



Flash Flooding Effects on Post-Harvest Physiological and Phytochemical Responses of the Phourel-Amubi Rice Cultivar of Manipur

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Flooding due to heavy rainfall is a major abiotic stress that significantly affects rice (*Oryza sativa* L.) production during the monsoon (Kharif) season, particularly in rain-fed lowland areas. This study evaluates the impact of flash flooding on the traditional rice cultivar Phourel-Amubi. The experiment was conducted by growing 15-day-old seedlings in plastic pots, followed by submergence stress treatments in a concrete tank for 5, 10, and 15 days at 30, 60, and 90 days after transplanting.

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Morpho-physiological, biochemical, and yield-related parameters were analyzed. Results indicated a gradual increase in total free amino acid content, total phenol content, and total proline content as flooding duration increased, indicating a biochemical stress response. However, key growth and metabolic indicators, such as plant height, total chlorophyll content, soluble protein, and total soluble sugars, declined significantly. Yield and its components at 120 days after transplant were also adversely affected, with a substantial decrease in panicle number (8 to 4.17 ± 0.14), grain number per panicle (135.83 to 95.17 ± 1.52), grain weight (20.69g to $16.19g \pm 0.62$), and overall grain yield per hill (25.08g to $11.67g \pm 0.64$). These findings suggest that Phourel-Amubi is highly susceptible to submergence stress, emphasizing the need for breeding flood-tolerant rice varieties or adopting effective water management strategies to mitigate yield losses in flood-prone regions.

Keywords: Chlorophyll; flash flooding; *Oryza sativa*; proline; yield; submergence stress.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple crop worldwide and is cultivated under diverse environmental conditions (Salgotra & Chauhan, 2023; Shah et al., 2024). While rice requires water for optimal growth, excessive water in the form of waterlogging or submergence disrupts key physiological and biochemical functions, ultimately reducing crop yield (Kumar et al., 2024; Wang et al., 2024). Submergence stress can be categorized into flash flooding and deep-water flooding based on the depth and duration of inundation (Bailey-Serres et al., 2010). Flash floods, triggered by heavy rainfall, are usually short-term but can cause severe physiological damage to plants (Hattori et al., 2011).

Flooding stress affects rice plants in multiple ways, including reduced light penetration, impaired gas exchange, and nutrient uptake limitations (Sarkar & Bhattacharjee, 2011). Oxygen depletion in flooded soils leads to anaerobic conditions, resulting in toxic accumulations of Fe^{2+} , Mn^{2+} , and H_2S , further exacerbating plant stress (Yumauchi et al., 2018; Mustroph, 2019). Although some rice varieties have evolved adaptations to cope with temporary flooding, prolonged submergence leads to growth inhibition, metabolic alterations, and yield losses (Sarkar et al., 2006).

Manipur, a northeastern state of India, relies heavily on rice as a staple food, with rain-fed agriculture as the predominant farming system. Traditional rice cultivars such as Phourel-Amubi are widely grown due to their adaptability to the region's agro-climatic conditions. However, these varieties may lack sufficient tolerance to prolonged submergence stress, which poses a threat to food security and farmer livelihoods. Despite the frequent occurrence of flash floods in Manipur, limited research has been conducted on

the physiological and biochemical responses of its indigenous rice varieties to flooding stress.

This study aims to investigate the impact of flash flooding on the growth, biochemical responses, and yield components of Phourel-Amubi rice. By assessing key parameters, including chlorophyll content, proline accumulation, soluble protein levels, total soluble sugars, and yield traits, the study provides valuable insights into the submergence tolerance mechanisms of this cultivar.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design

The experiment was conducted at the Plant Physiology Section, Manipur University, using the Phourel-Amubi rice cultivar. Seedlings were raised in a nursery and transplanted into plastic pots after 15 days. The plants were maintained under natural conditions for 30, 60, and 90 days before being subjected to flash flooding treatments.

2.2 Flooding Stress Treatment

Plants were submerged in a concrete tank filled with water up to 50 cm, ensuring complete plant submergence. Three treatment durations were applied: T1 (5 days submergence), T2 (10 days submergence) and T3 (15 days submergence). Control plants were maintained under normal conditions without submergence.

2.3 Measurement of Physiological and Biochemical Parameters

Various morpho-physiological and biochemical parameters were analyzed, including plant height

(cm), chlorophyll content, total soluble sugars (mg g^{-1} FW), soluble protein content (mg g^{-1} FW), total free amino acids (mg g^{-1} FW), total phenolic content (mg g^{-1} FW) and proline content (mg g^{-1} FW). Yield-related traits such as panicle number per hill, grains per panicle, thousand-grain weight, and total grain yield per hill were also recorded.

2.3.1 Plant height

The 30, 60, and 90-day-old plants were taken for plant height measurement using a scale from three different pots just before submergence (non-submergence) and after the submergence of 5, 10 and 15 days, and the heights were compared.

2.3.2 Estimation of chlorophyll content

Chlorophyll content was estimated following Witham et al. (1971) after extraction with 80 % acetone. The amount of chlorophyll present was expressed as mg g^{-1} fr. wt. using the following equation of (Witham et al. 1971).

$$\text{Chlorophyll}_a = [12.7(D_{663}) - 2.69(D_{645})] \times [V/1000 \times W]$$

$$\text{Chlorophyll}_b = [22.9(D_{645}) - 4.68(D_{663})] \times [V/1000 \times W]$$

$$\text{Chlorophyll (total)} = [20.2(D_{663}) - 8.02(D_{645})] \times [V/1000 \times W]$$

V = Volume made up in ml

W = Weight of leaf sample in mg

2.3.3 Estimation of total soluble sugar content

Total soluble sugar content is estimated based on the anthrone method (Hedge and Hofreiter 1962) and the concentration of soluble sugars was computed by using glucose as standard and expressed in mg g^{-1} fr. wt.

2.3.4 Estimations of soluble proteins

Estimations of phosphate buffer soluble proteins were done in fresh plant samples by Lowry's et al., methods (1951). The optical density was measured at 660 nm. Calculations were done from the standard curve prepared by using BSA (Bovine Serum Albumin) as the standard solution and expressed as mg g^{-1} fr. wt.

2.3.5 Estimation of total phenolics

The dried powdered plant samples were extracted in 10ml of methanol by intermittent

maceration for up to 48 hours, centrifuged and the supernatants were used for the estimations of total phenols. Total phenolic contents were determined by folin-ciocalteu method with sodium carbonate solutions following Donald et al., (2001). The absorbance was measured at 765nm using gallic acid as the standard.

2.3.6 Estimation of total free amino acid content

The total free amino acid was determined with ninhydrin reagent as per Yemm and Cocking (1955). The amount of total free amino acids was calculated and expressed in mg g^{-1} fr. wt. using a standard curve prepared from glycine.

2.3.7 Estimation of total proline content

The Proline accumulation in plant tissues was estimated according to Bates et al., (1973). The amount of proline in the samples were then calculated and expressed as mg g^{-1} fr. wt. using a standard curve prepared from analytical grade proline.

2.3.8 Panicle number

The number of panicles per hill, 3 pots in number for Phourel-amubi paddy cultivar was counted at 90 and 120 days after transplant i.e. at the reproductive stage of paddy cultivation.

2.3.9 Number of filled grains

Filled grain was measured by physical examination manually, in the presence of tested and mature seed. The number of filled grains per paddy plant was counted on 120 days after transplantation.

2.3.10 Weight of 1000 grains

One thousand cleaned and dried grains were counted from the collected seed stock after harvesting cultivation and weighed using an electronic balance.

2.3.11 Grain yield per hill

The matured threshed paddy grains were collected per hill at 120 days after transplantation. These grains were collected and oven-dried at $80^\circ\text{C} \pm$ for 48 h and weighed using an electronic balance.

3. STATISTICAL ANALYSIS

The experimental data were analysed statistically by following the standard procedure outlined by Gomez and Gomez (1984). Significance was tested by comparing the “F” value at a 5 percent level of probability and wherever the “F” values were found a significant, critical difference (CD) was worked out at a 5 percent level of probability, and the values were furnished.

4. RESULTS AND DISCUSSION

Rice (*Oryza sativa* L.), a member of the Poaceae family, exhibits resilience to various abiotic stresses, including flooding. In this study, the impact of flooding stress on the Phourel-amubi rice cultivar was assessed by evaluating morphological, physiological, and biochemical responses after subjecting the plants to flooding conditions for 5, 10, and 15 days at different growth stages.

4.1 Plant Growth and Morphology

4.1.1 Plant Height

A significant reduction in plant height was observed under flooding stress, with a progressive decline across all treatment durations (5, 10, and 15 days) at 30, 60, and 90 days after transplantation (DAT) (Table 1). This reduction aligns with findings from previous studies Jackson, 2008; Anandan et al., 2012; Wang et al., 2014), which attributed it to oxygen deficiency, anaerobic conditions, reduced root activity, and inhibited synthesis and transport of photosynthetic assimilates (Bedi et al., 2009; Promokhambut et al., 2010). Similar results were reported by Colmer and Voesenek (2009), who found that flooding restricts root elongation and decreases shoot height in rice. Additionally, Das et al. (2009) demonstrated that flood-induced hypoxia reduces plant height by altering hormonal balance, particularly gibberellins and ethylene levels.

4.1.2 Chlorophyll content

Chlorophyll content was highest under control conditions and decreased with increasing flood duration, with the lowest content recorded in plants subjected to 15 days of flooding (Table 1). Similar reductions in chlorophyll content due to submergence stress have been reported in rice (Deka and Baruah, 2000; Adak and Das Gupta, 2000), wheat (Collaku and Harrison, 2002), and

maize (Asha and Pandey, 2007). The decline in chlorophyll content under hypoxic conditions may be attributed to reduced synthesis and increased degradation of chlorophyll pigments (Ashraf, 2003). Recent studies by Fukao et al. (2019) and Ye et al. (2021) confirmed that chlorophyll degradation under submergence stress is regulated by oxidative stress and reactive oxygen species accumulation.

4.1.3 Total Soluble sugar content

A decreasing trend in total soluble sugar content was observed with increasing flood duration, with the lowest values recorded under the 15-day flooding treatment (Table 1). This decline may result from an increased demand for carbohydrates to sustain cell division, elongation, and overall plant growth under hypoxic conditions (Voesenek et al., 2006). Similar findings were reported by Yamada et al. (1995), who observed carbohydrate depletion in rice leaves under submergence stress. Kato et al. (2014) and Bailey-Serres et al. (2020) further noted that flooding disrupts carbohydrate metabolism, leading to altered sucrose and starch content, negatively impacting plant energy reserves.

4.1.4 Total soluble protein content

Flooding stress led to a significant decline in total soluble protein content, with the lowest values recorded under the 15-day treatment (Table 1). This reduction may be attributed to enhanced proteolytic activity and the reduced incorporation of free amino acids into proteins in aging tissues. Furthermore, flooding conditions often induce the expression of anaerobic proteins, many of which are involved in carbohydrate metabolism and alcoholic fermentation (Sarkar et al., 2006). Induction of key cytosolic enzymes such as alcohol dehydrogenase, aldolase, glucose phosphate isomerase, sucrose synthase, pyruvate decarboxylase, and glyceraldehyde phosphate dehydrogenase has been documented in rice and other crops under flooding stress (Miro and Ismail, 2013). Studies by Loreti et al. (2016) and Xie et al. (2022) further support these findings, highlighting how flooding-induced hypoxia triggers anaerobic metabolism in plants.

4.1.5 Total phenolic content

An increase in phenolic compound accumulation was observed with prolonged flooding, with the

highest levels recorded in the 15-day submergence treatment (Table 1). This increase may be attributed to incomplete oxidation of respiratory substrates under anaerobic conditions, as reported in previous studies (Mukherjee and Mondal, 1995). The accumulation of phenolic compounds under stress suggests their role in mitigating oxidative damage and enhancing plant defense mechanisms. Recent research by Jaiswal et al. (2021) and Nghi et al. (2023) indicates that phenolic accumulation is linked to antioxidant defense activation under flooding conditions.

4.1.6 Total free amino acid content

Flooding stress progressively increased total free amino acid content, with the highest accumulation observed in the 15-day flooding treatment (Table 1). This increase may be due to the rapid degradation of stored proteins (Perata, 1993) and enhanced proteolytic activity as part of the plant's stress response (Kamachi et al., 1991). The accumulation of free amino acids under flooding conditions highlights their role as osmoprotectants and metabolic intermediates, contributing to stress tolerance. Studies by Bailey-Serres and Colmer (2014) and Yang et al. (2020) also confirm that amino acid accumulation helps in osmotic balance under hypoxia.

4.1.7 Total proline content

Proline accumulation increased significantly across all flooding treatments, with the highest levels recorded in plants subjected to 15 days of flooding (Table 1). Proline plays a critical role in osmoprotection, stabilizing cellular structures, protecting membranes, proteins, and nucleic acids, and scavenging reactive oxygen species (Hayat et al., 2012; Mohammadrezakhani et al., 2019). Additionally, proline serves as a source of nitrogen, carbon, and energy under stress conditions, facilitating plant adaptation (Matysik et al., 2002). Studies by Hossain et al. (2021) and Barickman et al. (2023) support these findings, highlighting the role of proline in enhancing rice stress tolerance.

4.1.8 Panicle number, number of filled grains per panicle, and thousand-grain weight

Flooding stress resulted in a reduction in panicle number per hill, number of filled grains per panicle, and 1000-grain weight (Table 2). These reductions align with previous reports (Voesenek and Bailey-Serres, 2015; Sone et al., 2012). Yamauchi et al. (2020) found that flooding reduces spikelet fertility and grain filling by restricting assimilate translocation.

Table 1. Effect of flood stress on morpho-physiological and biochemical parameters of Phourel-amubi rice cultivar with 30, 60, and 90 days after transplant (DAT)

Parameters	DAT	Control (CON)	5 Days (5D)	10 Days (10D)	15 Days (15D)	*SE (m)	**SD±	***CD at 5%
Plant height (cm)	30	51.83	49.33	47.17	45.67	0.81	2.80	5.79
	60	108.30	106.04	102.85	100.60	1.86	2.63	5.44
	90	151.24	150.79	147.18	145.35	0.75	1.06	2.20
Total chlorophyll content (mgg ⁻¹ FW)	30	2.60	2.18	1.46	0.60	0.21	0.29	0.61
	60	1.59	1.15	0.94	0.84	0.001	0.001	0.003
	90	1.90	1.63	1.40	1.24	0.05	0.07	0.14
Total soluble sugar (mgg ⁻¹ FW)	30	16.29	12.20	9.06	6.79	0.21	0.72	1.48
	60	19.39	16.12	14.21	11.49	0.18	0.25	0.52
	90	39.49	36.31	34.29	31.71	0.06	0.08	0.17
Total soluble protein (mgg ⁻¹ FW)	30	1.92	1.77	1.42	1.25	0.09	0.30	0.61
	60	2.65	2.51	2.36	2.09	0.05	0.17	0.35
	90	3.83	3.60	3.32	2.91	0.08	0.27	0.56
Total phenols (mgg ⁻¹ FW)	30	0.68	2.07	3.98	5.64	0.15	0.52	1.07
	60	2.31	3.22	5.19	6.14	0.14	0.49	1.01
	90	3.06	3.80	5.65	6.56	0.11	0.39	0.81
Total free amino acid μgg ⁻¹ FW)	30	240	278	295	300	10.0	20.0	40.0
	60	310	364	395	399	10.0	30.0	60.0
	90	390	449	473	480	10.0	20.0	50.0
Total proline (μgg ⁻¹ FW)	30	68.00	81.50	98.90	113.80	3.60	12.50	25.90
	60	90.20	94.40	100.70	113.50	2.10	7.30	15.20
	90	95.20	120.90	141.50	154.00	0.10	0.10	0.30

*SE(m)=Standard error mean, **SD=Standard deviation, *** CD=Critical difference

Table 2. Effect of flood stress on yield parameters of Phourel-amubi rice cultivar at 90 and 120 days after transplant (DAT)

Parameters	DAT	Control (CON)	5 Days (5D)	10 Days (10D)	15 Days (15D)	*SE (m)	**SD±	***CD at 5%
Panicle number	90	6.83	6.00	5.16	3.00	0.16	0.55	1.13
	120	8.00	7.17	5.83	4.17	0.14	0.50	1.03
Number of filled grains per panicle	120	135.83	117.17	102.83	95.17	1.52	5.27	10.89
1000 grain weight (g)	120	29.69	24.86	19.79	16.19	0.62	2.15	4.45
Grain yield per hill (g)	120	25.08	23.49	16.18	11.67	0.64	2.21	4.57

*SE(m)=Standard error mean, **SD=Standard deviation, *** CD=Critical difference

4.1.9 Grain yield per hill

Flooding stress significantly affected grain yield per hill, with a progressive reduction observed as submergence duration increased (Table 2). The lowest yield was recorded under the 15-day flooding treatment. These findings agree with previous studies (Voeselek and Bailey-Serres, 2015; Grattan et al., 2002). Luo et al. (2019) and Zhao et al. (2023) further reported that flooding stress limits grain yield by inhibiting carbohydrate partitioning, reducing grain filling, and decreasing spikelet fertility.

5. CONCLUSION

The findings of this study demonstrate that flash flooding severely affects the growth, physiological processes, and yield performance of Phourel-Amubi rice. Reduced chlorophyll content, soluble sugars, and proteins and increased proline and phenolic accumulation indicate a biochemical stress response. Yield losses were substantial under prolonged submergence, emphasizing the need for flood-resilient rice varieties and improved water management strategies. Further research on breeding and agronomic interventions can aid in sustaining rice production in flood-prone areas.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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