

# Multivariate Evaluation of the Effect of Nanotechnology on Rice Development: A Multivariate Approach

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## ABSTRACT

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This study explores the impact of a nitrogen-based fertilizer enhanced with nanotechnology on the vegetative development of rice plants (*Oryza sativa*). As an emerging tool in agricultural innovation, nanotechnology offers promising advantages for increasing fertilizer efficiency by enabling the controlled and targeted release of nutrients. The main objective of this research is to evaluate whether this nano-formulated fertilizer produces measurable and beneficial effects on key rice growth parameters when compared to conventional fertilization methods. An experimental design was applied in which rice plants were divided into treatment groups: one receiving the nanotechnology-based fertilizer and others receiving standard nitrogen fertilizers commonly used in the region. Key indicators of plant development—such as plant height, root length and dry weight, tiller production, and leaf colorimetry—were monitored. The resulting data were statistically analyzed to determine significant differences among the treatments. The findings from this study are expected to provide valuable evidence on the effectiveness of nanofertilizers in enhancing crop performance. These insights may contribute to more sustainable and productive rice farming practices. Moreover, this research adds to the growing body of knowledge on the application of nanotechnology in agriculture, particularly in improving plant nutrition and increasing yields in essential food crops.

**Keywords:** Nanofertilizer; rice (*Oryza sativa*); Vegetative growth; Crop yield; Sustainable agriculture; Controlled nutrient release.

## 1. INTRODUCTION

Rice (*Oryza sativa*) is a vital crop for global food security, as it is the main food source for more than half of the world population (6). It is not only an essential part of the daily diet for millions of people, but also an important source of income for many countries, especially major exporters such as India, China, and Vietnam. In addition, the industrial use of rice by-products contributes to economic diversification (8). In Ecuador, especially in the coastal region and Guayas Province, rice cultivation is deeply integrated into both agricultural practices and rural livelihoods. However, increasing food demand, resource limitations, and the environmental impacts of conventional farming practices highlight the need for technological innovations that enhance the efficiency and sustainability of rice production systems.

In this context, nanotechnology has emerged as an innovative approach in agriculture, offering new opportunities to improve plant nutrition. It is based on the manipulation of materials at the nanometer scale (1–100 nanometers), which gives them unique properties such as increased reactivity and larger surface area. When applied to agriculture, nanotechnology enables the development of nano-insecticides, nanosensors, and bio-nanocapsules that support more precise and sustainable farming practices. One of the most promising applications is the formulation of nano-structured nitrogen fertilizers, specifically designed to release nutrients in a controlled and targeted manner. This controlled release can improve nutrient uptake by plants, reduce nutrient loss through leaching, minimize environmental impact, and encourage more efficient resource use. For instance, nitrogen is a key nutrient in plant

physiology, playing a vital role in photosynthesis and overall plant health. Nitrogen deficiency limits crop productivity, while excessive application can negatively affect the environment. For these reasons, it is essential to manage nitrogen efficiently to reduce losses through leaching and volatilization (9,10). As a result, nanotechnology also enables nitrogen to be encapsulated in nanocapsules, allowing its release to respond to environmental conditions. This improves enzyme activity related to nitrogen metabolism and reduces losses through runoff and volatilization (2,12). However, potential ecotoxicological risks must be considered, and long-term studies are needed to assess environmental impact (9). Furthermore, nanofertilizers have also shown potential to improve nutrient use efficiency, pest and disease control, and agricultural sustainability. Several studies have reported promising outcomes using oxide nanoparticles like zinc and copper report better germination, growth, and crop protection in maize and tomato (1). Research has also explored encapsulating conventional fertilizers in nanoparticles to reduce leaching and enable slow nutrient release (2). In addition, silica and chitosan nanoparticles have improved nutrient absorption and extended postharvest shelf life, leading to higher yields and better product quality (4,5).

However, it is crucial to produce strong scientific evidence of the actual effectiveness of nanotechnology under real field conditions, particularly in important crops like rice and in specific regions such as the coastal zone of Ecuador. Understanding the extent to which nanotechnology can influence rice growth is especially relevant in a context where agriculture must respond to the growing demands for efficiency, sustainability, and food security. Evaluating its impact on key physiological indicators of rice, in comparison with conventional fertilization methods, is essential to determine its real potential. Including biological fertilizers such as biol in these analyses also allows the exploration of organic alternatives to synthetic inputs, contributing to a more comprehensive perspective on agricultural sustainability and productivity.

Thus, the objective of this study is to assess the effect of a commercial nitrogen-based fertilizer, Nano Urea, on the vegetative development of the rice variety SFL 011, in comparison with conventional fertilization methods. The experiment was conducted using a comparative treatment design, evaluating key variables including plant height, tiller density, root length and dry weight, as well as leaf colorimetry as an indicator of nutritional status. The results are intended to provide applied and technical knowledge relevant to both farmers and agricultural innovation institutions, offering scientific evidence regarding the practical implications of using advanced fertilizer technologies in rice cultivation.

## 2. MATERIALS AND METHODS

### 2.1. Experimental design

This study follows an explanatory experimental design aimed at identifying causal relationships between the applied treatments and the vegetative development of the crop. The research was conducted on an agricultural site located in the Plan América sector, Daule canton, Guayas province (Ecuador), under open-field conditions during the rainy season, which represents the main rice production cycle in the region. The selected variety was *Oryza sativa* L. SFL 011, chosen for its relevance to local agricultural practices and its widespread commercial use. A completely randomized design (CRD) was implemented, with four distinct treatments (Table 1), each replicated four times, resulting in a total of 16 experimental units.

**Table 1.** Description of the fertilization treatments applied in the experimental design.

Treatment	Description
T1 (Negative control)	No fertilizer applied.
T2 (Conventional fertilizer)	Granular urea commonly used by local farmers in the region.
T3 (Organic fertilizer)	Liquid biol produced using sheep manure and plant residues, supplied by the local company Pumamaqui.
T4 (Nanotechnology-based fertilizer)	'Nano Urea', a nanotechnology-based formulation developed by Meghmani Organics Limited (India), applied following standardized technical protocols.

## 2.2. Population and Sampling

Each treatment was replicated four times and randomly distributed across homogeneous cultivation plots with similar soil and environmental conditions, resulting in a total of 16 experimental units. These units consisted of planting beds with uniform planting density, managed under the same irrigation schedule, weed control measures, and standardized agronomic practices. The experiment lasted 60 days, covering the entire vegetative growth phase of the crop, from sowing to its conclusion. During this period, fertilization treatments were applied according to a predefined technical schedule, and the selected response variables were monitored at the main phenological stages.

## 2.3. Study variables

As shown in Table 2, the selected variables were based on physiological, agronomic, and nutritional criteria, with the purpose of evaluating the overall effect of fertilization on rice plant development. All measurements were taken at the end of the vegetative stage, before panicle initiation, to ensure consistency and comparability across treatments.

**Table 2.** Description of observed variables

Variable	Description
Plant height (cm)	Distance from the base of the stem to the tip of the flag leaf.
Number of tillers	Total number of tillers per plant, recorded at peak tillering.
Root length (cm)	Measured after thoroughly washing and separating the root system.
Root dry weight (g)	Determined after drying roots in an oven at 65 °C for 72 hours.
Leaf colorimetry	Assessed using the Munsell color chart for rice (UCCE, California) as an indicator of nutritional status and chlorophyll content.

## 2.4. Statistical analysis

The effects of the different fertilization treatments on the physiological development of rice plants were assessed using both univariate and multivariate statistical methods. This combined approach allowed for a detailed evaluation of individual response variables as well as the overall treatment effects. The analysis was based on a completely randomized design with balanced replication.

The statistical workflow included the following stages:

- a) Descriptive statistics:** Summary statistics such as mean, standard deviation, skewness, and kurtosis were calculated to characterize the distribution of each variable and identify potential outliers or the need for data transformation.
- b) Univariate analysis (ANOVA):** One-way analysis of variance was performed for each response variable to determine whether significant differences existed among the treatment groups. Normality and homogeneity of variance assumptions were verified. Where significant effects were observed, Tukey's HSD post hoc test was applied to identify pairwise differences between treatments.
- c) Multivariate analysis of variance (MANOVA):** To evaluate the combined effect of treatments on all physiological variables, a MANOVA was conducted using Wilks' Lambda as the test statistic. This multivariate approach allowed for the detection of treatment effects that may not be evident when analyzing variables individually.
- d) MANOVA biplot visualization:** A multivariate biplot was generated based on the MANOVA model to facilitate visual interpretation of the relationships between treatments and physiological variables. This graphical tool provided a low-dimensional representation of the experimental space, illustrating patterns of association, variable contributions, and similarities among experimental units.
- e) Cluster analysis:** Hierarchical clustering using Euclidean distance and complete linkage was conducted as an exploratory method to identify potential groupings of treatments or experimental units based on their physiological response profiles.

f) Bootstrap validation: To verify the reliability and robustness of the multivariate findings, bootstrap resampling was used as a cross-validation method. This procedure provided additional confidence in the stability of the patterns observed in the MANOVA and cluster analyses.

## 2.5. Hypotheses

### a) General Hypothesis

H<sub>0</sub>: There are no significant differences in the morphophysiological development of rice plants among the applied treatments.

H<sub>1</sub>: There are significant differences in at least one physiological variable among the treatments.

### b) Specific Hypotheses

For ANOVA:

H<sub>0</sub>: There are no significant differences among treatments in plant height, root length, number of tillers, leaf colorimetry, or root dry weight.

H<sub>1</sub>: At least one of the physiological variables differs significantly among treatments.

For MANOVA:

H<sub>0</sub>: The set of physiological variables does not differ significantly among the treatments.

H<sub>1</sub>: The set of physiological variables differs significantly between at least two treatments.

## 2.6. Statistical tools

All statistical analyses were performed using the R programming language, employing the libraries listed in Table 3.

**Table 3.** R packages used for statistical analysis

Package	Main function in the study
readxl	Import of data from Excel (.xlsx) files
dplyr	Data manipulation and transformation using grammar-based syntax
ggplot2	High-quality data visualization through advanced plotting tools
car	Univariate ANOVA and post hoc significance testing
MANOVA.RM	Implementation of MANOVA models and extraction of multivariate components
factoextra	Visualization of multivariate analysis results (PCA, MANOVA, biplot, cluster analysis)
plotly	Generation of interactive 2D and 3D data visualizations
cluster	Calculation and visualization of hierarchical cluster analysis
dendextend	Enhancement and customization of dendrograms for hierarchical clustering
boot	Statistical validation using bootstrap resampling techniques

## 3. RESULTS

### 3.1. Descriptive analysis

A descriptive analysis was performed for the physiological variables assessed in the study (Table 3), including plant height, root length, number of tillers, leaf colorimetry, and root dry weight. For each variable, measures of central tendency (mean and median), dispersion (standard deviation and range), as well as skewness and kurtosis coefficients were calculated to assess data distribution. The results indicated that most variables were reasonably symmetrically distributed, with skewness values below  $\pm 1$  (Table 4). In particular, root length and root dry weight exhibited low skewness (ranging from 0.333 to 0.486) and moderate kurtosis, suggesting near-normal distributions. In contrast, number of tillers and leaf colorimetry showed slightly higher positive skewness (0.961 and 1.035,

respectively), though not at extreme levels. Overall, the statistical characteristics of the dataset indicate that the assumptions of normality and homoscedasticity required for parametric multivariate analyses such as ANOVA and MANOVA are reasonably met. These conditions support the validity of the inferential and comparative tests conducted in later stages of the study.

**Table 4.** Summary Statistics for Physiological Variables (n = 16)

Variable	Mean	SD	Median	Trimmed	MAD	Min	Max	Range	Skewness	Kurtosis	SE
PH	96,250	18,116	92,400	94,657	16,605	79,600	135,200	55,600	0,872	-0,759	4,529
RL	24,000	2,555	23,500	23,900	2,817	20,800	28,600	7,800	0,486	-1,115	0,639
NT	2,568	0,353	2,433	2,549	0,221	2,219	3,186	0,967	0,817	-1,084	0,088
LC	0,359	0,330	0,259	0,336	0,114	0,000	1,030	1,030	0,755	-0,901	2
RDW	22,175	12,521	19,842	21,621	14,675	8,692	43,408	34,716	0,333	-1,604	3,130

PH = plant height; RL = root length; NT = number of tillers; LC = leaf colorimetry; RDW = root dry weight. SD: standard deviation; MAD: median absolute deviation; SE: standard error. Trimmed mean excludes the highest and lowest 10% of values. Skewness and kurtosis were calculated to assess distribution symmetry and tail behavior.

### 3.2. Univariate Analysis (ANOVA)

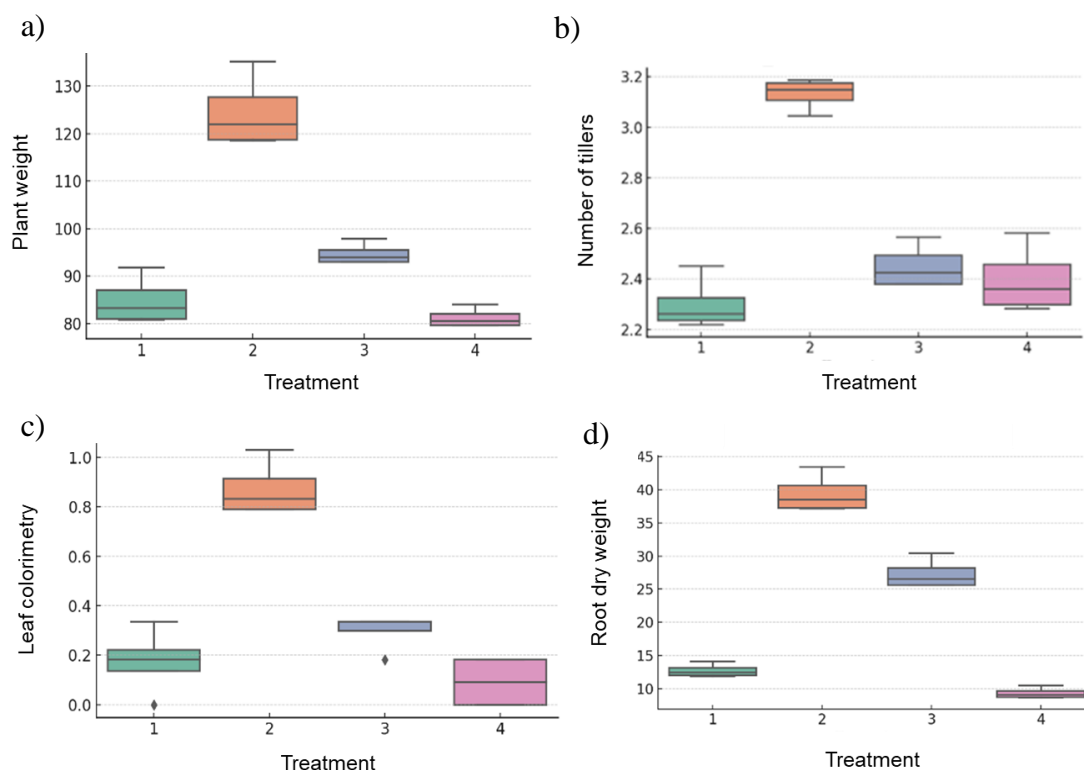
To identify significant differences in the physiological responses of rice plants subjected to different fertilizer treatments, a one-way analysis of variance (ANOVA) was performed for each dependent variable: plant height, root length, number of tillers, leaf colorimetry, and root dry weight. The independent factor was the fertilizer treatment, categorized into four experimental groups. As shown in Table 2, the ANOVA results revealed statistically significant differences ( $p < 0.05$ ) among treatments in the following variables: plant height ( $F = 63.80$ ,  $p < 0.000$ ), number of tillers ( $F = 55.83$ ,  $p < 0.000$ ), leaf colorimetry ( $F = 40.45$ ,  $p < 0.000$ ), and root dry weight ( $F = 197.16$ ,  $p < 0.00001$ ). In contrast, root length did not show significant variation between treatments ( $F = 0.74$ ,  $p = 0.550$ ). For the variables with statistically significant differences, Tukey's Honest Significant Difference (HSD) test was used for post hoc multiple comparisons. This allowed for the identification of specific differences between treatment groups. The results showed marked contrasts, particularly for root dry weight, where the nanotechnology-based treatment outperformed the conventional approaches significantly.

**Table 5.** ANOVA summary and Tukey HSD results

Variable	F value	p-value	Significant
Plant height (PH)	63,796	0.000	Yes
Root length (RL)	0,736	0.550	No
Tiller number (NT)	55,832	0.000	Yes
Leaf colorimetry (LC)	40,455	0.000	Yes
Root dry weight (RDW)	197,163	0.000	Yes

According to Figure 1, visual patterns align with the results obtained from the statistical analysis. In Fig 1a, plant height is noticeably greater in the nanotechnology-based treatment. Fig 1b shows a higher number of tillers under the same treatment. In Fig 1c, leaf colorimetry displays a higher average value, suggesting a more vigorous physiological state. Finally, Fig 1d, reveals a clear advantage of the nanofertilizer in terms of root dry weight, confirming the trend detected through the ANOVA. These findings support the initial hypothesis by demonstrating

that fertilizer treatments have distinct effects on various aspects of rice physiological development, highlighting the potential of nanotechnology-based approaches as a promising agronomic enhancement strategy.



**Figure 1.** Boxplots of the physiological variables that showed statistically significant differences according to the ANOVA results: (a) Plant height, (b) Number of tillers, (c) Leaf colorimetry, and (d) Root dry weight by treatment.

### 3.3. Multivariate analysis of variance (MANOVA)

To assess the combined effect of fertilizer treatments on the physiological variables of rice plants, a Multivariate Analysis of Variance (MANOVA) was conducted. This approach allows for the simultaneous evaluation of potential interdependencies among plant height, root length, number of tillers, leaf colorimetry, and root dry weight. The MANOVA results (Table 6) revealed that treatment had a highly significant effect on the set of physiological variables analyzed. Wilks' Lambda test yielded a value of 0.00002 with an F-statistic of 74.24 ( $p < 0.00$ ), indicating a strong overall difference among treatments. These findings were corroborated by additional multivariate criteria, including Pillai's Trace ( $F = 7.05$ ,  $p < 0.00$ ), Hotelling-Lawley Trace ( $F = 460.44$ ,  $p < 0.00$ ), and Roy's Largest Root ( $F = 1802.10$ ,  $p < 0.00$ ). Together, these results confirm that fertilizer treatments significantly influence the multivariate profile of rice physiological development. These findings support the application of complementary graphical tools to explore how each variable contributes to the observed differences, as further discussed in the following section through a MANOVA-Biplot.

**Table 6.** Resultados del análisis MANOVA aplicado a las cinco variables fisiológicas del arroz evaluadas en función del tratamiento aplicado.

Test	Value	Num DF	Den DF	F value	p-value
Wilks' lambda	1,98E-05	15	22,48588	74,23761	1,4E-15
Pillai's trace	2,337317	15	30	7,054102	3,15E-06

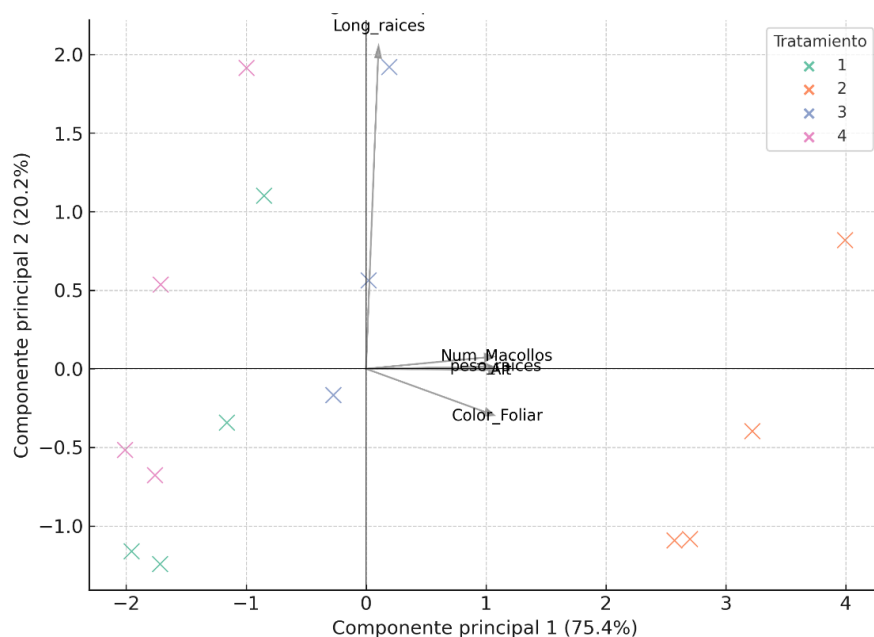


Hotelling-Lawley trace	936,0282	15	10,70423	460,4411	7,18E-13
Roy's greatest root	901,0517	5	10	1802,103	1,96E-14

\*All tests yielded highly significant results ( $p < 0.001$ ), indicating strong treatment effects on the multivariate physiological profile of rice.

### 3.4. MANOVA Biplot

As a visual complement to the multivariate analysis, a biplot of principal components was constructed using the standardized physiological variables. This approach allows for the simultaneous representation of both the observations (treatments) and the original variables in a two-dimensional space, facilitating the multivariate interpretation of the MANOVA results. As shown in Figure 2, there is a clear separation between treatment groups in the plane defined by the first two principal components, which together explain a substantial proportion of the total variance ( $PC1 = 75.4\%$ ,  $PC2 = 20.2\%$ , cumulative =  $95.6\%$ ). This distribution indicates consistent differences in the physiological profile of rice depending on the treatment applied. The vector arrows represent the contribution of each variable to the multivariate space. Notably, root dry weight, plant height, and number of tillers point in a similar direction, suggesting a positive association among these variables under the experimental conditions. In contrast, leaf colorimetry displays a partially divergent orientation, indicating a distinct response to the treatments. Overall, the biplot reinforces the previous statistical findings, highlighting that the nanotechnology-based treatment (Treatment 4) not only improves individual variables but also modifies the overall multivariate profile of rice physiological development, positioning itself in a distinct region of the factorial plane.

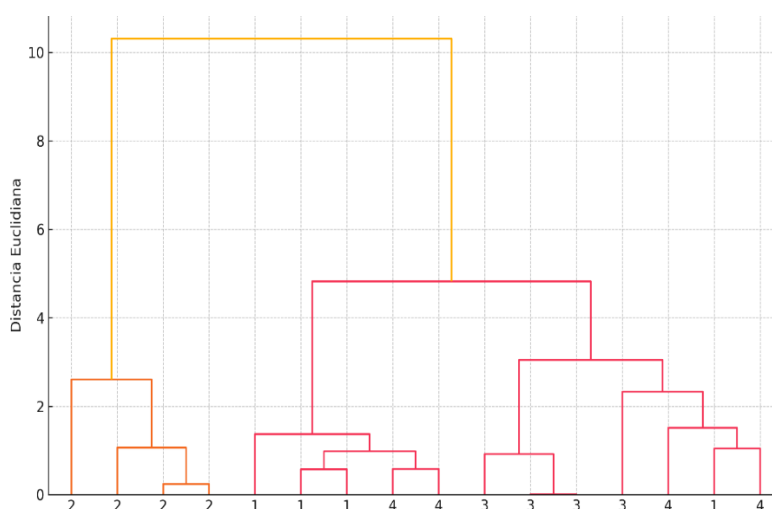


**Figure 2.** Principal component biplot derived from the MANOVA analysis. The plot displays the observations (fertilizer treatments) and the direction vectors of the evaluated physiological variables.

### 3.5. Análisis de clúster:

To explore the structural similarity among the fertilizer treatments applied to rice cultivation, a hierarchical cluster analysis was performed using Ward's method and Euclidean distance, based on the standardized physiological variables. The resulting dendrogram (Figure 3) reveals the formation of well-defined clusters among the treatments. The hierarchical structure allows for the identification of shared physiological behavior across treatments. Specifically, treatments with similar physiological profiles are grouped at lower dissimilarity levels, while others diverge early in the tree, indicating distinct agronomic responses. This analysis supports the findings from both the

ANOVA and MANOVA, providing additional evidence of differentiated effects among the treatments, both at the individual variable level and in the overall multivariate context.



**Figure 3.** Hierarchical dendrogram generated using Ward's method based on the standardized physiological variables of rice cultivation.

### 3.6. Statistical validation (Bootstrap)

To reinforce the statistical inference derived from the univariate analysis, a non-parametric bootstrap validation procedure was implemented by replicating the analysis of variance (ANOVA) for each significant variable. The procedure involved generating 1,000 resamples with replacement from the original dataset, creating an empirical distribution of the F-statistic. Based on this distribution, 95% confidence intervals were calculated for each variable in order to assess the stability of the initial results. As shown in Table 7, the observed F-values for each variable fall within their respective confidence intervals, indicating the robustness of the initial statistical inferences. Notably, root dry weight exhibited the widest interval yet consistently confirmed the statistical significance of the treatment effect, even under resampling.

**Table 7.** Results of the bootstrap procedure used to validate the significance of the physiological variables identified in the ANOVA.

Variable	Observed F	95% CI Lower	95% CI Upper
Plant height (PH)	63,8	40,81	342,89
Number of tillers (NT)	55,83	17,32	327,19
Leaf colorimetry (LC)	40,45	15,83	138,97
Root dry weight (RDW)	197,16	121,41	1131,17

### 3.7. Tested hypotheses

To evaluate the differential effect of fertilizer treatments on the physiological development of rice, both univariate (ANOVA) and multivariate (MANOVA) analyses were conducted. The results showed statistically significant differences among treatments in several physiological variables, indicating a relevant influence of the type of fertilizer applied. The univariate ANOVA identified significant differences ( $p < 0.05$ ) in plant height, number of tillers, leaf colorimetry, and root dry weight, while root length showed no significant variation between treatments. These findings suggest that the fertilizer type affects specific aspects of vegetative growth. Meanwhile, the multivariate analysis (MANOVA) confirmed overall significance ( $p < 0.001$ ) based on the Wilks Lambda, Pillai Trace, and Hotelling Trace statistics, demonstrating that the physiological profile of the crop varies with the treatment applied.



This reinforces the conclusion that differentiated fertilizer strategies have a comprehensive impact on rice morphophysiology.

## 5. CONCLUSIONS

This study assessed the impact of different fertilizer treatments, including a nitrogen-based nanofertilizer, on the morphophysiological development of rice (*Oryza sativa L.*, variety SFL 011) using a multivariate statistical approach. Techniques such as ANOVA, MANOVA, MANOVA-Biplot, cluster analysis, and bootstrap validation provided consistent evidence of treatment-related differences. Univariate analysis revealed significant effects on plant height, number of tillers, leaf colorimetry, and root dry weight, while root length remained unaffected. Multivariate analysis confirmed that the overall physiological profile varied significantly across treatments, with the nanotechnology-based fertilizer showing superior performance, particularly in root biomass and foliar vigor. These results were supported by clustering patterns and validated through resampling techniques. The findings highlight the potential of nanofertilizers to enhance vegetative growth more effectively than conventional alternatives. Further large-scale studies under real farming conditions, including productivity metrics and environmental–economic assessments, are recommended to support broader adoption within sustainable agriculture.

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