

Evaluation of Selected Rice Cultivars' Phenotypic Traits and Chlorophyll Content Under Sub-Mergence Stress

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Abstract

Currently, rice production in Nigeria is on the increase with the aim of meeting the nation's need but floods (mainly seasonal) has been among the major factors limiting rice production. Rice is generally a semi aquatic plant well adapted to partially flooded fields but flash flooding may leads to complete submergence there by resulting into huge economic losses mainly due to reduction in gases (hypoxia) and light intensity needed for photosynthesis. The research was conducted at the Biological Garden, Department of Biological Sciences, Federal University Birnin Kebbi. The experiment was set off in a Completely Randomized Design (CRD) consisting of four rice cultivars with control and three replications. Length of internodes, number of nodes per plant, plant height, root length and chlorophyll contents were evaluated using standard methods and protocols. The study analyzed the impact of flooding on rice cultivars, finding significant differences in node number, internode length, plant height, root lengths, and chlorophyll content. The highest number of nodes was found in CP Yartunga, followed by Faro with 6.33 per plant. The highest plant height was 15.50 cm in Faro, while the highest chlorophyll content was 0.41 mg/g in CP 44. The research reveals that rice cultivars recover from flooding stress, with CP Yartunga having the highest number of nodes per plant and Faro having the highest internode length. No significant differences were found in plant height, root length, or chlorophyll content among the evaluated cultivars. Faro had the lowest chlorophyll content. The study reveals that under stress, chlorophyll content in various locations decreases significantly, with a significant drop in recovery and node numbers. Lastly, node recovery is substantial, suggesting lower injury levels. The present study suggests that cultivars like Faro and CP 44 show better resilience, suggesting potential breeding programs focusing on structural traits.

Keywords: Flooding, sub mergence, stress, rice, cultivars

INTRODUCTION

Currently, rice production in Nigeria is on the increase with the aim of meeting the nation's need but floods (mainly seasonal) has been among the major factors limiting rice production. Rice is generally a semi aquatic plant well adapted to partially flooded fields but flash flooding may leads to complete submergence there by resulting into huge economic losses mainly due to reduction in gases (hypoxia) and light intensity needed for photosynthesis.

In recent times, despite support and interventions directed towards rice production in Nigeria, the uncertainty of climate prediction due to global climate change remain a stepping stone towards achieving the set objectives of attaining rice sufficiency in the near future. Recently in Nigeria, seasonal flooding accounted for more than 50% loss of rice produce as many rice fields are flood prone for fear of water shortage (Yoko et al., 2009).

Deep-water rice has the ability to elongate its internodes with increasing water depth. The non-deepwater rice cultivar African rice (*O. glaberrima*) shows little elongation and a monocarpic annunder deppwater condition (Yoko et al., 2009). Cultivars of *O. glaberrima* are roughly divisible into two ecotypes: upland and lowland, both highly adapted to deepwater inundation in some West African countries.

Flooding is one of the environmental stresses that constrain plant growth; it imposes hypoxia on plants and severely restricts aerobic respiration (Shunsuke et al., 2021). Despite the support and interventions of the Federal Government of Nigeria directed towards rice production in Nigeria, the uncertainty of climate prediction due to global climate change remains a stepping stone towards achieving the set objectives of attaining rice sufficiency in the near future. Hence the need for phenotyping rice genotypes for flood tolerance in the study area and the analysis of the submergence gene in rice (Lasse et al., 2021).

Flooding represents a significant abiotic stress affecting plant growth, survival, and productivity across various ecosystems. The detrimental effects of flooding are primarily attributed to hypoxia or anoxia conditions that disrupt cellular metabolism and energy production. Plants have evolved sophisticated mechanisms at physiological, molecular, and genetic levels to sense, signal, and respond adaptively to flooding stress (Waadt et al., 2022). The response of plants to combined or sequentially occurring stresses differs significantly from individual stress scenarios, emphasizing the need for comprehensive approaches to studying multi-stress resilience mechanisms. The post-flooding recovery phase poses additional challenges involving oxidative damage repair processes crucial for resuming normal growth after submergence episodes, highlighting ROS's dual role as both damaging agents and signaling molecules during recovery phases post-flooding events (Anwar et al., 2021). There are many research on abiotic stress of which only fucuses on water deficit (drought). To the best of our knowledge, this research will be the first to study submergence in rice grown in Kebbi State with the hope of identify flood resilient varieties suited for Kebbi state ecology.

MATERIALS AND METHODS

Description of Study Area

Kebbi State is located in north-western Nigeria, between latitude 12.45° N and longitude 4.20° E. It covers a geographical land area of 36,800 km² and borders the nation of Niger Republic to the west and Benin Republic to the south-east. It also borders Nigerian states: Niger, Sokoto, and Zamfara to the south, north, and east, respectively. The state was marked by a single rainy season from April to October with a mean annual rainfall of about 720 mm and a long dry season lasting for the remaining period of the year (Baba et al., 2014). The mean temperature range is 260 °C during the harmattan season (November to February) and 380 °C-400 °C during the months of April to June (Girma, 2008). Its vegetation is Northern Guinea savanna in the south and southeast and Sudan savanna in the north, covered with short grasses and small trees. Although the state faces desertification, it is still being altered in many areas by cultivation, grazing, cutting of fuel wood, excavation of soil, bush fire, and so on.

Plant Materials

A total of four most commonly cultivated rice cultivar seeds, including landraces and improved varieties, were collected across Kebbi State. The seeds were surface sterilized in 5% sodium hypochlorite and rinsed three times with distilled water. The seeds were sown in the nursery beds, and uniformly germinated seedlings were transferred into plastic pots containing the moisture of loamy soil and manure.

Determination of Number of Nodes, Internode/Shoot Elongation

The number of nodes and the length between the nodes (internodes) were measured using the centimeter rule. The shoot elongation was measured by measuring the length of the entire shoot of the plant. These parameters were measured before, during, and after the stress episode (Tran et al., 2020).

Determination of Chlorophyll Content and Injury Index

For the determination of chlorophyll content, the study adopted the methods described by Maclachlan and Zalik (2017) and Duxbury and Yentsch (2016). One (1) gram of ground leaf samples was added to 20 ml of 80% acetone and extracted after 15 minutes. The liquid portion was decanted into a 15-ml centrifuge tube and then centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred to a 100-ml volumetric flask, and the residue was diluted with 20 ml of 80% acetone. The mixture was decanted again into another test tube and centrifuged at 5000 rpm for 5 minutes. The procedure was repeated until the residue turned colorless, after which the absorbance was taken at 645 nm and 663 nm against 80% acetone using a spectrometer. The total chlorophyll was calculated using the formula below (equations 1, 2, and 3) (Duxbury & Yentsch, 2016).

$$= \text{Chlorophyll a (mgg}^{-1} \text{ FW)} = \frac{12.30D_{663} - 0.86D_{645} \times V}{(1000 \times W \times D)} \quad 1$$

$$\text{Chlorophyll b (mgg}^{-1} \text{ FW)} = \frac{19.60D_{645} - 3.60D_{663} \times V}{(1000 \times W \times D)} \quad 2$$

$$\text{Total chlorophyll content mg/g} = \text{chlorophyll a} + \text{chlorophyll b} \quad 3$$

Where V is the final volume of chlorophyll extract in 80% acetone (mL), W indicates the fresh weight of the sample (g), and D represents the path length of light (cm).

The plant injury index was calculated by measuring the plant stress damage, typically ranging from 0 (no injury) to 1 (complete injury or death). The computation was done using the following formulas:

$$\text{Injury Index (II)} = \left(1 - \left(\frac{T}{C}\right)\right) \times 100 \quad 4$$

Where :

T = treatment value (stress plant measurement)

C = control value (healthy plant measurement)

$$\text{Chrophyll content Injury Index (CII)} = \left(\frac{C-T}{C}\right) \times 100 \quad 5$$

Determination of Recovery Index

This is used to measure the effects of water stress on plants. A value of +1 shows that the stress has no effect on the plant, on the plant, and lower values show stronger effects (Lasse et al., 2021). The recovery index is calculated by the following formula:

$$\text{Recovery Index (RI)} = \left(\frac{R}{C}\right) \times 100 \quad 6$$

Where :

R = recovery value (measurement after stress relief)
 C = control value (measurement before stress)
 $RI = (R-S)/(C-S) \times 100$ (for measurements of chlorophyll)
 S = stress value (measurement during stress)
 C = control value
 R = recovery value

Data Analysis

The data obtained was subjected to one-way analysis of variance (ANOVA), and the means were separated using Duncan's multiple range using SPSS statistical software.

RESULTS AND DISCUSSIONS

Effects of Flooding on Vegetative and Chlorophyll Content of Rice Cultivars

The present study evaluated the effects of flooding on the vegetative and chlorophyll content of rice cultivars. The results show that there is a significant difference in the number of nodes between "Jirani Zawara" and the remaining four cultivars. However, there is no significant difference in the number of nodes between CP 44, Faro, and CP Yartunga (Table 1). The highest number of 6.67 nodes was recorded in CP Yartunga, followed by Faro with 6.33 nodes per plant (Table 2). Flooding stress significantly affected the length of internodes. The lowest length of internodes of 2.93 cm was observed in Jirani Zawara, and the highest length of internodes of 7.16 cm was recorded in the Faro cultivar (Table 2).

The highest plant height of 15.50 cm was recorded in Faro, which shows no significant difference from the plant height recorded in Faro of 13.47 cm (Table 1). Furthermore, the statistical analysis of this study revealed that the root lengths of the Faro and CP yartunga are the same, at 5.50 and 5.93 cm, respectively (Table 1). Flooding insignificantly affected the chlorophyll content of the rice cultivars evaluated. The highest chlorophyll content of 0.41 mg/g was observed in CP 44, while the lowest chlorophyll content of 0.13 mg/g was recorded in CP yartunga (Table 1).

Table 1: Effects of flooding on number of nodes, length of internodes, plant height, root length and chlorophyll content of four rice cultivars

Cultivars	Number of Nodes	Length of Internodes	Plant Height	Root Length	Chlorophyll Content
Jirani Zawara	3.00 ^a ±1.00	2.93 ^a ±0.35	9.43 ^a ±0.85	4.80 ^b ±0.20	0.19 ^a ±0.17
CP 44	6.00 ^b ±1.00	5.43 ^c ±0.47	13.47 ^c ±2.67	4.37 ^a ±0.84	0.41 ^a ±0.35
Faro	6.33 ^b ±0.570	7.16 ^d ±0.71	15.50 ^d ±1.08	5.50 ^{bc} ±0.55	0.25 ^a ±0.43
CP Yartunga	6.67 ^b ±1.52	4.27 ^b ±0.68	11.87 ^{ab} ±1.91	5.93 ^{bc} ±1.26	0.13 ^a ±0.12

Values presented in the table are express as mean±standard deviation values in a column with different superscript are significantly different.

Post-Flooding and Recovery Effects on Vegetative Parameters and Chlorophyll Content of Rice Cultivars

The present research reveals the extent to which the selected rice cultivars recover from flooding stress. The highest number of 8.00 nodes per plant was recorded in CP Yartunga, followed by Faro with 7.33 nodes per plant. However, Faro recorded the highest internode length of 7.70 cm, while Jirani Zawara recorded the lowest internode length of 3.67 cm (Table 2). Furthermore, there is no significant difference in plant height between Jirani Zawara and CP yartunga. The lowest plant height of 10.87 cm was observed in Jarani Zawara, followed by CP yartunga at 13.47 cm (Table 2).

The highest root length of 7.60 cm was observed in CP yartunga, followed by Faro with 5.93 cm. There is no significant difference in the content of chlorophyll among all the cultivars evaluated. However, the lowest chlorophyll content of 0.47 mg/g was observed in Faro, while the highest chlorophyll content of 0.87 mg/g was recorded in Jirani Zawara (Table 2).

Table 2: Post Flooding/ Recovery Effects on Number of Nodes, Length of internodes. Plant Height, Root length and Chlorophyll Content of Selected Rice Cultivars

Cultivars	Number of Nodes	Length of Internodes	Plant Height	Root Length	Chlorophyll Content
Jirani Zawara	4.00 ^a ±0.00	3.67 ^a ±0.50	10.87 ^a ±0.47	5.10 ^a ±0.20	0.87 ^a ±0.35
CP 44	6.67 ^b ±0.57	6.67 ^c ±0.11	14.77 ^b ±0.40	5.17 ^a ±0.70	0.70 ^a ±0.26
Faro	7.33 ^b ±0.57	7.70 ^d ±0.66	17.70 ^c ±1.96	5.93 ^a ±0.67	0.47 ^a ±0.70
CP Yartunga	8.00 ^b ±1.00	5.37 ^b ±0.30	13.47 ^b ±1.95	7.60 ^b ±0.75	0.60 ^a ±0.56

Injury Index and Recovery Index

Jirani Zawara Shows moderate chlorophyll content under stress (201) but a significant drop in recovery (27.77). The number of nodes also drops substantially (56 to 44.44), indicating a notable injury level. CP 44 has a high initial chlorophyll content (222) but extremely low recovery (12.85) and almost negligible node recovery (0.0083). This indicates a severe injury under submergence stress. Faro Despite the high chlorophyll content under stress (521), the recovery is very low (7.66). The number of nodes shows significant recovery (75.865), but the chlorophyll recovery suggests considerable injury. CP Yartunga has moderate chlorophyll content under stress (261) and moderate recovery (22.9). The number of nodes shows substantial recovery (72.72), indicating a relatively lower injury level compared to the others.

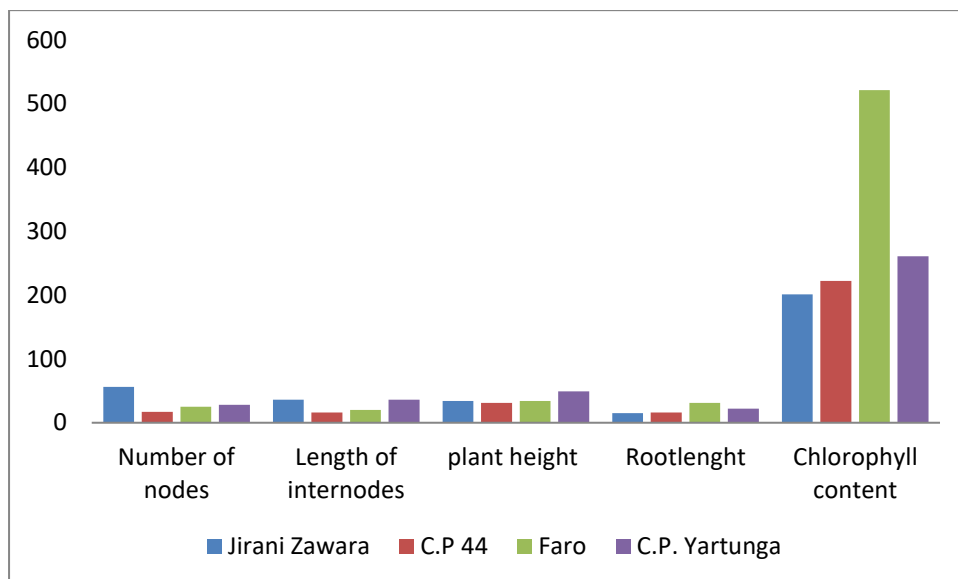


Figure 1: Injury Index of different rice cultivars subjected to submergence stress

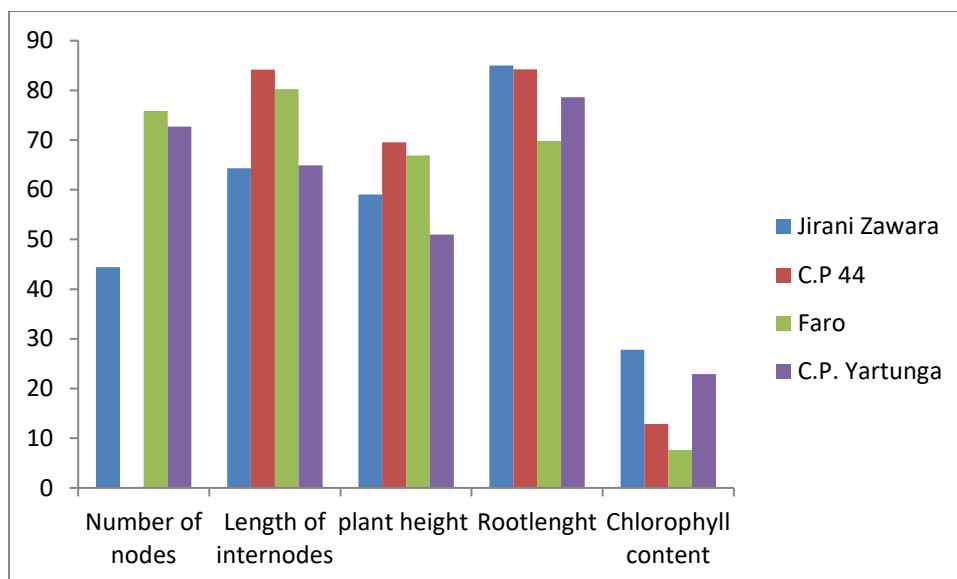


Figure 2: Recovery index of different rice cultivars after submergence stress

Effects of Flooding on Vegetative Growth

Flooding significantly influences the vegetative growth of rice plants. According to a study by Setter et al. (1997), flooding stress can lead to a reduction in plant height and the number of tillers, which are critical components of vegetative growth. The present study's finding indicated a significant difference in the number of nodes between “Jirani Zawara” and other cultivars, which aligns with the observation that different rice varieties exhibit varied responses to flooding.

The highest number of 6.67 nodes per plant was recorded in CP Yartunga, followed by Faro with 6.33 nodes per plant. This result is consistent with previous research indicating that some rice cultivars are more resilient to flooding and can maintain better vegetative growth (Bailey-Serres et al., 2020). There is no significant difference in the number of nodes between CP 44, Faro, and CP Yartunga, which suggests that these cultivars may share similar genetic traits conferring tolerance to flooding stress.

Internode length is another critical aspect of vegetative growth affected by flooding. Flooding often leads to internode elongation as a survival mechanism in rice, allowing the plant to reach above the water surface and continue photosynthesis (Colmer & Voesenek, 2019). The present study found that flooding stress significantly affected internode length, with the Jirani Zawara cultivar exhibiting the shortest internode length of 2.93 cm and Faro the longest with 7.16 cm. This variation can be attributed to the genetic diversity among rice cultivars. As described by Xu et al. (2019), rice cultivars with different genetic backgrounds show significant differences in their morphological responses to flooding. Faro's longer internodes might indicate a better adaptation mechanism, allowing it to survive and grow under flooded conditions more effectively than Jirani Zawara. The present study shows a decrease in chlorophyll content in the flooded plant. Chlorophyll content often decreases under flooding stress due to impaired photosynthesis and increased degradation of chlorophyll (Panda et al., 2018).

The highest plant height of 15.50 cm was recorded in the Faro cultivar, showing no significant difference compared to CP 44, which recorded a height of 13.47 cm (Table 2). Flooding generally influences plant height, often causing stunted growth due to hypoxia or anoxia conditions that impair root and shoot development (Sauter, 2013). However, certain rice cultivars exhibit enhanced elongation as a survival mechanism, as seen in the Faro cultivar.

This finding aligns with research by Hattori et al. (2019), which found that deepwater rice varieties can elongate their internodes to keep their leaves above water.

Root length is a critical trait affected by flooding. The results of this study shows no significant difference in root length between Faro with 5.50 cm and CP Yartunga 5.93 cm. Flooding can lead to the development of aerenchyma in roots, facilitating better oxygen transport and enhancing root length in some cultivars (Colmer & Voeselek, 2019). The similar root lengths of Faro and CP Yartunga suggest these cultivars may possess similar adaptive mechanisms to maintain root growth under flooded conditions.

The study found that flooding insignificantly affected chlorophyll content among the evaluated rice cultivars. The highest chlorophyll content was observed in CP 44 (0.41 mg/g), while the lowest was recorded in CP Yartunga (0.13 mg/g). Flooding can lead to reduced chlorophyll content due to oxidative stress and impaired chlorophyll synthesis (Panda et al., 2018). The variation in chlorophyll content among the cultivars suggests differing levels of resilience to flooding stress, with CP 44 potentially having better mechanisms to maintain chlorophyll levels.

The results presented in Table 2 provide a detailed comparison of the vegetative parameters and chlorophyll content under flooding conditions. Jirani Zawara showed the lowest performance across most parameters, indicating its sensitivity to flooding stress. In contrast, Faro and CP 44 exhibited better performance, suggesting greater resilience. CP Yartunga showed mixed results with a high number of nodes and moderate plant height but the lowest chlorophyll content.

Recovery of Vegetative Growth After Flooding

The study revealed that CP Yartunga had the highest number of 8.00 nodes/plant, followed by Faro with 7.33/plant after recovery from flooding (Table 2). This suggests that CP Yartunga has a robust recovery mechanism allowing for the regeneration of nodes post-flooding. According to Setter et al. (2019), the ability of a rice cultivar to recover vegetative growth after submergence is critical for overall yield, as it ensures that the plant can resume normal growth and development once floodwaters recede.

The internode length is another important indicator of recovery. Faro exhibited the highest internode length of 7.70 cm, while Jirani Zawara had the lowest with 3.67 cm. These findings align with research by Colmer & Voeselek (2019), which highlighted that internode elongation is a common adaptive response in rice cultivars to regain access to air during flooding. The ability to maintain longer internodes post-flooding indicates a stronger recovery mechanism, particularly in Faro.

There was no significant difference in plant height between Jirani Zawara and CP Yartunga, with the lowest height observed in Jirani Zawara at 10.87 cm. Recovery in plant height is crucial for ensuring that the plants can optimize light capture for photosynthesis. As noted by Sauter (2013), plant height can be severely affected by flooding, but some cultivars have adaptive traits that enable them to recover more effectively.

The highest root length post-flooding was observed in CP Yartunga (7.60 cm), followed by Faro (5.93 cm). Root length recovery is essential for the plant's ability to absorb water and nutrients efficiently. Xu et al. (2019) found that rice cultivars with longer roots post-flooding tend to show better overall recovery and resilience. The significant root length of CP Yartunga suggests that it has a strong root system capable of overcoming the adverse effects of flooding.

Chlorophyll Content Post-Flooding

The study found no significant difference in chlorophyll content among the cultivars post-flooding. However, the lowest chlorophyll content was observed in Faro with 0.47 mg/g, while the highest was in Jirani Zawara with 0.87 mg/g. Chlorophyll content is a vital indicator of photosynthetic activity and plant health. Flooding typically reduces chlorophyll content due to limited gas exchange and increased chlorophyll degradation (Panda et al., 2018). The ability of Jirani Zawara to maintain a higher chlorophyll content suggests a better photosynthetic recovery, which is crucial for the plant's energy production and growth post-flooding.

Chlorophyll Content Under Stress and Recovery Index

Jirani Zawara shows moderate injury index chlorophyll content under stress with 201.00 but a significant drop in recovery of 27.77. This significant reduction indicates that while the cultivar can maintain some level of chlorophyll during stress, its ability to recover post-stress is limited. Chlorophyll degradation under submergence is a common response due to oxidative stress and reduced photosynthetic efficiency (Panda et al., 2018). The significant drop in Jirani Zawara suggests that it suffers considerable injury during submergence, limiting its recovery capability.

CP 44 has high injury index initial chlorophyll content of 222.00 but an extremely low recovery of 12.85 and an almost negligible node recovery of 0.0083. This indicates a severe injury under submergence stress. High initial chlorophyll content may reflect the plant's potential photosynthetic capacity, but the drastic reduction in recovery suggests significant damage to the photosynthetic apparatus (Singh et al., 2019). The negligible node recovery further highlights its vulnerability to submergence.

Faro, despite the high chlorophyll injury content under stress (521.00), this shows very low recovery (7.66). The high chlorophyll content during stress suggests an ability to maintain photosynthesis under adverse conditions. However, the low recovery index indicates that the damage incurred during submergence impedes its post-stress recovery. Studies have shown that high chlorophyll content during stress does not always correlate with better recovery if the damage to cellular structures is severe (Setter et al., 2019; Tran et al., 2020). However, the significant recovery in the number of nodes (75.865) suggests that Faro can regenerate vegetative structures despite the chlorophyll loss.

CP Yartunga has a moderate injury index of chlorophyll content under stress of 261.00 and a moderate recovery of 22.9. The number of nodes shows a substantial recovery of 72.72, indicating a relatively lower injury level compared to the others. This balanced response suggests that CP Yartunga has mechanisms to protect its chlorophyll and vegetative structures better than the other cultivars (Colmer & Voesenek, 2019). The substantial recovery in the number of nodes reflects its ability to resume growth post-stress, which is crucial for yield.

The number of nodes is a critical parameter for assessing vegetative growth and recovery. Jirani Zawara shows a drop from 56 to 44.44, indicating notable injury. The reduction in nodes suggests that the plant's ability to develop new growth points is compromised during stress (Sauter, 2013). CP 44 shows negligible recovery in the number of nodes with 0.0083 indicates severe injury. This lack of recovery underscores its poor resilience and potential yield loss under submergence stress (Bailey-Serres et al., 2010).

Faro despite low chlorophyll recovery, the number of nodes shows significant recovery of 75.865, it is suggested that Faro can re-establish its vegetative growth post-stress, which is essential for overall plant recovery and productivity (Xu et al., 2016). CP Yartunga shows substantial recovery in the number of nodes with 72.72, indicating a robust recovery mechanism. The ability to maintain and develop new nodes post-stress is a key trait for resilience in flooded conditions (Setter et al., 1997).

CONCLUSION

The study highlights the impact of flooding on rice cultivars, highlighting the importance of genetic diversity in developing flood-tolerant varieties. Differences in plant height, root length, and chlorophyll content highlight the need for further research to understand flood resilience in rice. Cultivars like Faro and CP 44 show better resilience under flooding, suggesting potential use in breeding programs. The injury index and recovery index provide insights into the resilience and adaptability of different rice cultivars under submergence stress. Plant height and root length are critical indicators of recovery post-flooding, while chlorophyll content is less critical. These insights can guide breeding programs to improve flood resilience in rice by prioritizing structural traits.

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