Development and validation of diagnostic SNP markers for quality control genotyping in a collection of four rice (*Oryza*) species

Arnaud Comlan Gouda[1*], Gustave L. Djedatin[2], Sèdjro Bienvenu Kpeki[1], Karlin Gnikoua[1], Tia Dro Daniel[1] & Marie Noelle Ndjiondjop[1]

1Africa Rice Center (AfricaRice), 2Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), Abomey, Benin

*Corresponding author: A.Gouda@cgiar.org

Introduction

- One of the major challenges faced by genebank managers and plant genetic resource scientists is taxonomic misclassification/mislabelling of conserved accessions.
- In rice, our team recently reported 339 diagnostic SNPs that can be used in correcting taxonomic misclassification of *O. barthii, O. glaberrima*, *O. longistaminata* and the two *O. sativa* subspecies (Fig.1&2). These diagnostic markers require conversion for use in KASP assays and validation prior to use for quick and low-cost QC analysis.
- This study presents the custom KASP assays created from the DArTseq-based *Oryza* species and subspecies diagnostic SNPs and the results of their validation. The aim was to recommend a smaller set of the best diagnostic SNPs for routine use in taxonomic curation of the four rice species and two subspecies conserved in different rice genebanks.

Fig.1 Kim et al.,2020

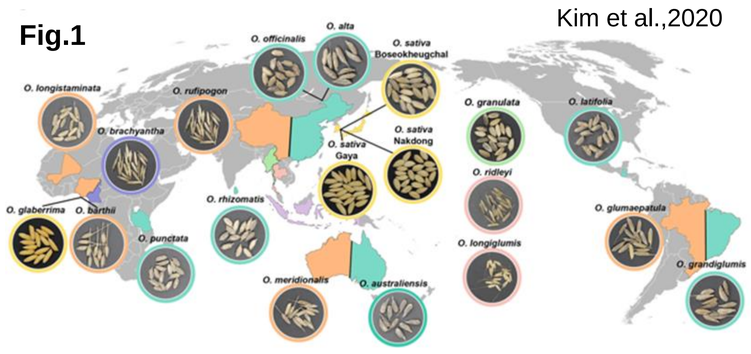


Fig.2

Materials and methods

1. SNP conversion to PCR-based markers

- Based on the LGC Genomics KASP markers design criteria, 224 SNPs out of the 339 DArTseq-based SNPs were selected and submitted to conversion in LGC Biosearch Technologies service laboratory, (Hoddesdon, UK).
- LGC genomics lab designed KASP oligonucleotide assays for 158 (in red color, Fig.3) out of the 224 SNPs submitted.

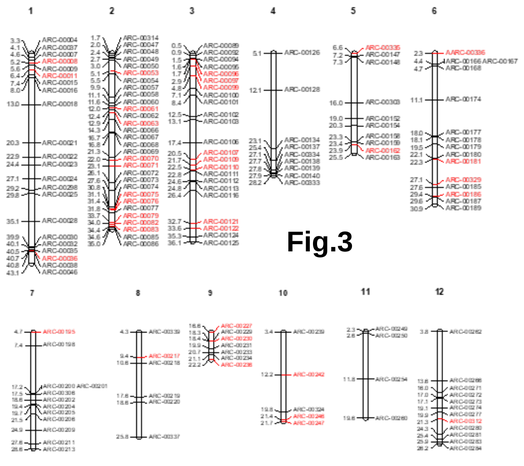


Fig.3

2. KASP markers testing and validation

- Converted KASP markers were tested using 80 DNA samples (green color in Fig.4)
- Genotypic data showed that (i) 93 SNPs out of the 158 were monomorphic or not taxonomically diagnostic and (ii) remaining 65 seems diagnostic.
- Selected markers (65 SNPs) were then validated using 625 DNA samples (bleu color in Fig.4). Leaves sampling details are showed in Fig.5
- Various statistical analyses were performed as described by Gouda *et al*., (2021)

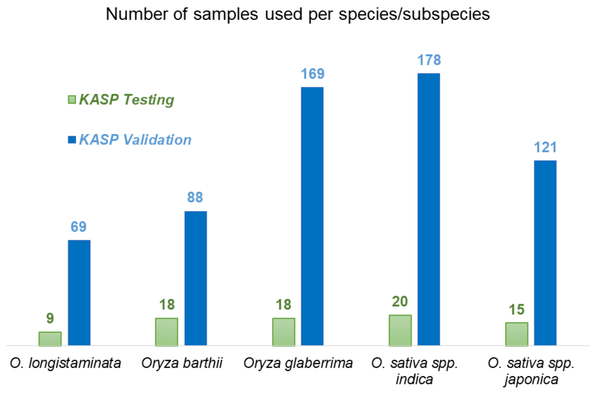


Fig.4

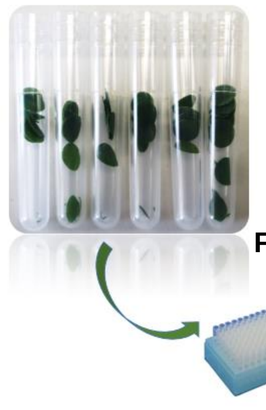
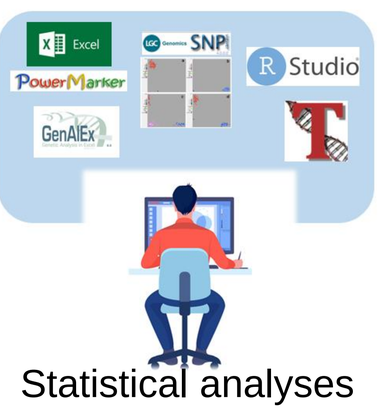


Fig.5



Statistical analyses

Results

1. Selection of a diagnostic SNP set

- 80 accessions tested were divided into five groups according to their taxon (Fig.6)
- Six (6) group-specific sets of SNPs were designed from the 65 KASPs markers as showed in Fig.7 (light blue color)

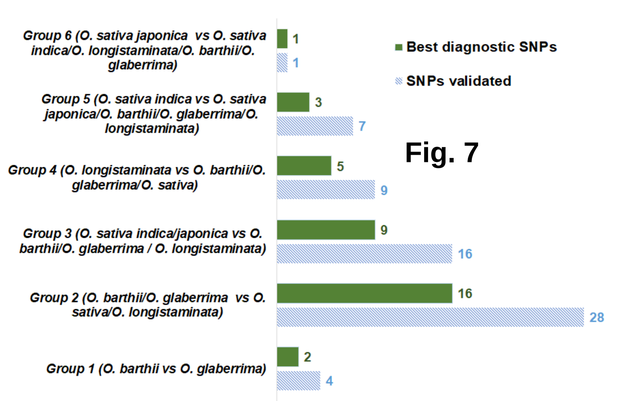
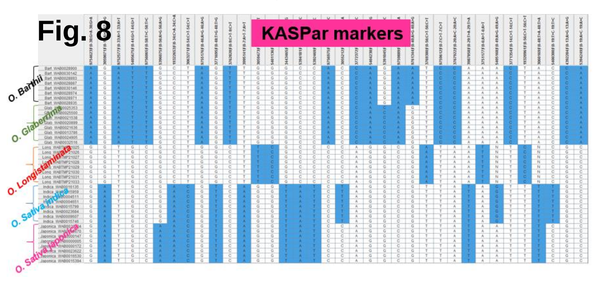


Fig. 7



Cluster I: *O. longistaminata* accessions
Cluster II: *O. barthii* accessions,
Cluster III: *O. glaberrima* accessions
Cluster IV: *O. sativa* spp *indica* accessions
Cluster V :*O. sativa* spp *japonica.*

Fig. 6

2. Validation of a diagnostic SNP subset

625 accessions were also divided in 5 groups according to their taxon (Fig.8).

Fig.8

Cluster I: *O. longistaminata* accessions
Cluster II: *O. barthii* accessions,
Cluster III: *O. glaberrima* accessions
Cluster IV: *O. sativa* spp *indica* accessions
Cluster V :*O. sativa* spp *japonica.*

- Thirty-six (36) KASP markers were strongly diagnostic for the six (6) group-specific sets (green color, Fig.7). Haplotype of the 36 SNPs presented in Fig.8

Conclusion

Based on these validation test results, we recommend a panel of 36 SNP markers that clearly delineate *O. barthii*, *O. glaberrima*, *O. longistaminata*, *O. sativa* spp. *indica* and *japonica*. The KASP assays provide a flexible, rapid turnaround and cost-effective tool to facilitate germplasm curation and management of these four *Oryza* AA genome species across multiple genebanks.

References

Semagn, K. *et al.* Quality control genotyping for assessment of genetic identity and purity in diverse tropical maize inbred lines. Theor. Appl. Genet. 125, 1487–1501 (2012).

- **Ndjiondjop, M. N. *et al***. Development of species diagnostic SNP markers for quality control genotyping in four rice (Oryza L.) species. Mol. Breed. 38, 131 (2018).
- **Gouda, A. C. *et al.*** Comparisons of sampling methods for assessing intra- and inter-accession genetic diversity in three rice species using genotyping by sequencing. Sci. Rep. 10, 13995 (2020).
- **Gouda, A. C. *et al***. "Development and validation of diagnostic SNP markers for quality control genotyping in a collection of four rice (Oryza) species." Scientific reports 11.1 (2021): 1-11.