Molecular analysis of gamma irradiated rice cultivar FARO 44 for submergence tolerance using RAPD markers

Mohammed Jiya[1,2*], Falusi Ahmed Olamide[2], Daudu Oladipupo Abdulazeez Yusuf[2], Abubakar Abdulhakeem[2]

[1]Ahman Pategi University, Patigi, Kwara State, Nigeria
[2] Federal University of Technology Minna, Niger State, Nigeria

Submitted: 10th April, 2025; Accepted: 19th April, 2025; Published online: 30th June, 2025
DOI: https://doi.org/10.54117/jcbr.v5i3.1
**Corresponding Author: Mohammed Jiya; mjiya0811308016@gmail.com*

Abstract

Rice (Oryza sativa L.) is an essential crop supporting over 60% of the global population, with rainfed lowland and deepwater ecosystems accounting for approximately 33% of global rice cultivation. However, submergence stress caused by seasonal flooding - exacerbated by climate change - significantly threatens rice productivity. This research sought to develop variability in submergence tolerance in FARO 44, a popular Nigerian rice variety, using gamma irradiation. The seeds were given gamma rays at 0, 50, 100, 150, and 200 Gy and submerged entirely for five days at the 21-day seedling level. Plant height and survival rate were some of the measured agro-morphological traits. The highest survival rate (93.75%) was observed at both 150 Gy and 200 Gy doses. To evaluate the genetic diversity within these lines of irradiated rice, molecular characterization was conducted with six primers of Random Amplified Polymorphic DNA (RAPD). The results suggest that 150 Gy and 200 Gy doses had marked increases in survival rates, which indicated improved tolerance. Analysis of the polymorphism showed significant results in the RAPD analysis, where primer B04 had the most considerable polymorphic information content of 0.82 and genetic diversity of 0.84, indicating genetic differentiation and effective change induction. These results confirm that generating rice genotypes tolerant to submergence using gamma irradiation is feasible, while the genetic diversity caused by irradiation can easily be monitored using RAPD markers.

Keywords: FARO 44; Gamma irradiation; Submergence tolerance; RAPD markers; Genetic variability

Introduction

Approximately 60% of the worldwide population depend on rice (Oryza sativa L.) as a basic diet source, making it the second most crucial cereal crop (Asma et al., 2023). These putatively cultivated rice varieties, at roughly 33 percent of the globe's deep-water rice fields, account for nearly fifty million hectares out of 150 million worldwide. (Asma *et al*., 2023; Abeysekara *et al*., 2020). Ajibade *et al*. reported in 2024 that flooding in Nigeria can cause annual crop losses ranging between 10% and complete loss, which profoundly impacts rice production.

Submergence tolerance refers to the ability to completely submerge the Rice plant in water for several days with the intent of allowing it to survive, use energy, and even commence growth consolidation, and later use this latent energy for growth purposefully (Oladosu et al., 2020)

Gamma irradiation is an alternative crop-breeding technique that enhances crop quality by inducing novel genetic variations (Bharat *et al.,* 2024). Induced rice mutants are valuable for the molecular and functional assessment of yield-limiting features in rice (Benavente & Giménez, 2021). Notably, characteristics like semi-dwarfism, maturity at an early stage, increased panicle numbers per plant, and improved fruitfulness have been identified through mutant studies (Ariharasutharsan *et al*., 2025).

Molecular markers have proven effective in identifying genetic variation and managing plant genetic resources (Salgotra & Chauhan, 2023). They offer advantages over morphological traits by detecting differences between genetic constitution at the molecular level, offering a further direct, dependable, and practical approach for germplasm characterisation, preservation, and utmost management (Oginyi *et al.,* 2024).

Enhancing submergence tolerance has been a significant breeding intention for rain-fed lowland and deep-water rice regions (Panda *et al*., 2022). Despite this, progress in improving submergence tolerance in African countries, particularly Nigeria, has been limited (Akinkuolie *et al*., 2025). In order to address this issue, submergence tolerance of local cultivars is a primary focus of ongoing rice enhancement programs in Nigeria. (Mwakyusa, *et al.,* 2023). Developing submergence-tolerant varieties is a primary approach to mitigating the submergence vulnerability of lowland rice farms

(Mwakyusa et al., 2023). Random Amplified Polymorphic DNA markers were selected for diversity analysis in this study owing to their great applicability in assessing crop genetic diversity. RAPD markers have been used in rice, maize, wheat, and barley diversity studies. (Arabzai *et al.,* 2021). This research aimed at enhancing submergence tolerance by inducing genetic diversity in FARO 44 through gamma ray exposure and evaluating the genetic variation through RAPD analysis.

Materials and methods

Collection and Treatment of Seeds

The seeds (FARO 44) used in this investigation were procured from the National Cereal Research Institute (NCRI) in Badeggi, Nigeria. At the same time, the exposure to gamma radiation was conducted at the Centre for Energy Research and Training at Ahmadu Bello University, Zaria, Nigeria. The seeds were divided into five groups. Four experimental groups were irradiated with gamma-rays at doses of 50, 100, 150, and 200 Gy using a cesium-137 source, while an additional unirradiated group (0 Gy) served as the control. The control group underwent identical handling and environmental conditions as the irradiated groups, but received no radiation exposure. Subsequently, the seeds were planted in 10-liter experimental pots containing a mixture of sandy-loam soil with a soil volume of 4 liters. At the Experimental Garden of the Department of Plant Biology, Federal University of Technology Minna, Nigeria, the trials were replicated in a randomised complete block design (RCBD) four times for each treatment. Following the modified Akinwale *et al.* (2012) method, the seedlings were submerged for five days during the 21-day seedling stage. All essential agronomic practices were followed throughout the experiment, and meticulous recordkeeping was maintained for the agro-morphological information documented.

Molecular investigation

Molecular analysis of the gamma-irradiated FARO 44 rice mutants was conducted with a modified protocol based on Warwick and Gugel (2003) and further built upon by Pham *et al.* (2011). Freeze-dried (-80C) young seedlings (21-28 days) were kept whole and milled in liquid nitrogen. A genomic DNA sample was obtained using powdered tissue using the standard CTAB technique. DNA value and concentration were evaluated using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific) with a diluent of 25ng/µl for further application.

After quantification, the intact DNA was polymorphically partitioned through RAPD markers utilizing decamers to determine the degree of genetic deviation (diversity and polymorphism) across the three lines of mutants. There is little need for genomic sequence foreknowledge, so RAPD was effective within its cost yet still reliable in detecting variations.

PCR amplification

An Applied Biosystems thermal cycler was used to execute the Polymerase Chain Reaction methodology. Each PCR reaction included an initial denaturation at 94°C for 3 minutes, followed by 30 cycles consisting of denaturation at 94°C for 1 minute, annealing at 40°C for 2 minutes, and extension at 72°C for 2 minutes. A final extension was executed at 72°C for 5 minutes, followed by a hold at 4°C.

Amplified products were resolved on 1.8% agarose gels stained with ethidium bromide. A 100 bp molecular weight ladder was used as a reference. Electrophoresis was carried out in 1× TBE buffer, and bands were pictured and documented using a gel documentation system (Bio-Rad Gel Doc™ XR+). The molecular experiments were conducted at the Bioscience Center, the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Data analysis

Data on agro-morphological parameters were collected, including plant height before and after submergence and survival rate following submergence. An analysis of variance (ANOVA) was performed using SPSS version 25 to assess the significance of differences among treatment means. Significant differences ($p < 0.05$) were analyzed using Duncan's Multiple Range Test (DMRT) to separate the means.

For molecular data, each unique DNA band was recorded as either present (1) or absent (0), resulting in a binary matrix for each RAPD primer. The genetic similarity among the mutant lines was evaluated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). A dendrogram was created with NTSYS-pc software version 2.11 to identify genetic relationships and cluster diversity (Tamura *et al.,* 2013).

Results and discussion

Impact of gamma irradiation on the submergence tolerance parameters of FARO 44

Plant Height

In FARO 44, the lowest heights (12.75) were documented prior to submergence for the plant exposed to 100 Gy of gamma doses over 5 days of submergence. The plant subjected to 150 Gy of gamma radiation exhibited a maximum height of (15.45), markedly distinct from all other doses. Following DE submergence over 5 days, the plant height showed significant differences. Plants subjected to 150 Gy of gamma irradiation exhibited the most significant height (23.13). The 50 Gy dose exhibited a markedly distinct outcome compared to all other doses, recording the lowest height at (15.08), which is marginally decreased from the control and notably differs from the remaining doses. This pattern indicates that gamma irradiation can cause morphological alterations in rice plants, with elevated doses (up to 150 Gy) enhancing plant development under submerged conditions. These observations align with the discoveries of Yasmine *et al.* (2019), who recounted that rice seedlings exposed to cumulative gamma doses of 67 and 162 Gy exhibited increased plant height, indicating a stimulatory effect of low to moderate radiation doses on growth parameters. The enhanced growth at 150 Gy may be attributed to the radiation-induced activation of growth-promoting genes or hormonal pathways, leading to improved cell elongation and division.

In contrast to the Mushtaq & Rakesh (2015) findings, which report a reduction in plant height with increasing radiation doses and the control plants exhibiting the most significant height, the current study differs in that the control plants did not show the most significant height. This discrepancy may be attributed to the changes in rice cultivars, environmental factors, or methodological differences in the earlier experiments. The increase in plant height with greater irradiation doses suggests that FARO 44 may show greater sensitivity to moderate levels of gamma irradiation, resulting in better growth under submergence stress.

Survival Percentage (%)

The proportion of survivors from the FARO 44 rice variety subjected to 5-day submergence stress showed a pronounced change with different irradiation dosages. The maximum surviving proportion was achieved at 150 Gy and 200 Gy; statistically, no difference was found between these two treatments. The control where no irradiation was done (0 Gy) and 100 Gy treatment were significantly lower than expected, showing the lowest survival rate.

Ali *et al*. (2020) observed that flooding moderately enhances the physiological resilience of rice plants to stress at gamma irradiation doses between 150 and 200 Gy, which aligns with this study's findings. The effects of gamma irradiation are believed to induce beneficial genetic mutations that increase chlorophyll, root, and carbohydrate metabolism during post-submergence recovery, which is critical for recovery post-submergence.

Moreover, Iftekharuddaula *et al.* (2011) reported that improved submergence tolerance in rice varieties like *FARO 44* can be attributed to enhanced expression of the SUB1A gene, which regulates ethylene and gibberellin responses under stress. While some studies (Kwon *et al.*, 2022) suggest that gamma irradiation might mimic stress-response pathways similar to those regulated by SUB1A, this hypothesis requires validation through gene expression assays.

This observation contradicts reports by Animasaun *et al.* (2014), who indicated that untreated plants exhibited higher survival rates than those subjected to gamma irradiation. The observed discrepancy could be due to genotype-specific responses to radiation and submergence or differences in experimental design, including submergence duration and water turbidity.

Molecular characterization

This study employed six RAPD primers (H10, T06, B04, T16, T05, and T17) to distinguish between FARO 44 and its potential mutants, selected based on their reproducibility and documented efficacy in crop diversity studies (Al-Asmari *et al.,* 2021). The polymorphism analysis revealed that primer H10 generated 8 fully polymorphic bands, while T06 produced 6 bands (5 polymorphic, 1 monomorphic). Primer B04 showed high discriminatory power with 9 bands (8 polymorphic), T16 yielded 6 bands (3 polymorphic), T05 generated 9 bands (8 polymorphic), and T17 amplified 10 polymorphic bands. The mean Polymorphism Information Content (PIC) value of 0.62 across all primers fell within the high-polymorphism range (0.5–1.0) recommended for robust diversity studies, confirming the markers' reliability (Al-Asmari et al., 2021). Primer B04 stood out with exceptional PIC (0.82) and genetic variation (0.84), comparable to findings in potato mutants (Humera & Javid, 2012), validating its utility for detecting genotype-level polymorphisms.

The dendrogram analysis grouped the samples into three main clusters: Cluster 1 included the control (untreated) and 200 Gy-treated sample 4 (200s4), with subclusters further segregating other mutants (200s3, 150s1); Cluster 2 comprised 150s2 and 200s5; and Cluster 3 contained 150s4 and 200s1. These groupings elucidated genetic linkages between irradiated mutants and the control, with polymorphic bands—particularly from B04—reflecting significant gamma irradiation-induced divergence. The results align with Daudu *et al*. (2016), who demonstrated RAPD-PCR's effectiveness in establishing genetic relationships in *H. sabdariffa*, reinforcing that the observed polymorphisms are reliable indicators of mutagenic effects.

Although RAPD is a cost-effective method for initial diversity assessment, its reproducibility and resolution are often considered limited compared to more advanced markers like SSRs or SNPs (Benavente & Giménez, 2021). Thus, these findings should ideally be validated with higher-resolution marker systems

Table 1: Impact of gamma irradiation on submergence tolerance parameters of FARO 44 over a 5 day duration

Dose (Gy)	Height prior to submergence (cm)	Height post de-submergence (cm)	Survival rate (%)
0	14.60 ± 0.12^{ab}	16.55 ± 1.58^{ab}	68.75 ± 6.25^{a}
50	14.00 ± 0.57^{ab}	15.08 ± 2.33^{a}	75.00 ± 10.21^{ab}
100	12.75 ± 1.27^{a}	18.20 ± 1.71^{abc}	68.75 ± 23.66^{a}
150	15.45 ± 0.37^{b}	23.13 ± 2.09^{c}	93.75 ± 6.25^{b}
200	14.83 ± 0.13^{b}	21.80 ± 0.54^{bc}	93.75 ± 6.25^{b}

Values are expressed as Mean ± Standard Error. Different superscript letters within a column indicate statistically significant differences ($p < 0.05$) according to Duncan's multiple range test. Means sharing common letters are not significantly different.

Table 2: Polymorphic Bands Generated by RAPD Primers

Primers	Monomorphics band	Polymorphics band	Total number of bands	Percentage polymorphism
H10	0	8	8	100
T06	1	5	6	83.0
B04	1	8	9	88.0
T16	3	3	6	50.0
T05	1	8	9	88.0
T17	0	10	10	100

Table 3: *Major Allelic Frequency, Number of Alleles, Genetic Diversity, and Polymorphic Information Content*

DNA Primers	Major Allelic Frequency	Sample size	Number of observation	Allele number	Availability	Genetic diversity	PIC
H10	0.40	10.0	10.0	6.0	1.0	0.76	0.73
T06	0.70	10.0	10.0	2.0	1.0	0.42	0.33
B04	0.30	10.0	10.0	8.0	1.0	0.84	0.82
T16	0.70	10.0	10.0	3.0	1.0	0.46	0.41
T05	0.40	10.0	10.0	5.0	1.0	0.74	0.70

T17	0.40	10.0	10.0	6.0	1.0	0.76	0,73
MEAN	0.48	10.0	10.0	5.0	1.0	0.66	0.62

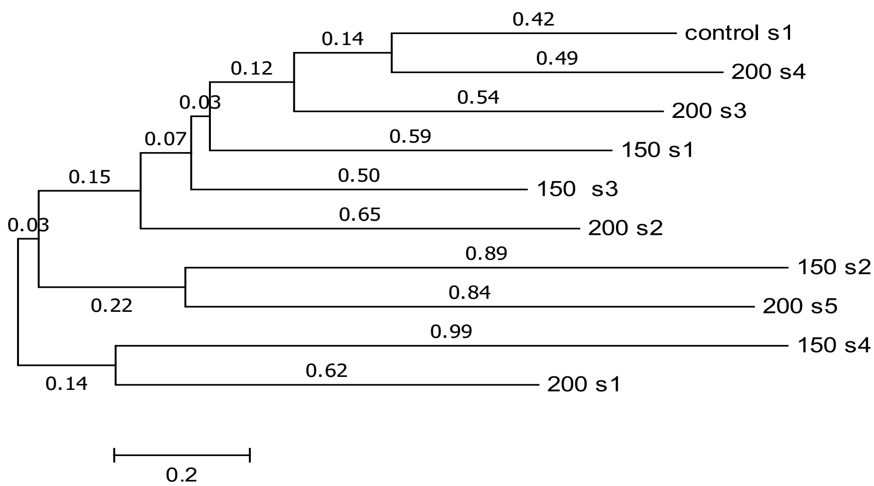
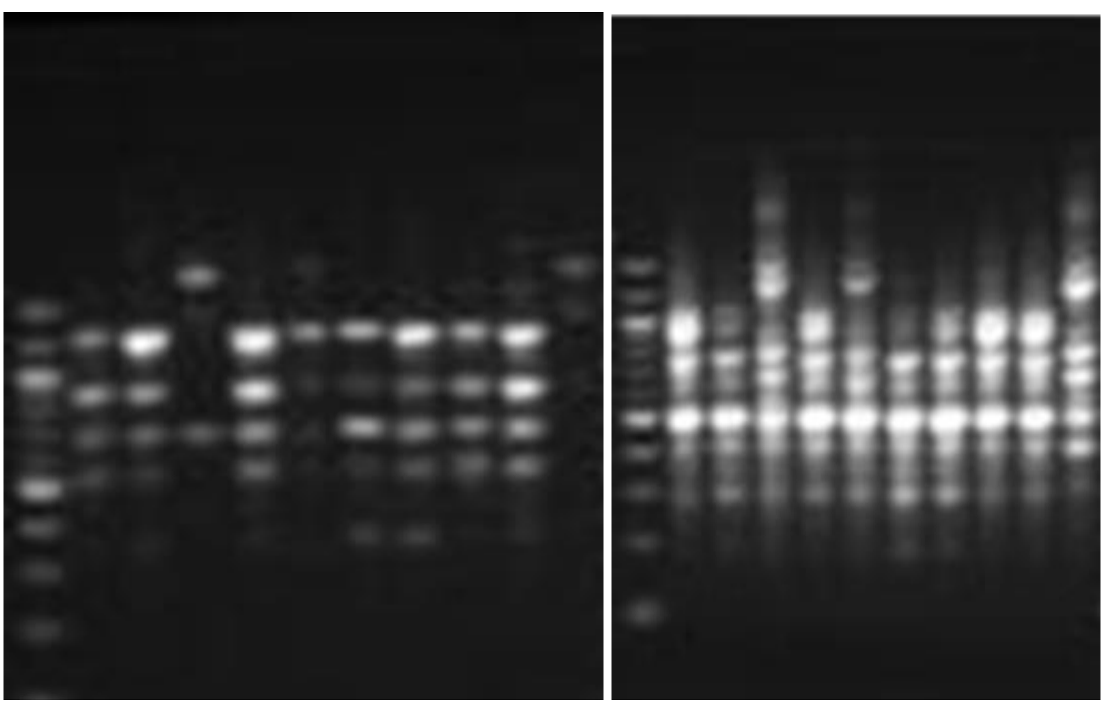
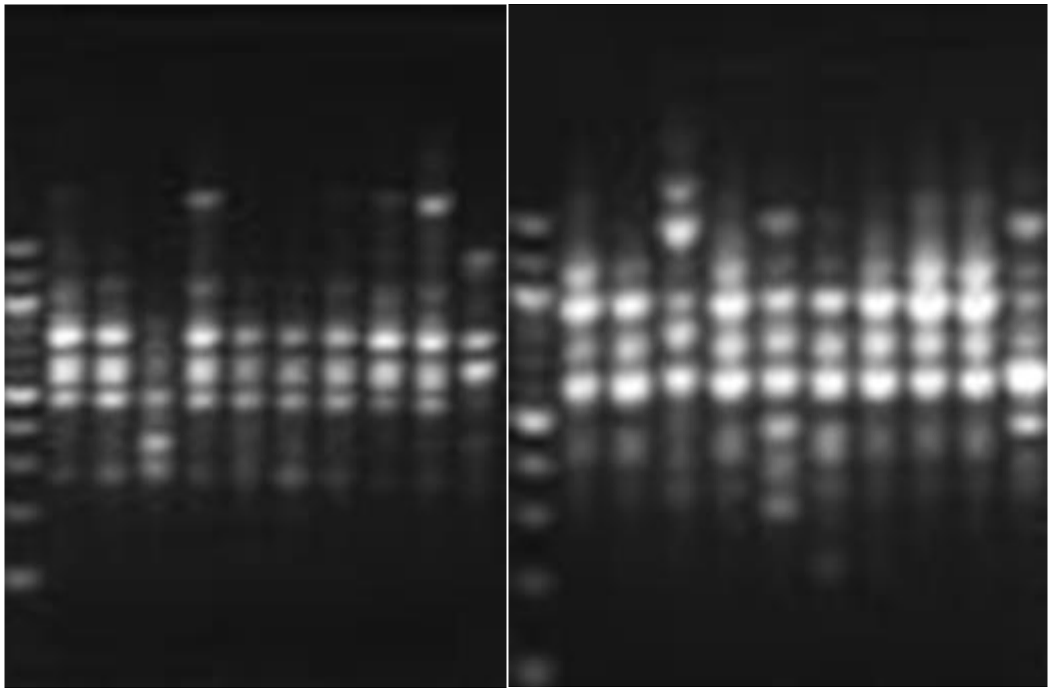


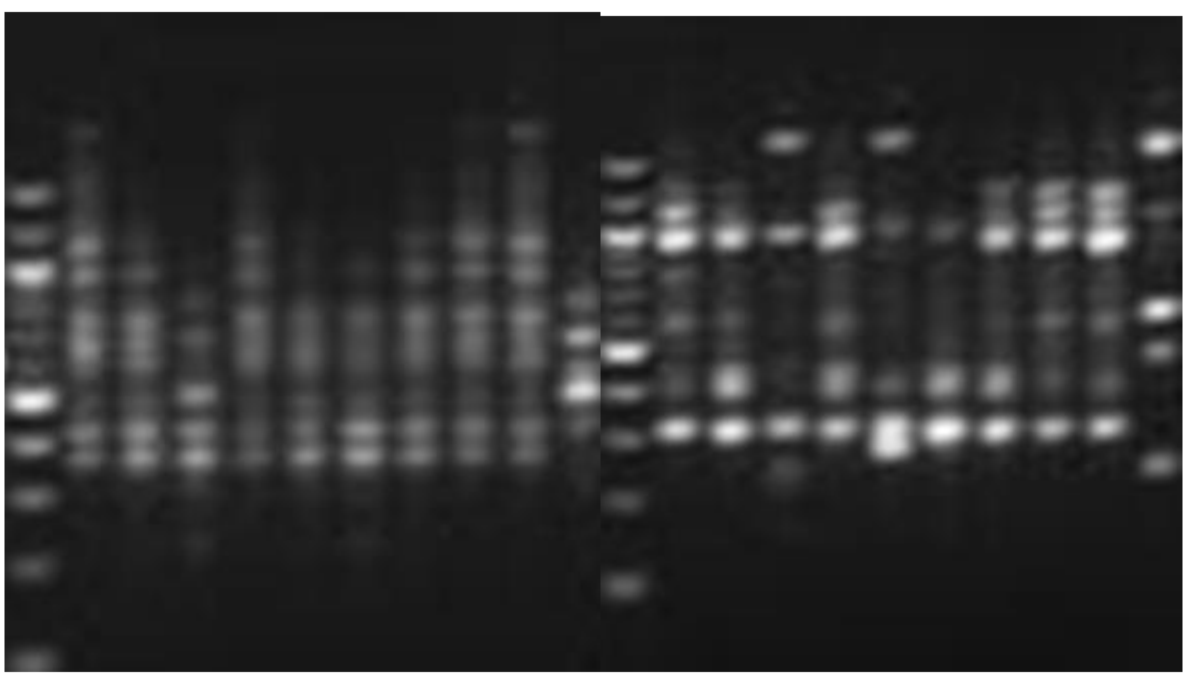
Fig 1. *Clustered dendrogram of Faro 44 samples*



H10 **T06**



B04 T16



T05 T17

Fig 2. *RAPD banding pattern obtained in gamma-irradiated faro 44 with six primers*

Conclusion

This study demonstrates that gamma irradiation can induce submergence tolerance in FARO 44 rice. Moderate to high doses (particularly 150–200 Gy) enhanced survival rates and plant height post-submergence. Molecular analysis using RAPD markers confirmed significant genetic variation, with primer B04 proving most effective in distinguishing irradiated mutants from the control. These results underscore the potential of induced mutagenesis in developing rice varieties that are better adapted to flood-prone regions. Further research employing field trials and advanced molecular tools such as SSR or SNP markers is recommended to validate and expand upon these findings.

Acknowledgments

We express our gratitude to God the Creator for His infinite mercy in sustaining our lives and enabling us to conclude this scientific undertaking.

Disclosure of potential conflicts of interest

The authors of this work stated that there is no conflict of interest.

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