



# Induce genetic variability in rice (*Oryza sativa* L.) under Tissue Culture condition

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## ABSTRACT

This study investigated the induction of genetic variability in rice through tissue culture techniques using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Two rice accessions underwent varying soak durations (12 and 24 hours) and concentrations (0%, 20%, 40%, 60%, 80%, and 100% H<sub>2</sub>O<sub>2</sub>). Following soaking, the seeds were inoculated into Murashige and Skoog (MS) media in a completely randomized design with three replications under in vitro conditions. Phenotypic traits, including the number of shoots, shoot length, number of roots, and root length, were analyzed through ANOVA with a significance threshold of  $\leq 0.05$ . Results indicated significant differences among the treated plantlets and the control group, with specific treatments demonstrating superior performance in various physical parameters. Notably, the highest mean number of shoots (2.06) was observed in the control group (12 hours) for accession NGB00789, followed by 2.00 in the control group (12 hours) for NGB00792. Conversely, the lowest mean value (0.17) was recorded for NGB00792 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours. Regarding shoot length, NGB00789 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours exhibited the highest mean value (4.44 cm), while NGB00792 soaked with 60% H<sub>2</sub>O<sub>2</sub> for 24 hours reached a mean value of 4.36 cm. For the number of roots, the highest mean value (6.67) was observed in NGB00789 under the control condition, followed by 6.22 in NGB00792, also in the control group. The lowest mean value (0.56) was recorded in NGB00792 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours. Concerning root length, the highest mean value (6.01 cm) was achieved in NGB00792 treated with 60% H<sub>2</sub>O<sub>2</sub> for 24 hours, while NGB00792 soaked with 20% H<sub>2</sub>O<sub>2</sub> for 12 hours reached a mean value of 5.99 cm. The lowest mean value (0.32 cm) was recorded in NGB00789 treated with 80% H<sub>2</sub>O<sub>2</sub> for 24 hours. In conclusion, this study not only sheds light on the practical applications of hydrogen peroxide in rice tissue culture but also underscores the necessity for further research in this domain to fully harness the potential of mutagenesis as a tool for crop improvement.

## INTRODUCTION

Rice (*Oryza sativa* L.), a staple crop worldwide, possesses untapped genetic potential for significantly higher grain yields in various rice-growing ecosystems. However, realizing this potential remains a challenge, primarily due to the crop's sensitivity to abiotic stresses, with salinity being a prominent stressor (Grover *et al.*, 2000). In Nigeria, rice cultivation, largely led by smallholder farmers utilizing traditional methods, constitutes over 80% of the national production (Omoare and Oyediran, 2020). Despite being the top rice producer in Africa, challenges such as outdated technology, low productivity, and inadequate infrastructure hinder sustainable production (Afiukwa, 2016; Rosenzweig *et al.*, 2001). Abiotic stresses, including salt toxicity, drought, and nutrient deficiency, present significant threats to rice production in Nigeria (Umego *et al.*, 2020). The impact of drought stress, affecting approximately 23 million hectares of rain-fed rice globally, is expected to exacerbate with climate change (Serraj *et al.*, 2011; Ahmad *et al.*, 2020).

To mitigate these challenges, it is essential to develop effective strategies to protect rice production from the adverse effects of abiotic stresses. A cost-effective approach for enhancing crop species resilience against production challenges involves employing chemical mutagenesis. While various chemicals have been utilized for inducing mutagenesis in crop species, the potential of hydrogen peroxide in this context remains largely unexplored.

Hydrogen peroxide functions as a mutagen in plant breeding experiments, fostering genetic variability for diverse traits in crops such as cowpea, mung bean, and sesame (Susrama *et al.*, 2022; Ablaku *et al.*, 2021). It is also implicated in various gene expressions, DNA damage, and programmed cell death across plant species. In *Arabidopsis thaliana*, hydrogen peroxide treatment induced significant changes in gene expression patterns related to light signaling, nutrient status, and temperature, highlighting its role in regulating cellular processes. Studies, exemplified by Takáč *et al.* (2016), reveal that hydrogen peroxide positively influences flax adventitious root formation by regulating auxin levels, suggesting its potential application for enhancing flax regeneration capacity. While hydrogen peroxide is extensively used in various plant studies, specific experiments on its role in inducing genetic variability in rice are limited, with most rice research concentrating on its response to oxidative stress and the activation of defense mechanisms.

This study aimed to determine the efficacy of hydrogen peroxide for inducing genetic variability in rice under tissue culture conditions. It sets the stage for a comprehensive examination of the mutagenic effects of hydrogen peroxide and its implications for rice genetic diversity and agronomic traits.

## MATERIALS AND METHODS

### Plant materials

Seeds from two rice accessions, namely NGB 00789 and NGB 00792, recognized for their upland characteristics and higher yield potential, were obtained from the Seed GeneBank Department at the National Centre for Genetic Resource and Biotechnology (NACGRAB) in Ibadan, Nigeria.

### Seed surface preparation

Healthy seeds underwent a thorough cleaning process, involving washing with a 0.02% (v/v) tween-20 solution, followed by rinsing under running tap water. Surface sterilization was carried out by immersing seeds in 0.15% (v/v) NaOCl<sub>2</sub> for 10 minutes, followed by subsequent treatment with 70% (v/v) ethanol for 5 minutes. These steps were performed under aseptic conditions within a laminar airflow chamber. Sterilized seeds were meticulously rinsed with sterile distilled water five times and then air-dried on sterile filter sheets within 90 mm Petri dishes.

### Inoculation and culture

Upon surface sterilization, seeds were introduced onto fresh culture media composed of Murashige and Skoog (MS) salts and vitamins (Murashige and Skoog, 1962). These sterilized seeds underwent treatment with varying concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) — 0%, 20%, 40%, 60%, 80%, and 100% — for different durations: control (untreated), 12 hours, and 24 hours. After treatment, the seeds were meticulously washed with sterile water before placement on the prepared MS media. To ensure sterility, the media's pH was adjusted to 5.80 using 0.1 N HCl or NaOH, followed by autoclaving at 121°C for 15 minutes. Cultures were then maintained under controlled conditions in a growth room, featuring a temperature of 27 ± 2°C, a photoperiod of 16 hours of light (at an intensity of 10,000 lux), and 8 hours of darkness.

### Experimental design

The research study was carried out using experimental design a completely randomized design (CRD) with three replications under in vitro conditions. It is important to note that the number of seeds or samples per replication was [insert specific number], and randomization procedures were employed during the experiment.

### Data collection and analysis

After two weeks of inoculation, various physical parameters were assessed to monitor the growth and development of the cultures. Regeneration frequency, denoting the number of surviving plantlets resulting from

H<sub>2</sub>O<sub>2</sub> treatment, was determined. Data obtained were subjected to statistical analysis using AGRES software, employing analysis of variance (ANOVA) to assess the impact of H<sub>2</sub>O<sub>2</sub> on mutagenesis induction and regeneration.

## RESULTS AND DISCUSSIONS

The study aimed to induce genetic variation in rice under tissue culture conditions using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a chemical mutagen. The impact of H<sub>2</sub>O<sub>2</sub> treatment on desirable phenotypic traits, including the number of shoots, shoot length, number of roots, and root length, was evaluated. The experiment focused on the direct regeneration of plants through embryogenesis in Murashige and Skoog (MS) media treated with H<sub>2</sub>O<sub>2</sub>. Analysis of variance (ANOVA) was conducted to assess the significance of observed variations, with significant levels determined at a probability threshold of  $\leq 0.05$  (Table 1).

### Effect of H<sub>2</sub>O<sub>2</sub> treatment on phenotypic traits

A notable decrease in all assessed traits was observed with increasing duration of mutagenic treatments, both

for the 12-hour and 24-hour treatments with full-strength MS media (Table 2). Significant differences ( $p \leq 0.05$ ) were identified among the treated plantlets and the control group. Certain treatments performed better in terms of physical parameters. The number of shoots showed the highest mean value (2.06) in the control group (12 hours) for rice accession NGB00789, followed by a mean value of 2.00 in the control group (12 hours) for NGB00792. In contrast, the lowest mean value (0.17) was recorded for NGB00792 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours. For shoot length, the highest mean value (4.44 cm) was achieved in NGB00789 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours, while NGB00792 soaked with 60% H<sub>2</sub>O<sub>2</sub> for 24 hours reached a mean value of 4.36 cm. Regarding the number of roots, the highest mean value (6.67) was observed in NGB00789 without H<sub>2</sub>O<sub>2</sub> treatment (control), followed by a mean value of 6.22 in NGB00792, also in the control group. The lowest mean value (0.56) was recorded in NGB00792 soaked with 100% H<sub>2</sub>O<sub>2</sub> for 12 hours. The highest mean value (6.01 cm) for root length was achieved in NGB00792 treated with 60% H<sub>2</sub>O<sub>2</sub> for 24 hours, while a mean value of 5.99 cm was observed in NGB00792 soaked with 20% H<sub>2</sub>O<sub>2</sub> for 12 hours. The lowest mean value (0.32 cm) was recorded in NGB00789 treated with 80% H<sub>2</sub>O<sub>2</sub> for 24 hours.

**Table 1: Analysis of variance of H<sub>2</sub>O<sub>2</sub>-induced phenotypic traits in rice under tissue culture conditions**

Source of variation	DF	SHTN	SHTL	RTN	RTL
Treatment	5	0.018*	0.024*	0.039*	0.041*
Hr	1	1 <sup>ns</sup>	0.063 <sup>ns</sup>	0.161 <sup>ns</sup>	0.234 <sup>ns</sup>
Treatment x Hr	5	0.021*	0.027*	0.072 <sup>ns</sup>	0.078 <sup>ns</sup>
Error	24				

Probability of significant level when  $p \leq 0.05$

NS: Number of shoots; SL: Shoot length; NR: Number of roots; RL: Root length

**Table 2: Mean and standard deviation of H<sub>2</sub>O<sub>2</sub>-induced phenotypic traits in rice under tissue culture conditions**

Treatment	Number of shoots	Shoot length (cm)	Number of roots	Root length (cm)
H <sub>2</sub> O <sub>2</sub> 20% (12hr) NGB 00789	1.33 ± 0.97bc	3.28 ± 2.56abc	4.72 ± 3.71bcd	3.21 ± 2.44def
H <sub>2</sub> O <sub>2</sub> 40% (12hr)	1.11 ± 0.90cde	3.07 ± 2.54bc	4.06±3.54cdef	4.17±3.05bcd
<b>H<sub>2</sub>O<sub>2</sub> 60% (12hr)</b>	<b>0.00±0.00h</b>	<b>0.00±0.00h</b>	<b>0.00±0.00j</b>	<b>0.00±0.00i</b>
H <sub>2</sub> O <sub>2</sub> 80% (12hr)	0.67±0.97defg	1.54±2.25def	2.50±3.84efgh	1.79±2.61fgh
H <sub>2</sub> O <sub>2</sub> 100% (12hr)	1.72±0.57ab	<b>4.44±2.07a</b>	5.17±2.09abcd	3.38±1.59de
CONTROL (12h)	<b>2.06±0.54a</b>	3.38±1.27abc	<b>6.67±3.41a</b>	<b>5.62±1.62ab</b>
H <sub>2</sub> O <sub>2</sub> 20% (24hr)	1.06±0.94cdef	1.31±1.14efg	2.28±2.22fghi	1.19±1.06ghi
H <sub>2</sub> O <sub>2</sub> 40% (24hr)	1.89±0.32a	3.54±1.45abc	<b>5.67±1.61abc</b>	4.43±1.38bcd
H <sub>2</sub> O <sub>2</sub> 60% (24hr)	1.67±1.28ab	2.34±1.77cde	4.28±3.18cde	3.83±3.12bcd
H <sub>2</sub> O <sub>2</sub> 80% (24hr)	0.33±0.49gh	0.70±1.02fgh	0.67±0.97hij	0.32±0.49hi
<b>H<sub>2</sub>O<sub>2</sub>100% (24hr)</b>	<b>0.00±0.00h</b>	<b>0.00±0.00h</b>	<b>0.00±0.00j</b>	<b>0.00±0.00i</b>
CONTROL (24hr)	1.83±0.38ab	2.28±0.24cde	3.67±1.81def	3.11±1.43def
H <sub>2</sub> O <sub>2</sub> 20% (12hr) NGB 00792	1.83±0.38ab	3.24±1.29abc	5.17±2.43abcd	5.81±2.10a
H <sub>2</sub> O <sub>2</sub> 40% (12hr)	0.67±0.97defg	1.44±1.67efgh	1.11±1.67ghij	2.21±3.11fgh
H <sub>2</sub> O <sub>2</sub> 60% (12hr)	1.17±0.99cd	2.55±2.33cd	2.78±2.51efg	3.09±2.75def
H <sub>2</sub> O <sub>2</sub> 80% (12hr)	0.61±0.91efg	1.33±2.15efg	1.39±2.19ghij	1.93±3.01efg
H <sub>2</sub> O <sub>2</sub> 100% (12hr)	0.17±0.38gh	0.08±0.19h	0.56±1.29ij	0.68±1.57ghi
CONTROL (12h)	<b>2.00±0.00a</b>	2.96±1.59bc	<b>6.22±2.69ab</b>	5.53±2.03ab
H <sub>2</sub> O <sub>2</sub> 20% (24hr)	1.78±0.65ab	2.73±1.39bc	5.44±2.66abcd	<b>5.99±2.24a</b>
H <sub>2</sub> O <sub>2</sub> 40% (24hr)	1.72±0.67ab	<b>3.84±1.75ab</b>	4.94±2.53abcd	4.63±2.19abcd
H <sub>2</sub> O <sub>2</sub> 60% (24hr)	1.89±0.32a	<b>4.36±1.96a</b>	5.56±2.97abcd	<b>6.01±2.18a</b>
H <sub>2</sub> O <sub>2</sub> 80% (24hr)	0.56±0.92fg	1.13±1.87efgh	1.83±3.19ghij	0.99±1.66ghi
H <sub>2</sub> O <sub>2</sub> 100% (24hr)	0.22±0.55gh	0.14±0.35gh	0.67±1.64hij	0.69±1.59ghi
CONTROL (24hr)	<b>1.94±0.23a</b>	3.27±1.56abc	5.39±2.30abcd	4.97±2.07abc

## DISCUSSION

Mutation induction has been recorded for a long period. Nevertheless, many improvements have been made to increase the mutation frequency from modern technology. Rice is an important cereal crop species for economic values and value addition chain globally, and since it presents a small genome and displays synteny with other crops, many achievements in rice structural and functional genomics have been expanded to other crop species. Rice was already objective from the initial findings of mutation induction of recent research work, which includes more than the 9<sup>th</sup> century of research. Much has been gained agronomically and conservatively through the increase in rice variability. Chemical mutagenesis offers several advantages, including ease of handling, cost-effectiveness, and specificity compared to physical mutagenesis methods. However, it is crucial to exercise caution due to the potentially carcinogenic

nature of chemical mutagens. Research in crop mutagenesis has demonstrated the superiority of chemical mutagens over ionizing radiations, as they induce milder genetic effects and fewer chromosome breaks. Rapoport (1966) made significant contributions to the field by introducing the concept of "microgenetics." This concept elucidates gene structure, function, mutagen mode of action, mutation origin, and fixation in progeny.

This review summarized the data related to the role of hydrogen peroxide that we have published during the past 15 years using rice (cultivar TN1) as a model plant. It is clear that, in rice plants, hydrogen peroxide not only acts as a toxic compound but also as a signaling molecule associated with basal salt media. In considering the relationships between hydrogen peroxide production and basal salt media and response to mutation, we would also remember that these stresses almost occur in the field together with other abiotic stress

conditions. Such combinations could include phenotypic and temperature factors. How these different stress combinations affect hydrogen peroxide production and basal salt media is a subject of active research that should be taken into reward. Chloroplasts are one of rice cells' most significant sources of hydrogen peroxide. Extensive insight mutagens play an important role in cereal crops especially rice by using hydrogen peroxide in signaling seems to be necessary. However, it would be a great admire and demand to notify all the changes in gene expression regulated by phenotypic expression or abiotic stresses in rice using transcriptomic analyses. The combined use of mutagenic treatment with *in vitro* culture was applied to play the role of alteration in genetic variations. This was delineated by best growth value and green plant regeneration frequency of irradiated Hydrogen peroxide-adapted affected chlorophyll content of the plant and some formed callus induction as compared with non -non-mutagenized control shown in (Tables 1 and 2). Some results were recorded by other authors (Bhagwat and Duncan, 1998; Cheema, *et al.*, 2002; Lee, *et al.*, 2003). It was believed that minimal stress or proper stress on the callus by irradiation might not induce irreversible genotypic changes but could stimulate chlorophyll pigmentation reduction or callus formation and plant regeneration. Furthermore, the haploid induction technique can nowadays be efficiently combined with several other plant biotechnological techniques, enabling several novel breeding achievements, such as hybrid breeding, improved mutation breeding, reverse breeding, and genetic transformation.

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