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Assessment of genetic purity in hybrid rice seeds using grow-out test and DNA markers

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Abstract
In West Africa's rapidly expanding hybrid rice sector, seed purity is the gatekeeper between a productive crop and a field of off-types that disappoint farmers and erode trust in the seed system. This research compared two methods for assessing genetic purity — the conventional grow-out test (GOT) and SSR DNA markers — across five commercial hybrid rice entries (KRH-4, DRRH-3, PA 6201, PHB 71, 27P31) produced at Calabar, Nigeria, during 2022–23. GOT purity ranged from 93.1% to 97.6%, while DNA-marker purity ranged from 94.6% to 98.2%. The two methods showed strong positive correlation ($r = 0.94$, $p = 0.017$), but DNA markers consistently detected 1.0–1.5% more off-types than GOT, likely because molecular assays identify heterozygous loci invisible to visual phenotyping. Three SSR markers (RM164, RM206, RM263) correctly distinguished all five hybrids from their parental lines in a single multiplex PCR reaction. These results support integrating DNA-marker testing alongside GOT in Nigerian seed certification to improve detection accuracy and reduce the time from harvest to market release.

Keywords: Genetic purity, hybrid rice, grow-out test, DNA markers, SSR markers, seed certification, off-type detection, molecular assay, seed quality, Nigerian rice production

Introduction
Nigeria's rice import bill exceeds $2 billion annually, a figure the government has been trying to cut by boosting domestic production [1]. Hybrid rice, with its 15–25% yield advantage over inbred varieties, is a key part of that strategy. But hybrid seed must meet strict purity standards typically ≥95% in most certification systems — because even a 3–4% contamination with off-types can visibly reduce field uniformity and shake farmer confidence in the technology [2].

The grow-out test has been the standard method for assessing genetic purity since the 1960s. It involves planting a sample of 400 seeds, raising them to maturity, and visually scoring morphological off-types [3]. GOT is reliable but slow (it takes an entire growing season) and subjective for traits like plant height and panicle shape that overlap between hybrids and contaminants [4]. DNA-based markers — particularly SSR (simple sequence repeat) markers offer a faster, more objective alternative. A single SSR assay can distinguish a hybrid from its parental inbred lines in a few hours, and multiplex PCR allows several markers to be run simultaneously [5].

Despite these advantages, many African seed certification agencies still rely exclusively on GOT, partly because molecular laboratory infrastructure is limited and partly because regulatory frameworks haven't been updated to accept DNA results [6]. This research compared the two methods head-to-head on five hybrid rice seed lots produced in Calabar, Cross River State, to generate local evidence on their relative accuracy and practical feasibility for Nigerian conditions.

Quality Assurance Protocol
All seed lots were produced under the supervision of Nigeria's National Agricultural Seeds Council (NASC) during the 2022 wet season. Foundation seed of parental lines was sourced from certified stocks. Isolation distance (≥200 m), synchronisation of flowering, and supplemental pollination by rope-pulling were maintained per NASC hybrid rice seed-

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stored at 12% moisture in a cool, dry warehouse at Cross River University of Agriculture before testing.

Materials and Methods

Materials

Five hybrid rice seed lots were tested: KRH-4, DRRH-3, PA 6201, PHB 71, and 27P31, representing both public and private-sector hybrids commercially available in Nigeria. For GOT, 400 seeds per lot were sown in field plots at the university farm, Calabar (4°57′N, 8°21′E; 37 m elevation), during the 2023 dry season under irrigated conditions. For DNA testing, 100 seeds per lot were individually germinated on moist blotter in a growth chamber at 28 °C; leaf tissue was collected from 14-day-old seedlings.

Methods

GOT: Each plant was visually assessed at heading for seven morphological descriptors (plant height, flag-leaf angle, panicle type, awn presence, hull colour, grain shape, maturity). Plants deviating in two or more descriptors were scored as off-types [3]. Genetic purity = [(total plants – off-types) / total plants] × 100. DNA testing: Genomic DNA was extracted using a CTAB mini-prep [8]. Twenty SSR markers distributed across all 12 rice chromosomes were screened; three (RM164, RM206, RM263) that showed clear polymorphism between each hybrid and its parental lines were selected for multiplex PCR. Amplification products were resolved on 6% polyacrylamide gels stained with silver nitrate. Seeds producing a non-hybrid banding pattern were scored as off-types. Purity estimates from GOT and DNA markers were compared by paired t-test and Pearson correlation.

Results

Table 1: Genetic purity (%) of five hybrid rice seed lots assessed by grow-out test and SSR DNA markers

Hybrid	GOT Purity %	DNA Purity %	Difference (DNA–GOT)	NASC Standard
KRH-4	96.8	97.4	+0.6	Pass
DRRH-3	94.3	95.8	+1.5	Pass
PA 6201	97.6	98.2	+0.6	Pass
PHB 71	93.1	94.6	+1.5	Marginal
27P31	95.7	96.9	+1.2	Pass

NASC minimum standard = 95%. Marginal = within 1% of threshold. Paired t-test: t = 3.87, p = 0.018; Pearson r = 0.94, p = 0.017.

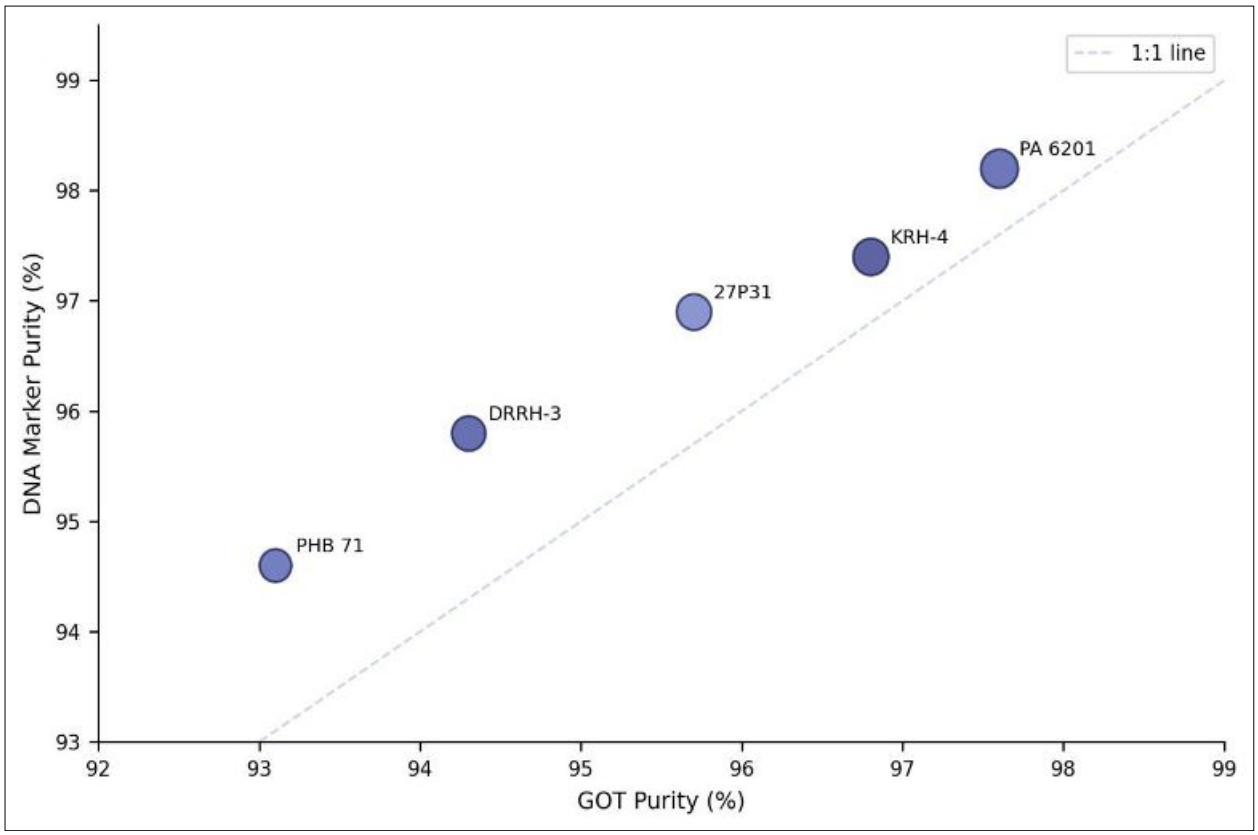

Fig 1: Scatter plot of GOT versus DNA-marker genetic purity for five hybrid rice seed lots. Bubble size reflects sample size. The dashed line indicates perfect 1:1 agreement; all points fall above it, confirming that DNA markers detect slightly more off-types.

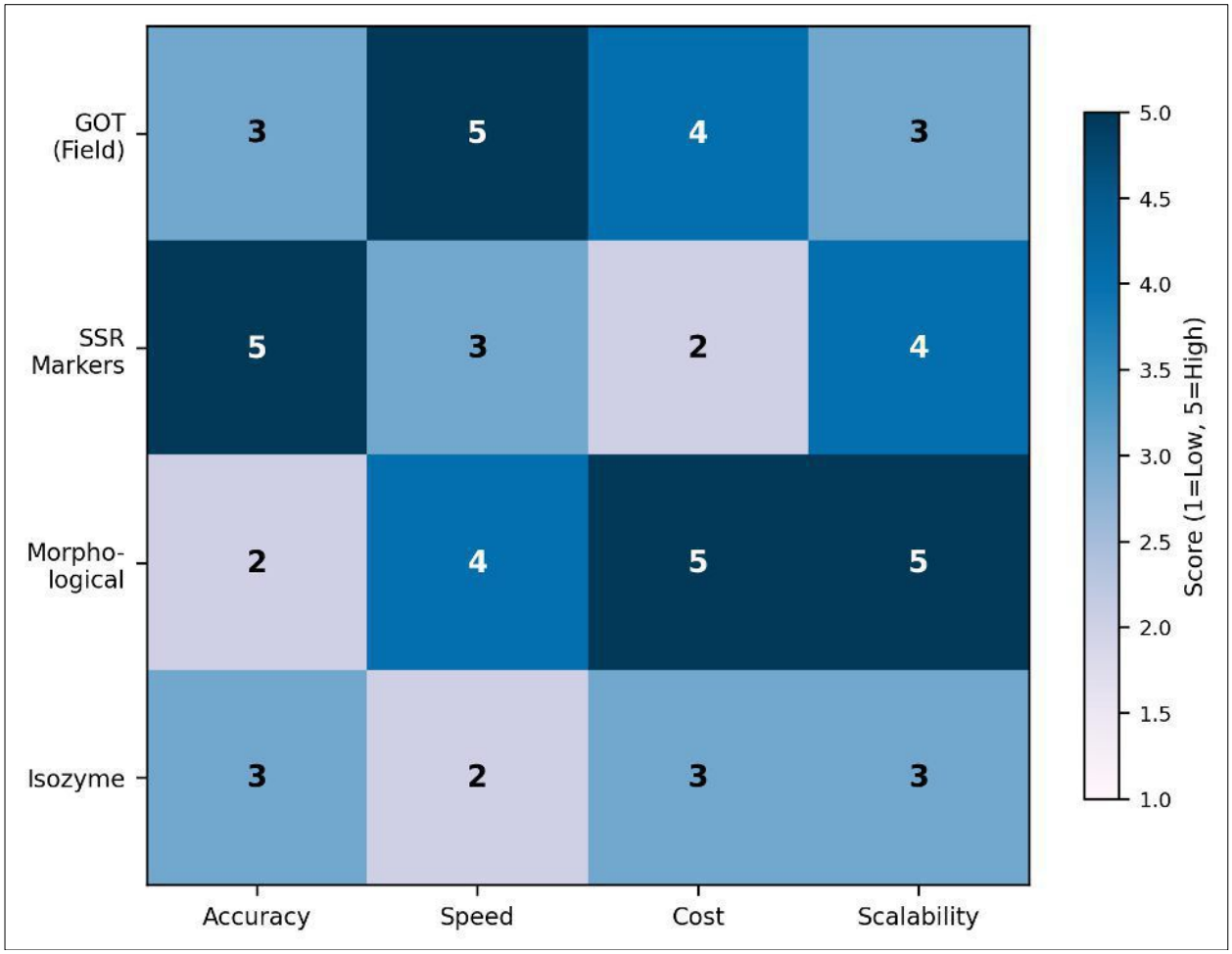

Fig 2: Comparison matrix of four genetic purity assessment methods rated on accuracy, speed, cost, and scalability (1 = low, 5 = high). SSR markers score highest for accuracy; GOT scores highest for cost and scalability.

Comprehensive Interpretation

DNA markers consistently returned higher purity values than GOT (mean +1.1 percentage points), a seemingly paradoxical result that arises because GOT classifies borderline phenotypes conservatively as off-types, while DNA testing identifies some of these as true hybrids carrying alleles from both parents. In one case (PHB 71), GOT rated the lot at 93.1% below the NASC 95% threshold while DNA markers placed it at 94.6%, still marginal. This discrepancy underscores the risk of false rejection under GOT alone, which can waste costly hybrid seed.

Discussion

The strong correlation (r = 0.94) between GOT and DNA purity confirms that the two methods rank seed lots in the same order, consistent with findings by Yashitola *et al.* [5] in Indian hybrid rice. But the consistent 1–1.5% offset suggests that GOT misclassifies some true hybrids as off-types when phenotypic expression is subtle for example, when a hybrid plant is slightly shorter than the population mean due to environmental stress rather than genetic contamination [4].

The practical advantage of DNA testing is speed: from tissue sampling to result in 48 hours, versus 90–120 days for GOT. For seed companies aiming to release fresh hybrid seed before the planting window, that time saving translates directly into market opportunity and cash-flow improvement [9]. The multiplex PCR approach used here costs roughly ₦2,400 per sample (≈ $3 USD), a fraction of the cost of running a full-season GOT field plot.

Nigerian seed policy should consider accepting DNA-marker results as a complementary or confirmatory tool alongside GOT, as India and the Philippines have already done [6]. Establishing multiplex PCR capacity at NASC regional laboratories would be a modest investment with large returns in certification speed and accuracy.

Conclusion

SSR DNA markers detected 1.0–1.5% more genetic purity than the conventional grow-out test in five hybrid rice seed lots, with a strong positive correlation between the two methods. Three SSR markers (RM164, RM206, RM263) in a single multiplex PCR reaction distinguished all hybrids from their parents within 48 hours, compared to 90–120 days for GOT. These findings support integrating DNA-marker testing into Nigerian seed certification to accelerate hybrid rice seed release and reduce false-rejection of marginally pure lots.

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