

Analysis of the Application of Vitamin B1 on the Response of Salinity Stress Resistance in Several Varieties of Rice (*Oryza Sativa* L.)

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Received: November 30, 2023

Revised: July 20, 2024

Accepted: August 25, 2024

Published: August 31, 2024:

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DOI: [10.29303/jppipa.v10i8.6324](https://doi.org/10.29303/jppipa.v10i8.6324)

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Abstract: One of the most common stresses in rice cultivation is salinity. Rice plants stressed by salinity exhibit changes such as yellowing leaves, drying tips, and chlorosis. The efforts made by the government and farmers so far include implementing cultivation scheduling techniques, planting patterns, and using stress-resistant varieties, as well as improving soil to increase water-holding capacity through lime application. Each of these efforts comes with its own risks. Another approach to enhance the growth and yield of rice plants is the application of vitamins. Providing vitamins can stimulate the growth of plant organs, as they play a crucial role in the growth process by acting as catalysts for metabolism. Research has indicated that vitamin B1 can significantly promote plant growth under stressful conditions. This study aims to investigate the positive effects of various concentrations of vitamin B1 on the growth and yield of rice plants while also reducing salinity stress. The method used involved planting three varieties of rice – IR-46, Inpari-32, and Pokkali – in planting buckets using the TABELA system. Vitamin B1 was applied at concentrations of 0, 5, and 10 mM during the peak vegetative phase, with salinity stress of 6 dS/m introduced one day after vitamin application. The plants were maintained under salinity stress conditions until harvest, during which morphological and phytochemical analyses were conducted. Morphological analysis included measurements of plant height, number of tillers, number of productive tillers, number of grains per panicle, and percentage of healthy grains. Biochemical parameters measured included total chlorophyll and electrolyte leakage analysis. The results indicate that vitamin B1 can effectively reduce stress in plants affected by salinity.

Keywords: Active compound; Local rice; Vitamin B1.

Introduction

Rice plants (*Oryza sativa* L.) are the most important crops in Indonesia, as they serve as the staple food for the population. According to data from BPS (2021), rice production in various regions of Indonesia shows inconsistent yields. This variability is due not only to the differing land areas in each region but also to various issues that arise during cultivation. One significant problem in rice production is global warming, which causes stress to the plants. The impacts of global

warming include drastic climate changes such as drought, flooding, waterlogging, high temperatures, and saline land (Mamurung, 2019). Stress refers to conditions that fall outside the optimum range. One of the most common stresses in rice cultivation is salinity. Salinity stress results from climate change, where rising sea levels encroach upon land, causing stress to the plants (Berlinasari, 2019).

As an archipelagic country, Indonesia has many tidal lands that could be utilized for agriculture; however, high salinity levels render these tidal areas less

How to Cite:

Ratnasari, T., Handoyo, T., Dewanti, P., & Restanto, D. P. (2024). Analysis of the Application of Vitamin B1 on the Response of Salinity Stress Resistance in Several Varieties of Rice (*Oryza Sativa* L.). *Jurnal Penelitian Pendidikan IPA*, 10(8), 6260–6266. <https://doi.org/10.29303/jppipa.v10i8.6324>

productive. According to Zannati (2015), rice plants under salinity stress will show changes such as yellowing leaves, drying tips, and chlorosis. The emergence of saline soils, or soils invaded by seawater and containing iron, is a result of widespread land degradation. So far, rice production on saline land has not reached its maximum productivity, yielding only 2 tons/ha compared to the national rice standard of 6 tons/ha (BPS, 2015).

According to Surmaini (2011), the efforts made by both the government and farmers so far include implementing cultivation techniques such as scheduling and planting patterns (Makarim, 2005). However, there are gaps in planting time, and while stress-resistant varieties are planted, these varieties often have less favorable agronomic conditions, such as susceptibility to lodging. Soil improvement to enhance water retention through lime application can create residues that harden the soil (Adnyana, 2013), each with its own risks.

Another approach to improving the growth and yield of rice plants is through the application of vitamins. Providing vitamins can stimulate the growth of plant organs. Vitamins play a crucial role in the growth process as catalysts for metabolism. Moreover, research by Rika (2016) and Hasegawa (2000) indicates that thiamine, or vitamin B1, significantly affects plant resilience, as it helps prevent stress. Jabeen (2020) also noted that vitamin B1 can significantly enhance plant growth under stress conditions. In plants, vitamin B1 helps improve hormone activity within plant tissues and accelerates the distribution of new cells (Amalia, 2013). According to Garuda (2015), vitamin B1 also facilitates the rapid division of cells in root meristems. It acts as a catalyst and coenzyme in metabolism, particularly in energy production, including carbon absorption and respiration (Munir, 2016). Without energy, processes such as the formation of new tissues, cell division, and root growth cannot occur (Fitzpatrick, 2020).

This research aims to investigate the positive effects of various concentrations of vitamin B1 on the growth and yield of rice plants, as well as its potential to reduce stress in rice plants under salinity stress conditions.

Method

Seed planting is done directly or using the direct seed planting system (Tabela) in soil media within buckets. The number of seeds for each bucket is three. Seven days after planting, one well-growing seed will be selected to serve as the research sample, while the other two seeds will be removed. After that, irrigation is performed by filling the planting medium with water to a height of 2-3 cm above the soil surface. Irrigation is continued until ten days before harvesting.

Plant maintenance involves weeding and fertilizing. Fertilization is carried out based on recommendations from the Agricultural Research and Development Agency. Fertilizers are applied at 10 days after planting (hsp), 21 hsp, and 42 hsp, with amounts of 175 kg/ha NPK (Phonska), 150 kg/ha Urea, and 100 kg/ha ZA. Each bucket contains one planting hole and one plant, so the amount of fertilizer applied per bucket is 0.89 g NPK, 0.77 g Urea, and 0.51 g ZA.

Pest and disease control is conducted when attacks occur, using pesticides while considering economic thresholds. Insect pest control is done using insecticides (Antracol and Regent), while fungal pests are controlled with fungicides. Fungicide spraying (Syngeta Filia 525SE) at a dosage of 1 ml/liter of water is carried out at 24 hsp, as brown planthopper infestations were found to have reached economic thresholds at that time.

Vitamin B1 is applied using Thiamine Hydrochloride. The method of administering Vitamin B1 involves spraying it on the rice plants one day before the first NaCl stress application, and then every week thereafter at concentrations of 0 mM (control), 5 mM, and 10 mM.

NaCl stress treatment is applied the day after the vitamin B1 application until harvest. The NaCl is administered by pouring it into the buckets one day after the first vitamin B1 application, with a concentration of 100 mM.

The physical parameters measured include plant height, number of tillers, number of productive tillers, number of grains per panicle, and percentage of filled grains. These will be measured ten days before harvest and at harvest.

Electrolyte leakage analysis is conducted when the rice enters the generative stage. This stage is characterized by elongation of the upper stem internodes, a decrease in the number of tillers formed, and the growth of flag leaves and flowers. The EL analysis is performed to assess plant tolerance to stress by testing membrane stability and chlorophyll content. The testing method follows Ueda (2013). Rice leaves are cut into pieces measuring 0.5-1 cm with a weight of 0.5 g and gently shaken with 20 ml of distilled water in a flat-bottomed Makarthy tube for 24 hours at room temperature. The electrolyte conductivity of the water is measured using a conductivity meter (Horiba Scientific), and the result is expressed as EC1. The tube containing the mixture of water and leaves is autoclaved at 120°C for 15 minutes, and the electrolyte conductivity is then measured again to obtain the total electrolyte conductivity, expressed as EC2. Chlorophyll content in the plant leaves is measured using the SPAD method.

Results and Discussion

Thiamine is an essential nutrient for converting carbohydrates into the energy needed by plant tissues. Vitamin B1/thiamine can be obtained synthetically or naturally. Like citric acid and ascorbic acid, thiamine is sometimes used as an antioxidant to prevent or reduce browning or darkening of explants. The addition of thiamine to plants can accelerate growth because thiamine belongs to a group of phytohormones, which are substances that can stimulate or trigger growth, thus addressing issues of delayed growth (Rika et al., 2016). According to Suyanto (2022), thiamine can act as a regulator in transforming carbohydrates into energy required by plant tissues for cell division, tissue formation, and root growth. Research by Lukman et al. (2023) indicates that the application of thiamine can control stress in plants, as the addition of thiamine can enhance metabolic activity, thereby converting carbohydrates into energy. The carbohydrate and sugar content accelerate metabolic increases, leading to enhanced plant growth and cell differentiation.

Table 1. Summary of ANOVA Results (F-values) for All Observation Variables

Observation Variable	Thiamine (A)	Variety (B)	F-value Interaction A x B
Plant Height	7.74**	1001.89**	8.56**
Number of Offspring	7.72**	16.57**	3.54*
Productive Offspring	7.89**	11.31**	3.65*
Number of Panicles	7.89**	11.31**	3.65*
Panicle Length	113.33**	110.77**	3.76*
1000 Grain Weight	0.97ns	1.19ns	0.96ns
Weight of Filled Grains	273.78**	127.00**	5.83**
Chlorophyll	18.04**	30.13**	1.12ns
Electrolyte Leakage	81.00**	205.60**	0.52ns

Table 1 shows that the interaction of thiamine treatment with the Pokkali and Inpari 32 varieties has a significantly different effect on all observation variables, except for the weight of 1000 grains, chlorophyll, and electrolyte leakage. The effect of thiamine application on all variables shows a significant difference, except for the

weight of 1000 grains. The effect of rice variety on all variables shows a significant difference for all variables, except for the weight of 1000 grains.

Based on the research results, the control treatment positively influences plant height growth, as the plants do not experience salinity stress under those conditions. This aligns with the study by Muttaqien and Rahmawasti (2020), which found that plants adapt to salinity stress by exhibiting stunted height. The results indicate that the application of thiamine at 0 mM (T1), 5 mM (T2), and 10 mM (T3) affects plant height because the thiamine applied enables the plants to develop properly even under salinity stress conditions. T0 represents the positive control plants.

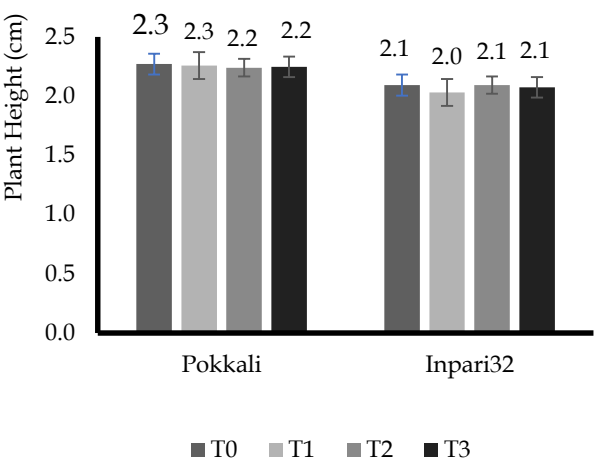


Figure 1. Morphological Characteristics of Rice Plants of Pokkali and Inpari32 Varieties with Thiamin (Vitamin B1) Administration.

The variable number of tillers shows that thiamin administration affects the number of tillers. According to Fitzpatrick et al. (2020,) the increasing thiamin content has the potential to increase harvest quality, yield, and plant metabolic resistance during climate change.

In line with the number of tillers, the number of productive tillers also indicates that thiamine affects the number of productive tillers. Productive tillers are those that produce panicles, which can influence the yield of rice plants. Not all tillers will produce panicles, as this depends on the available nutrients. The number of productive tillers in each treatment is directly proportional to the number of tillers that grow. The more tillers that grow, the more productive tillers are produced, which will affect the plant's yield (Tajudin and Sungkawa, 2020).

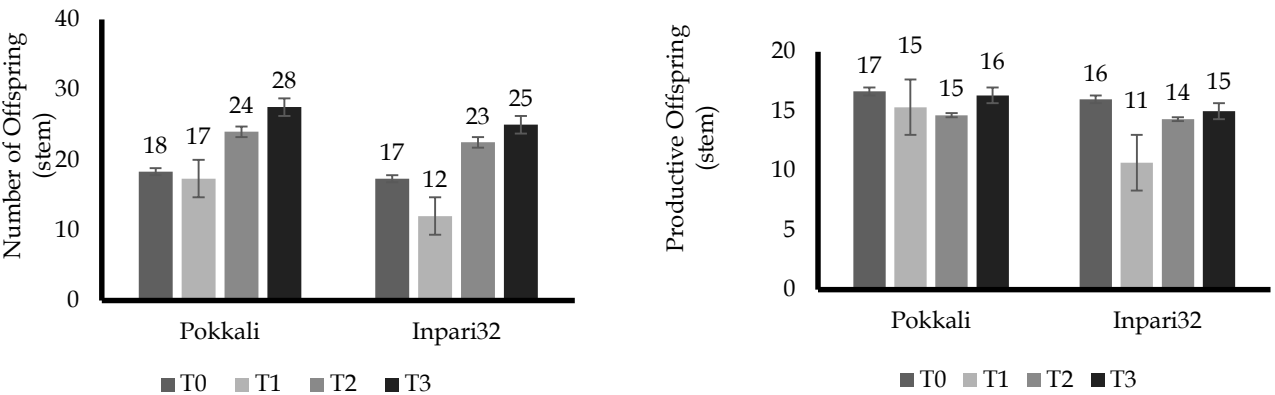


Figure 2. Number of offspring and number of productive offspring

Pokkali rice is tolerant to salinity stress, so there is no stress under saline conditions, and the role of B1 in alleviating stress conditions is not necessary. In contrast, the Inpari 32 variety shows an increase in the number of tillers with B1 application under salinity stress. This aligns with the observation that the number of productive tillers is also greater compared to Inpari 32 without B1 treatment. This indicates that vitamin B1 can alleviate salinity stress by restoring the metabolism of plants disrupted by NaCl. Vitamin B1 plays a role in stimulating plant metabolism.

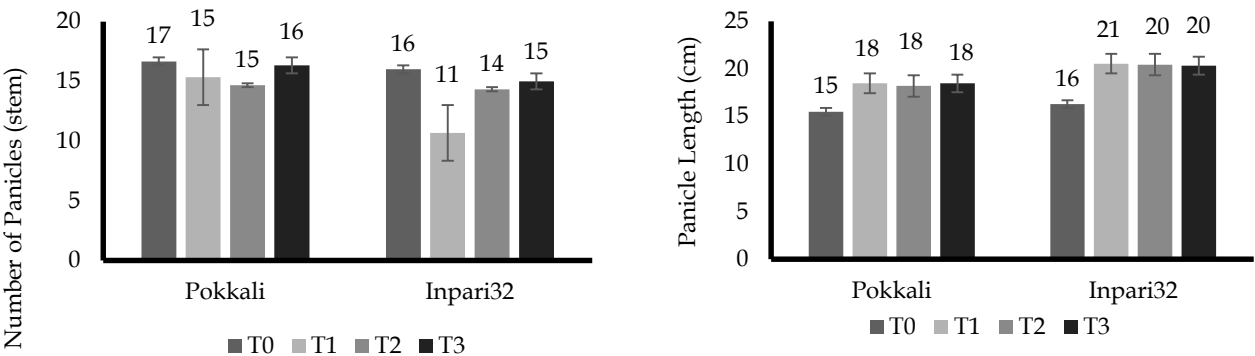


Figure 3. Number of panicles and panicle length.

In the Pokkali variety, the panicle length for each treatment shows no significant differences because Pokkali is one of the rice varieties resistant to salinity stress. However, this is not the case for Inpari 32, which is sensitive to salinity stress; the negative control without thiamine treatment has shorter panicle lengths compared to the thiamine treatments. This indicates that thiamine can help recover plants from stress.

The observation of the percentage of filled grains shows no significant effect across all treatments. Grain formation is influenced by the assimilates produced during photosynthesis in the generative phase and the remobilization of photosynthesis products stored as food reserves during the vegetative phase. The allocation of these assimilates is what leads to filled grains (Babu, 2007; Ubudiyah, 2013).

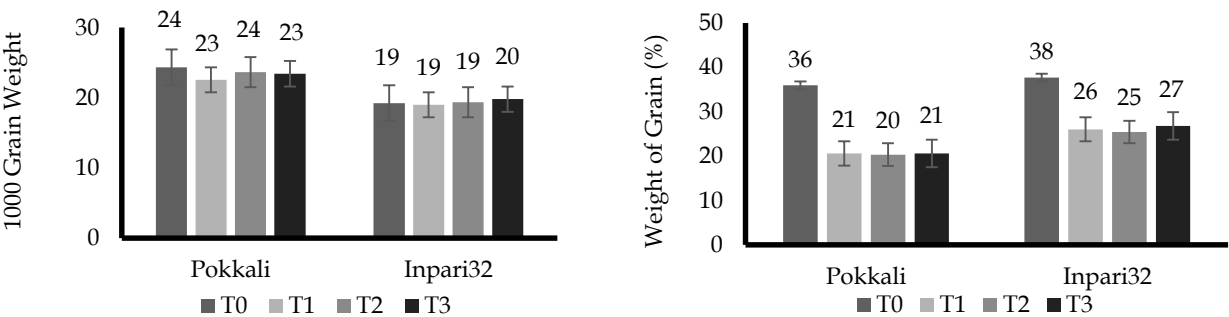


Figure 4. Weight of grains per hundred grams and percentage of filled grains

The results of the study show that the application of B1 on Inpari 32 under salinity stress resulted in the same number of grains and percentage of filled grains as without treatment. This may occur due to failure in grain filling. Both photosynthesis and respiration metabolism will influence the yield from stored photosynthates and the amount of ATP, which are essential for growth and development (Handayani, 2018; Widiastoety, 2019). In line with this, salinity stress affects plant metabolism. Initially, thiamine was able to alleviate stress while maintaining the number of tillers and productive tillers; however, subsequent storage of food reserves in the form of fruits and seeds was still not successful.

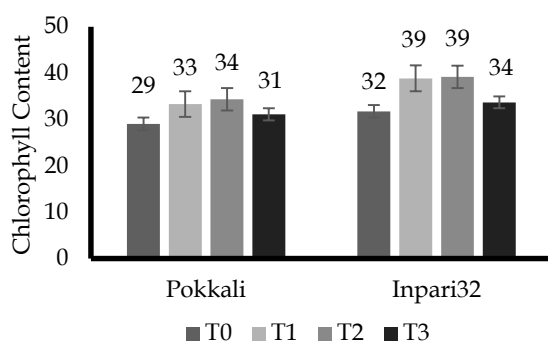


Figure 5. Chlorophyll

Chlorophyll is the green pigment in leaves and is an important protein in the process of photosynthesis (Amirjani, 2011). Its protein structure means that chlorophyll must be produced or synthesized from the metabolic activities of the plant (Alipoor, 2012; Arifiani, 2018). B1 can alleviate stress, allowing metabolism to function optimally with good chlorophyll formation, which enhances photosynthesis. The products of photosynthesis are then used for growth, development, and production in plants (Ahn, 2007; Kosová, 2013). However, this study shows that chlorophyll content does not differ significantly among the treatments. This may be due to insufficient concentrations of thiamine provided.

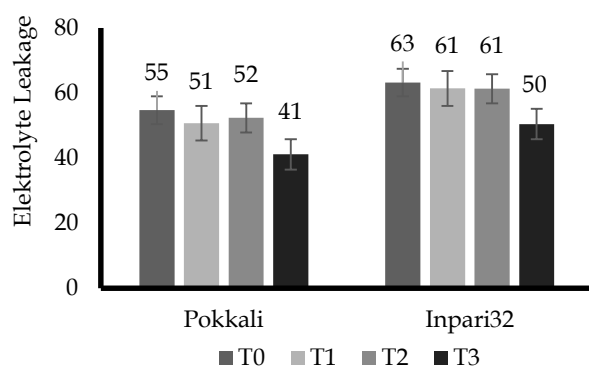


Figure 6. Electrolyte leakage

The application of NaCl at a concentration of 10 dS/m, applied every three days a week to the Inpari 32 and Pokkali varieties, was less effective at mitigating stress, resulting in plant stress. In this study, thiamine influenced rice plants by showing lower electrolyte leakage in the Pokkali variety at a concentration of 10 mM, while the Inpari variety exhibited significantly higher leakage compared to Pokkali. This indicates that increasing thiamine concentration further reduces electrolyte leakage in both the Pokkali and Inpari 32 varieties. NaCl in salinity stress leads to cell damage due to the inability of cells to maintain membrane permeability, causing the membranes to rupture and undergo lysis when the concentration of the solution inside and outside the cell is not equal (Ghafar, 2019; Ueda, 2013).

Conclusion

The combination of thiamine application on the Pokkali and Inpari 32 rice varieties under salinity stress has a very significant effect on the variables of plant height and the weight of filled grains, as well as a significant effect on the number of tillers, the number of productive tillers, the number of panicles, and panicle length. It also shows no significant effect on other variables. Vitamin B1 can reduce stress in plants affected by salinity. The best concentration of B1 is 10 mM, as it effectively reduces stress in the plants.

Acknowledgments

We would like to express our gratitude to the internal research grant for beginner lecturers from LP2M Universitas Jember, which funded this research until completion.

Roles of Each Author

The contributions of the authors include: research idea, Tri Ratnasari; methodology, Tri Ratnasari; software, Tri Ratnasari; validation, Tri Ratnasari, Tri Handoyo, Parawita Dewanti, and Didik Pudji Restanto; formatting, Tri Ratnasari; data analysis, Tri Ratnasari; writing, Tri Ratnasari; review, Parawita Dewanti; supervision, Tri Handoyo; project administration, Didik Pudji Restanto; funding acquisition, LP2M Universitas Jember.

Funding

This research was funded by DIPA Universitas Jember through the beginner lecturer research grant from LP2M Universitas Jember.

Conflicts of Interest

There are no conflicts of interest whatsoever between the funders and the authors. The authors are staff members at Universitas Jember who received funding from an internal grant, specifically the beginner lecturer research grant.

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