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Assessment of genetic diversity in *Aspergillus carbonarius* through random amplified polymorphic DNA (RAPD) marker analysis

[1]Thomas, B. T., *[1]Coker, M. O., [1]Popoola, O. D., [1]Adesetan, T. O., [1]Banjo, O. A., [1]Taiwo, O. S., [1]Popoola, K. E., [2]Thomas, A. N., [1]Adegbe, G. M., and [3]Owolabi, S. L.

[1]Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
[2]Department of Animal Production, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
[3]Department of Science Laboratory Technology (Microbiology Unit), Gateway Polytechnic, Saapade, Ogun State, Nigeria
*Correspondence to: olawunmi.coker@oouagoiwoye.edu.ng; +2349054006625;
ORCID ID: https://orcid.org/0000-0003-0937-7755

Abstract:

Background: The importance of RAPD in revealing genetic diversity among ochratoxigenic strains of *Aspergillus carbonarius* lies in its ability to provide critical information that can support the development of effective strategies to limit ochratoxin A contamination in agricultural produce. This study therefore focused on evaluating the genetic diversity of 20 *A. carbonarius* strains previously isolated in an earlier investigation.
Methodology: This study focused on evaluating the genetic diversity of 20 *A. carbonarius* strains previously isolated in an earlier investigation. A total of 26 RAPD primers were initially screened to determine optimal annealing temperatures and to identify those that produced clear, consistent, and reproducible banding patterns. Primers meeting these criteria were subsequently employed in genetic analysis, following standard RAPD protocols.
Results: The obtained results revealed that 7 RAPD primers gave clear and good polymorphisms at the optimal PCR amplification conditions between 36°C and 40°C. These 7 primers generated 18 distinctive RAPD loci from the 20 *A. carbonarius* isolates. The analysis of population structure at the geopolitical region of Ogun State revealed that the intra-site genetic variation (range=0.02 to 0.33) made up most of the genetic variation observed (82.0%). The magnitude of the overall variation arising from site-level divergence (G_{ST}) ranged from 0.01 to 0.42, suggesting minimal site-level population structure. The phylogenetic relationships among the 20 *A. carbonarius* isolates revealed that none of the isolates exhibited genetically distinct clusters.
Conclusion: The high genetic variation detected among ochratoxigenic *A. carbonarius* strains underscores the biotechnological importance of RAPD-based molecular characterization for strain-level discrimination, biosafety assessment, and targeted ochratoxin A risk management, thereby supporting informed strain selection and application in fungal biotechnology.

Keywords: *Aspergillus carbonarius*; Ochratoxin; Genetic Diversity; RAPD

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Évaluation de la diversité génétique chez *Aspergillus carbonarius* grâce à l'analyse de marqueurs d'ADN polymorphe amplifié aléatoirement (RAPD)

[1]Thomas, B. T., *[1]Coker, M. O., [1]Popoola, O. D., [1]Adesetan, T. O., [1]Banjo, O. A., [1]Taiwo, O. S., [1]Popoola, K. E., [2]Thomas, A. N., [1]Adegbe, G. M., et [3]Owolabi, S. L.

[1]Département de Microbiologie, Université Olabisi Onabanjo, Ago-Iwoye, État d'Ogun, Nigeria
[2]Département de Production Animale, Université Olabisi Onabanjo, Ago-Iwoye, État d'Ogun, Nigeria
[3]Département de Technologie de Laboratoire Scientifique (Unité de Microbiologie), Polytechnique Porte, Saapade, État d'Ogun, Nigeria
*Correspondance à: olawunmi.coker@oouagoiwoye.edu.ng; +2349054006625;
ORCID: https://orcid.org/0000-0003-0937-7755

Résumé:

Contexte: L'importance de la technique RAPD pour révéler la diversité génétique des souches ochratoxigènes d'*Aspergillus carbonarius* réside dans sa capacité à fournir des informations cruciales pour l'élaboration de stratégies efficaces de limitation de la contamination des produits agricoles par l'ochratoxine A. Cette étude s'est donc concentrée sur l'évaluation de la diversité génétique de 20 souches d'*A. carbonarius* isolées lors d'une précédente étude.
Méthodologie: Cette étude a porté sur l'évaluation de la diversité génétique de 20 souches d'*A. carbonarius* isolées lors d'une précédente étude. Au total, 26 amorces RAPD ont été initialement testées afin de déterminer les températures d'hybridation optimales et d'identifier celles produisant des profils de bandes clairs, cohérents et reproductibles. Les amorces répondant à ces critères ont ensuite été utilisées pour l'analyse génétique, selon les protocoles RAPD standard.
Résultats: Les résultats obtenus ont révélé que 7 amorces RAPD présentaient des polymorphismes clairs et satisfaisants dans des conditions d'amplification PCR optimales, entre 36°C et 40°C. Ces 7 amorces ont généré 18 loci RAPD distincts à partir des 20 isolats d'*A. carbonarius*. L'analyse de la structure de la population dans la région géopolitique de l'État d'Ogun a révélé que la variation génétique intra-site (de 0,02 à 0,33) représentait la majeure partie de la variation génétique observée (82,0%). L'amplitude de la variation globale due à la divergence au niveau du site (GST) variait de 0,01 à 0,42, suggérant une structure de population minimale au niveau du site. Les relations phylogénétiques entre les 20 isolats d'*A. carbonarius* ont révélé qu'aucun des isolats ne présentait de groupe génétiquement distinct.
Conclusion: La forte variation génétique observée chez les souches d'*A. carbonarius* ochratoxigènes souligne l'importance biotechnologique de la caractérisation moléculaire par RAPD pour la discrimination des souches, l'évaluation de la biosécurité et la gestion ciblée des risques liés à l'ochratoxine A, contribuant ainsi à une sélection et une application éclairée des souches en biotechnologie fongique.

Mots-clés: *Aspergillus carbonarius*; Ochratoxine; Diversité génétique; RAPD

Introduction:

Aspergillus carbonarius is a species of filamentous fungus considered one of the most economically important members of the black Aspergilli due to its frequent occurrence in food, plant, and soil environments (1). The predilection of *A. carbonarius* for different pre- and post-harvest food commodities as well as their known ability for producing ochratoxins including ochratoxin A has been well emphasized (2,3). This ochratoxin A (OTA) which is classified as a group 2B human carcinogen by the International Agency for Cancer Research in 2002, is known to be hepatotoxic, cytotoxic, teratogenic, neurotoxic, mutagenic, and carcinogenic in addition to inhibiting DNA and RNA synthesis (4,5).

Despite the widespread presence of this significant fungal pathogen and its impact on various food products, including ready-to-eat foods (3,6), studies focused on understanding its genetic diversity which is crucial for managing its extensive epidemiological distribution (7) remain limited. Traditionally, phenotypic methods such as the assessment of morphological characteristics, including colony diameter, color, conidial size and texture, and hyphal structure, have been employed (8). Additionally, investigating somatic cell fusion among isolates by screening for vegetative compatibility has also served as a method to examine fungal diversity (9).

These morphological parameters exhibit high variability (10), which limits their resolution and reduces their effectiveness in accurately distinguishing genetic diversity among isolates (11,12) of this fungus. It is therefore important to note that the application of reliable analytical procedures alongside sound quantitative analyses is essential for fungal diversity research (13) to preclude downward bias of genetic variation scores (12). One method that is increasingly gaining recognition is the application of random amplified polymorphic DNA (RAPD) marker analysis because of their documented efficiencies in deciphering the genetic diversities of different biological specimens (14,15). This molecular marker has not only revolutionized and simplified microbial diversity studies (16,17,18), but has also circumvented previous limitations associated with pedigree-based analyses and morphological characterization.

However, despite its advantages such as being cost-effective, rapid, and not requiring prior genomic information, RAPD also presents certain limitations. These include low reproducibility due to sensitivity to reaction conditions, difficulty in distinguishing homologous from non-homologous bands, and challenges in data standardization across laboratories, which can affect the reliability of inter-study comparisons. (18), thereby enhancing the fine resolution required to score appropriate diversity of organisms (12,19). This high throughput technique which is contingent upon amplification of nucleic acid (19,20) is a well understood *in vitro* process (21) with several tools aimed at optimizing their products (22) and thus simplifying the highly complex fungal taxonomy through the determination of the level of polymorphism and simila-

rity amongst fungal strains. This technique has shed light on both the detection of fungal isolates and their genetic relationships (23).

The significant recent advancements in molecular biology tools and technologies have impacted genetic diversity studies (24) and RAPDs, which are random 10-bp primers (decamers) are used in PCR to detect polymorphisms when the DNA sequence is unknown (25). However, there may be variation in the capability of different RAPD markers in the resolution of different fungal isolates. Therefore, the RAPD markers need to be optimized for different fungal isolates. Generally, the RAPD technique is commonly employed in ecological genetics to identify fungal species, estimate genetic patterns of association, analyse composite DNA samples, and develop targeted probe. Therefore, this study aimed to determine the genetic diversity of *A. carbonarius* strains isolated from different sources using the RAPD technique.

Materials and method:

Source of *Aspergillus carbonarius* isolates:

Aspergillus carbonarius isolates utilized in this study were obtained from one of our previous studies (26). The source of the isolates, the code, and the geopolitical zones from which they were isolated in Ogun State, Nigeria, are properly noted in Table 1. The climate of Ogun State is tropically wet and dry with average annual rainfall of 1,340 mm across a total cropland area of 693.21k ha.

All the isolates were obtained between 2018 and 2022 and were appropriately stored at 4°C in a refrigerator (Haier Thermocool, Model HTF-610DM7, Indonesia) in a 50% glycerol stock for future use. These *A. carbonarius* isolates from 'garri' samples, were identified through both phenotypic characterization and molecular analysis, were preserved on Sabouraud Dextrose Agar (SDA) slants and stored at 4 °C under refrigerated conditions for long-term maintenance.

Random amplified polymorphic DNA analysis (RAPD):

Genomic DNA was extracted from the ochratoxigenic *A. carbonarius* isolates using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's recommended protocol. To ensure reproducibility and generate clear, polymorphic DNA banding patterns, RAPD-PCR conditions were systematically optimized. A total of 26 decamer primers (Operon Technologies, USA) were initially screened using the extracted genomic DNA. Each primer was tested under a range of reaction conditions to evaluate its amplification efficiency and reproducibility. Optimization involved systematic variation of key PCR parameters, including primer concentration (0.2–1.0 µM), $MgCl_2$ concentration (1.5–3.0 mM), and template DNA quantity (20–80 ng per reaction). A temperature gradient PCR was used to identify the optimal annealing temperature for each primer, ranging from 36 °C to 40 °C, given the low-stringency binding characteristics of decamer RAPD primers.

Amplification reactions were performed in a total volume of 25 µL, following standard RAPD protocols, with 40 amplification cycles. Primers that consistently produced distinct, reproducible, and polymorphic banding patterns were selected for further analysis. Based on these criteria, 7 primers; OPX07 (GAGCGAGGCT), OPR16 (CTCTGCGCGT), OPR19 (CCTCCTCATC), OPR11 (GTAGCCGTCT), OPV06 (GAACGGACTC), OPA01 (CAGGCCCTTC), and OPA04 (AATCGGG CTG), yielded clear and scorable polymorphic bands. Optimal annealing temperatures for the primers ranged from 36°C to 40°C, with reproducibility confirmed across independent replicates. The final PCR reaction mix (25 µL) contained 12.5 µL of 2×PCR master mix [0.0545 U/µL Taq DNA polymerase in reaction buffer containing 4 mM $MgCl_2$, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP)], 40 pmol of a single decamer primer, and 1 µg of template DNA. The thermal cycling profile was as follows; initial denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 15 sec, annealing starting at 40°C and gradually reduced to 35°C in 4-cycle increments (1 min each), and extension at 72°C for 2 min.

Amplification products were resolved by electrophoresis on 3% agarose gels prepared in 1× TBE buffer, stained with ethidium bromide (0.5 µg/mL), and visualized under UV light using a gel documentation system. Banding patterns were manually analyzed following the criteria of Roodt et al., (27), based on the total number of bands and amplification consistency. Each lane was scored by recording the presence (+) or absence (–) of bands.

All RAPD-PCR reactions were performed in duplicate to assess reproducibility. For each primer and DNA sample, two independent PCR amplifications were carried out under the optimized conditions. Banding patterns were visually compared across replicates, and only bands that were consistently present in all the replicates were scored as true positives. This approach ensured that only reproducible and reliable polymorphic markers were considered for downstream analysis. No numerical averaging of band intensities was performed, as RAPD data

were scored qualitatively based on the presence (1) or absence (0) of bands.

Statistical analysis of data:

All statistical analyses of data were performed using GenAlEx version 6.5. Genetic data were summarized using frequencies, percentages, and diversity indices. RAPD markers were scored as binary data (presence/absence), and allele frequencies were computed for each locus across populations. Genetic diversity parameters (H_S, H_T, and G_{ST}) were estimated following Nei's method. Mean genetic variation was expressed as mean ± SEM.

Analysis of molecular variance (AMOVA) was used to partition genetic variation among and within populations. Chi-square (χ^2) test of independence was used to assess whether the distribution of variable loci and unique genotypes differed significantly among populations from Yewa, Egba, Remo, and Ijebu. Observed frequencies were organized into contingency tables, and expected frequencies were calculated under the null hypothesis of no association between population and genetic category. The degrees of freedom were determined as $(r-1) \times (c-1)$, where r represents the number of populations and c represents the number of categories. Statistical significance was set at $p < 0.05$.

Results:

Seven primers yielded 18 distinct RAPD loci from the studied *A. carbonarius* isolates. The proportion of variable loci observed among the isolates differed across the four populations; Egba (14.3%), Yewa (21.4%), Ijebu (28.6%), and Remo (35.7%) (Table 1). This indicates an increasing trend in genetic variability from Egba to Remo. Similarly, the proportion of unique genotypes identified at these loci also varied among populations, with Egba showing 16.6%, Yewa 22.2%, Ijebu 27.8%, and Remo 33.3% (Table 1). These percentages represent the proportion of genotypes that were exclusive to each population, suggesting increasing genotypic diversity from Egba to Remo. The Simpson's measure of diversity and genotypic evenness for the isolates were all at equilibrium at 0.95 and 1.00, respectively (Table 1).

The allele frequencies of most loci varied significantly ($p<0.05$) among the four populations, with the exception of OPX 07:100 and OPA04:700, which showed no significant variation. Intrapopulation genetic diversity (HS) ranged from 0.02 to 0.33 across loci, while total genetic diversity (HT) ranged from 0.02 to 0.49. The coefficient of genetic differentiation (GST) ranged from 0.01 to 0.42 across all loci (Table 2).

Table 1: Genetic characteristics of *Aspergillus carbonarius* isolates across four regions of Ogun State, Nigeria

Characteristics	**Sample collection sited**			
	YEWA	**EGBA**	**REMO**	**IJEBU**
Number of isolates	5	5	5	5
Number of loci	4	3	6	5
Percentage of variable loci	21.4	14.3	35.7	28.6
Percentage of unique genotypes	22.2	16.6	33.3	27.8
Simpson's Index of Diversity	0.95	0.95	0.95	0.95
Genotype evenness	1.00	1.00	1.00	1.00

Using Chi square test of independence, no significant differences were observed among populations for the proportion of variable loci (χ^2=1.905, df=3, p=0.59) and unique genotypes (χ^2=1.60, df=3, p=0.66)

Table 2: RAPD-based genetic diversity of twenty *Aspergillus carbonarius* isolates from Ogun State, Nigeria

Locus number	**Frequency of the marker allele**				**Genetic Diversity**		
	YEWA=5	**EGBA=5**	**REMO=5**	**IJEBU=5**	**H_S**	**H_T**	**G_{ST}**
OPX 07:100	1.00	1.00	1.00	1.00^{ns}	0.02	0.02	0.03
OPX 07:150	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:200	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:220	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:290	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:310	0.00	0.20	1.00	0.80*	0.16	0.19	0.01
OPX 07:500	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:520	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:700	0.00	0.00	0.00	1.00*	0.11	0.12	0.01
OPX 07:710	0.00	0.00	0.00	1.00*	0.11	0.12	0.01
OPX 07:980	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:1100	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPR16:100	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:150	0.80	0.80	0.80	0.60*	0.12	0.25	0.42
OPR16:200	0.20	0.20	0.20	0.40*	0.32	0.41	0.01
OPR16:210	0.80	0.80	0.80	0.60*	0.12	0.25	0.42
OPR16:220	0.80	0.80	0.80	1.00*	0.12	0.20	0.02
OPR16:290	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:310	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:400	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:450	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:555	0.20	0.20	0.20	0.40*	0.32	0.41	0.01
OPR16:560	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:700	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:900	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:1000	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:1300	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR16:1600	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR11:270	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:290	0.40	0.20	0.20	0.20*	0.26	0.31	0.07
OPR11:350	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:400	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:450	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR11:500	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR19:300	0.20	0.40	0.20	0.60*	0.13	0.24	0.02

OPR19:375	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR19:500	0.80	0.80	0.20	0.60*	0.12	0.18	0.03
OPR19:800	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR19:900	0.40	0.60	0.20	0.60*	0.19	0.34	0.09
OPV06:500	0.20	0.80	0.20	0.60*	0.33	0.49	0.33
OPV06:550	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPV06:600	0.80	0.20	0.20	0.20*	0.19	0.39	0.13
OPV06:700	0.60	1.00	0.20	0.60*	0.15	0.46	0.17
OPV06:750	0.40	1.00	0.20	0.60*	0.13	0.24	0.01
OPA01:400	0.20	1.00	0.20	0.60*	0.18	0.31	0.18
OPA01:450	0.20	0.60	0.20	0.20*	0.24	0.41	0.05
OPA01:560	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPA01:700	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPA01:800	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPA04:380	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPA04:390	0.40	0.20	0.20	0.20*	0.26	0.31	0.07
OPA04:700	0.20	0.20	0.20	0.20ns	0.20	0.20	0.03
OPA04:750	0.60	0.60	0.60	0.20*	0.13	0.29	0.44
OPA04:800	0.40	0.80	0.20	0.20*	0.04	0.05	0.09
OPA04:825	0.00	0.00	0.00	0.60*	0.14	0.17	0.01

H_S = intrapopulation genetic diversity; H_T = total genetic diversity; G_{ST}= coefficient of genetic differentiation; ns = not statistically significant; * = statistically significant

Table 3 shows the mean genetic diversity indices of the studied *A. carbonarius* isolates across the four geopolitical zones. The mean intrapopulation genetic diversity (HS) values were 0.28±0.01, 0.21±0.00, 0.27±0.00, and 0.31±0.02 for isolates from Yewa, Egba, Remo, and Ijebu, respectively. The corresponding total genetic diversity (HT) values were 0.32±0.00, 0.28±0.03, 0.36±0.01, and 0.45±0.00.

The proportion of total genetic diversity within populations (HS/HT) was approximately 88%, 75%, 75%, and 69% for Yewa, Egba, Remo, and Ijebu, respectively. The mean GST values were 0.21±0.01 (Yewa), 0.12±0.01 (Egba), 0.17±0.01 (Remo), and 0.23±0.00 (Ijebu).

Table 3: Mean genetic variation of the studied *Aspergillus carbonarius* isolates

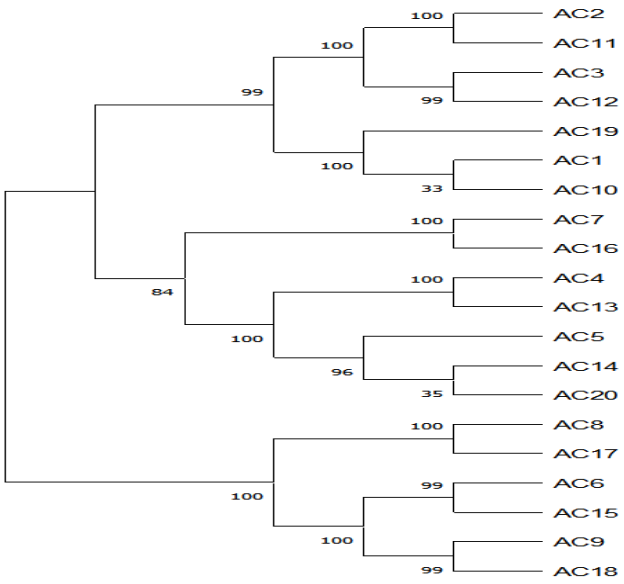
GEPZ/OS	**Number of loci**	**Mean ± SEM**		
		H_S	**H_T**	**G_{ST}**
YEWA	22	0.28 ± 0.01	0.32 ± 0.00	0.21 ± 0.01
EGBA	23	0.21 ± 0.00	0.28 ± 0.03	0.12 ± 0.01
REMO	35	0.27 ± 0.00	0.36 ± 0.01	0.17 ± 0.01
IJEBU	30	0.31 ± 0.02	0.45 ± 0.00	0.23 ± 0.00

GEPZ/OS=Geopolitical Zones of Ogun State, SEM=Standard Error of Mean

Table 4: Analysis of Molecular Variance (AMOVA) for the loci of *Aspergillus carbonarius* isolates

Sources of variation	df	SS	MS	F
Between-treatments	3	10.2727	3.4242	0.81178
Within-treatments	40	168.7273	4.2182	
Total 43 179				

F-ratio of 0.81178; *p*-value of 0.494904; result not statistically significant at $p<0.05$



AC1-AC5 = Yewa isolates, AC6-10 = Egba isolates, AC11-15 = Remo isolates, AC16-AC20 = Ijebu Isolates

Fig 1: Phylogenetic characterization of ochratoxigenic *Aspergillus carbonarius*

The analysis of molecular variance validated that even though the genetic diversity of isolates within samples from specific geopolitical zones accounted for majority of the total genetic diversity, the observations when compared between different zones were not statistically significant at $p<0.05$ (Table 4).

The phylogenetic relationships among the 20 *A. carbonarius* isolates are depicted in Fig 1. None of the isolates formed location-designated genetic clusters, while 3 of the 5 isolates from the Yewa zone formed a cluster with 99% similarity, while the remaining 2 Yewa isolates grouped with isolates from Egba, Remo, and Ijebu, showing 84% similarity. Similarly, 3 of the 5 isolates from Egba formed clusters with Remo and Ijebu at a 100% level of similarity, but both Remo and Ijebu are interspersed by isolates sharing different levels of similarities with isolates from all the geopolitical zones (84-100%).

Discussion:

The importance of RAPD in deciphering the genetic characterization of living organisms has been well documented (28,29,30). In this study, the strains of ochratoxigenic *A. carbonarius* are between 14.3% to 35.7% of variable loci, 16.6 to 33.3% of unique genotypes, Simpson's diversity index of 0.95 and genotypic evenness of 1 to highlights extensive genetic diversity within and between the isolates (7).

The genotypic evenness of 1 could further delineate that the population of the isolates yielded equally abundant genotypes. The observed values for total gene diversity (HT), within-subpopulation diversity (HS), and genetic differentiation among subpopulations (GST) are consistent with those reported for fungi possessing known sexual reproduction cycles. This is because several studies have documented that fungi

that reproduce through sexual means demonstrate wider genetic diversity than those with asexual means of reproduction (7,31,32). In another vein, it has been reported that some fungal isolates known to reproduce asexually have a genetic diversity index higher than expected, while the importance of parasexuality of some fungi in influencing diversity under controlled field conditions has also been emphasized (7).

Genotypic diversity analysis, nested analysis of molecular variance, and phylogenetic analysis consistently indicated that majority of the genetic differences within the organisms is attributable to differences across unique isolates. These analyses also revealed significant differentiation among isolates from each of the geopolitical zones, and such spatial community arrangement is possible through gene flow (33, 34). The occurrence of this type of gene flow within and between geographic populations of *A. carbonarius* can be accomplished by wind-aided spore dispersal, sexual and asexual reproduction (34). Most of the RAPD allele frequencies of the studied *A. carbonarius* except for two strains, shows apparent variation within the studied populations, to further connote that there might be frequent interbreeding events among these strains of *A. carbonarius* (35)

The comparatively low genetic divergence among the studied isolates (GST) suggests that genetic material (alleles) is being exchanged between and within populations across a broad spatial range, resulting in increased genetic similarity. There may be a need to do more intensive sampling of *A. carbonarius* at the geopolitical zones to examine if the aforementioned inter-collection sites variation reflects the actual disparities affecting how populations exchanged genetic material.

The fact that none of the isolates formed site-specific genetic clusters is an indication that allele frequencies among the studied *A. carbonarius* populations are very similar (34). It is thus imperative to note that any efforts aimed at controlling the ochratoxigenic *A. carbonarius* should take into account their extensive genetic diversities as well as factors prompting such high levels of diversity.

Conclusion:

In conclusion, the detection of high genetic variation among ochratoxigenic *A. carbonarius* strains emphasizes the biotechnological relevance of RAPD marker analysis as a rapid and effective tool for strain differentiation. In biotechnology, such genetic information is critical for biosafety evaluation, the identification of highly toxigenic genotypes, and the selection of appropriate strains for industrial, agricultural, or environmental applications. The findings, therefore, provide a molecular framework that supports informed decision-making in fungal biotechnology and mycotoxin risk management.

Contributions of authors:

BTT and ODP were involved in the study conceptualization and design; BTT, MOC, OST, KEP, ANT, GMA and ODP were involved in the methodology and data analysis; BTT, TOA, and SLO wrote the original draft; BTT, MOC, OAB and ODP reviewed and edited the manuscript. The final version of the manuscript was reviewed and approved by all authors.

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