC) methods using low cost, high throughput and user-friendly SNP markers. We recently identified in several *Oryza* species/subspecies 224 diagnostic single nucleotide polymorphic (SNPs) markers based on DArTseq-based, next-generation sequencing technology, which can be used to rectify taxonomic/mislabeling errors. We converted them into Kompetitive allele-specific PCR (KASPar or KASP) assays and validated a subset of them for low-cost routine genotyping quality control (QC) analysis. Among the 224 diagnostic SNPs submitted to LGC Biosearch Technologies' service laboratory (Hoddesdon, UK). One hundred and fifty-eight SNPs produced working KASP assays and were tested on 80 accessions from five species/subspecies. Among these, 87 (55 %) clearly differentiated the species/subspecies, indicating the utility of these SNPs as diagnostics markers. The remaining 45 % were either monomorphic (20 SNPs), or polymorphic, but with no clear haplotype pattern, making them ineligible to serve as diagnostic markers. We validated these results using 65 of the diagnostic SNPs in 625 accessions representing the five species/subspecies. In sum, we recommend subsets of 24 and 36 SNP markers be employed for "rapid" and "broad" diagnostic activities, respectively, to clearly delineate *O. barthii, O. glaberrima, O. longistaminata, O. sativa* spp. *indica* and *japonica*.

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