CHILEAN JOURNAL
of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Foliar application of chitosan and brassinosteroids on glutinous rice ('RD6'): Alteration in growth, agronomic trait, antioxidant capacity, elemental composition and aroma compound, 2-acetyl-1-pyrroline (2AP) in rice grain

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Received: 17 April 2024; Accepted: 12 July 2024, doi:10.4067/S0718-58392024000600739

ABSTRACT

The 'RD6' glutinous rice (*Oryza sativa* L.), developed from the popular aromatic rice 'KDML105' through gamma irradiation, is a key economic cultivar widely consumed in Thailand and exported throughout Asia. However, cultivation of the 'RD6' faces challenges due to its low grain yield and growth performance. The objective of this study was to use biostimulant chitosan and brassinosteroids to promote growth performance, nutrition, and yield of the 'RD6' rice. Chitosan (2.5, 5.0, 7.5, and 10.0 mg L^{-1}) and brassinosteroids (0.00005, 0.0005, 0.001, and 0.002 mg L^{-1}) were sprayed twice at 40- and 75-d-old rice plants in paddy fields. The results demonstrated that foliar spraying with 7.5 mg L^{-1} chitosan can promote leaf growth of 'RD6' and improve grain yield by 24% as compared with the control. More pronounced effects were observed with 0.002 mg L^{-1} brassinosteroid, improving not only growth but also 'RD6' yield by 41.8% compared to the control. Moreover, 0.002 mg L^{-1} brassinosteroids can enhance antioxidant capacities (total phenolic contents), elemental K and Ca contents, and the aroma compound 2-acetyl-1-pyrroline (2AP) contents in the rice grains. Furthermore, the use of 0.002 mg L^{-1} brassinosteroids proved to be a financially sound investment, yielding superior net returns and an advantageous benefit-cost ratio compared with 7.5 mg L^{-1} chitosan. Therefore, the use of exogenous brassinosteroids on glutinous rice in this study offers promising advantages for promoting the growth and physiological performance of rice plants.

Key words: Brassinosteroids, chitosan, glutinous rice, grain yield, nutrition, *Oryza sativa*, 2AP.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop that sustains nearly half of the global population (Yuan et al., 2021). It contributes to over 21% of the worldwide human caloric intake, and in Southeast Asia, this figure rises to as much as 76% (Yuan et al., 2021; Mohidem et al., 2022). Asia is responsible for more than 90% of the world's rice production (Mohidem et al., 2022), with the crop being cultivated across approximately 135 million hectares, yielding around 516 million tons annually (Bunnag and Suwanagul, 2017). For 2023/2024 cycle, China remains the top rice producer in Asia, according to the US Department of Agriculture (USDA), followed by India and Bangladesh. These countries produce approximately 28% (144.62 million tons), 26% (134.0 million tons), and 7% (36.30 million tons) of the global rice output, respectively (United States Department of Agriculture, 2024). In Thailand, rice farming cover roughly 11.11 million hectares, predominantly in the northern and northeastern regions of the country (Bunnag and Suwanagul, 2017). In 2023/2024, Thailand is ranked 6th in worldwide rice production, accounting for about 4% of the total or 20.00 million tons (United States Department of Agriculture, 2024).

Rice 'RD6', developed from 'KDML105' through gamma irradiation, ranks among the most popular glutinous rice cultivars in northern and northeastern Thailand. Owing to its superior cooking qualities, flavour, and texture, the demand for 'RD6' rice has grown over time (Thanasilungura et al., 2020). However, the yield of 'RD6' rice is significantly affected by biotic and abiotic stresses (Bunnag and Suwanagul, 2017; Wongkhamchan et al., 2018; Thanasilungura et al., 2020), as well as nutrient deficiencies and imbalances that hinder growth and development (Sangwongchai et al., 2023). Consequently, there is a need to explore the use of alternative agrochemicals or plant growth regulators to improve the growth, development, and yield of rice plants, particularly for 'RD6'.

Increasing crop yield remains a significant challenge in modern agriculture. The widespread use and overuse of chemical fertilizers on farms is a global crisis, leading to a number of adverse effects such as soil fertility erosion, water quality degradation, biodiversity loss, nutrient and pesticide runoff, and significant environmental and human health impacts (Ahmed et al., 2020a). Therefore, to mitigate the negative impacts of agriculture on the environment, it is crucial to reduce reliance on fertilizers. In this context, there is a growing demand for effective and eco-friendly plant growth regulators to enhance crop production. Numerous plant growth regulators have been applied for this purpose such as plant phytohormones (salicylic acid, abscisic acid, brassinosteroids) and natural polymer (chitosan) (Chen et al., 2022; 2023).

Chitosan is a mucopolysaccharide and naturally occurs as a linear polymer composed of 1,4-glycosidically linked glucosamine (2-amino-2-deoxy-D-glucopyranose) (Román-Doval et al., 2023). It has become a focal point in the agricultural sector, especially in advancing organic agriculture that poses no harm to the environment (Ahmed et al., 2020a). The effect of exogenous application of chitosan (such as foliar spraying and seed pretreatment) to promote plant growth and disease resistance has been recognized in a variety of crop plants (Ahmed et al., 2020a; Quitadamo et al., 2021; Chen et al., 2023). Besides, chitosan helps increase the effectiveness of nutrient absorption by plants and also reduces the rate of fertilizer application (Ahmed et al., 2020a).

Brassinosteroids, recognized as the sixth class of phytohormones, are a relatively newly discovered group of naturally occurring plant steroid hormones. They play a crucial role in normal plant growth, development, including regulation of cell elongation and division, photomorphogenesis, leaf growth and senescence, reproduction, proton pump activity, and gene expression (Nolan et al., 2020; Li et al., 2023). In addition, brassinosteroids can induce environmental stress tolerance by inducing a range of metabolic changes (Li et al., 2023). Exogenous application of brassinosteroids, e.g., foliar spraying, seed pretreatment, and root soaking at certain concentration can promote plant growth and increase crop yield, crop quality and improved crop stress tolerance (Li et al., 2021; 2023).

As chitosan and brassinosteroids are considered to have significant potential for crop improvement, the objective of this study was to evaluate the effectiveness of chitosan and brassinosteroids in stimulating growth and agronomic traits, as well as in altering the antioxidant properties, elemental composition, and the key aroma compound, 2-acetyl-1-pyrroline (2AP), in the grains of the glutinous rice 'RD6'.

MATERIALS AND METHODS

Plant material and chemicals

The rice (*Oryza sativa* L.) 'RD6' was obtained from the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Kantarawichai District, Maha Sarakham, Thailand. The moisture content of rice seeds is about 14%. Both chitosan (molecular weight 50 000-190 000 Da) and brassinolide (> 80%) used in this study were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Experimental design and plot culture

The experiment was conducted in a randomized complete block design (RCBD) replicated four times with nine treatments as follows: Water (control), 2.5, 5.0, 7.5, and 10.0 mg L^{-1} chitosan, 0.00005, 0.0005, 0.001, and 0.002 mg L^{-1} brassinosteroid.

The experiment was performed in August-December 2022 at Agricultural Research Field, Mahasarakham University (16°12'14" N, 103°17'06" E), Thailand. The average temperature was 28.5 ºC (day/night 33/24 °C). Experimental plots were prepared by ploughing the soil, adding 1000 kg ha^{-1} manure, water and sub-soiling thoroughly. The plot was divided into 2 × 4 m^2 plots, resulting in 32 plots. Seeds of 'RD6' glutinous rice were surface-sterilized with 1% sodium hypochlorite for 10 min before washing three times with sterile distilled water. The sterilized seeds were immersed in distilled water for 24 h. After 5 d germination, uniform-sized seedlings were planted in the plot at a spacing of 25 × 25 cm. Rice was sprayed twice at 40 d (vegetative stage) and 75 d (reproductive stage) after sowing. During the foliar application, the soil surface was covered with plastic sheet to prevent the solution dripping into the soil. Measurements of growth and physiological parameters were conducted at 55 and 85 d after spraying.

Measurement of plant growth

The rice plant height (cm) was measured from ground level to the tip of the longest leaf using a measuring tape. Roots were excavated from the experimental plot and carefully washed several times with tap water. The length of the root (cm) was measured from base to the tip of root using the standard ruler.

To determine stem and root dry weight, stems and roots were excavated from the plot and thoroughly washed with tap water to remove soil and dust particles before rising with distilled water. Then, stems and roots were cut into small fragments (*ca*. 10-15 cm). To obtain constant dry weight, the fragments of stem and root samples were put in a paper bag and oven-dried at 80 °C for 48 h before weighting using a digital balance with a resolution of 0.0001 g.

Measurement of leaf growth and photosynthetic parameters

Leaf growth was determined in terms of leaf area, length and width. The measurement was performed between 09:00-11:30 h on the fully expanded third leaf from the lower part of two plants using the handheld leaf area meter CI 203 (CID-Bio-Science, Camas, Washington, USA).

The photosynthetic and transpiration rates were determined on the thirdly fully expanded leaf from the lower part of two plants using the Ultra Compact Photosynthesis System (LCi-SD, ADC BioScientific Ltd., Herts, England). Measurement was performed in the morning between 09:00 and 11:30 h to avoid high vapor pressure deficit and photo-inhibition at midday.

Yield component and grain yield

Agronomic characteristics of rice were determined at 120 d after planting using the method of Baloch et al. (2002) including tiller number $hill^{-1}$, seeds number $panicle^{-1}$, grains filling (%), 100 seeds weight (g), 100 seeds volume (mL), and yield (kg ha^{-1}).

Elemental analysis of rice grain

Elemental analysis of Ca, Fe, Mg, Mn and Zn in rice grain was determined using atomic absorption spectrophotometry according to the method of Tyagi et al. (2020). For elemental K, flame photometry was performed according to the method described by Thunus and Lejeune (1994).

Determination of antioxidant capacity

Total phenolic content (TPC) was determined according to the method described previously by Singleton et al. (1999) using 0.1 mL extract, distilled water, Folin-Ciocalteu reagent, and sodium carbonate solution at room temperature for 90 min. Then the absorption of light at 760 nm was measured. Gallic acid was used as a standard. The result was the total amount of phenolic compounds (milligrams of gallic acid per 100 g dry weight).

Ferric reducing ability power (FRAP) solution consists of Fe^{3+} and 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ) in an acidic state, and the absorbance was measured at 593 nm. The reducing ability was based on the increased absorption of light at wavelengths being used as a measure of the reaction, using the method of Benzie and Strain (1996).

Determination of 2-acetyl-1-pyrroline (2AP) in grain

At maturity stage, the fresh grain samples were frozen in liquid nitrogen and immediately stored at -20 °C for the determination of 2AP content. The grain samples were ground using pestle and mortar and passed through a 0.297 mm mesh sieve (N°50 mesh sieve) to obtain the fine power. After that, 2.0 g powder was weighed for measurement of 2AP content using the gas chromatography-mass spectrometry (GC-MS) (Shimadzu Corporation, Kyoto, Japan) according to the method of Hien et al. (2006). The 2AP content was expressed as ng g^{-1} dry weight (DW).

Economic value of foliar application of chitosan and brassinosteroids on the ‘RD6’ rice plant

The treatment cost (USD ha^{-1}) is referred to how much the treatment costs (SEPWA, 2016). Other economic values, i.e., the total cost, gross return, net return and benefit cost ratio (BCR) were calculated based on the formula provided by Vercuiel and Botha (2023) and expressed as USD ha^{-1}.

Statistical analysis

A randomized complete block design (RCBD) with four replicates and nine treatments was conducted. The ANOVA was performed using SPSS v.20 (IBM, Armonk, New York, USA). Comparisons of means between different treatments were performed using the Duncan’s multiple range test (DMRT) at a 5% probability level.

RESULTS AND DISCUSSION

Chitosan and brassinosteroid hormones showed differential roles on promoting plant growth

Upon treating rice plants with chitosan and brassinosteroid hormones, both biostimulants induced substantial changes in plant height, root length, stem dry weight, and root dry weight (Table 1). However, chitosan did not exhibit beneficial effects on plant growth, despite numerous studies suggesting that its application could enhance plant height, branch and leaf number per plant, and total dry mass per plant (Al-Hassani and Majid, 2019; Ahmed et al., 2020b). The lack of effectiveness in this study could be attributed to the insufficient concentration of chitosan used, which might not have been adequate to stimulate plant development. Ullah et al. (2020) noted that plant growth parameters increased significantly with high concentrations of chitosan. In contrast, brassinosteroid hormones, particularly at 0.002 mg L^{-1}, resulted in increased plant heights, root lengths, stem and root dry weights. These hormones are known to regulate various processes including cell elongation, cell division, photomorphogenesis, xylem differentiation, and reproduction (Nolan et al., 2020).

Table 1. Effects of foliar application of chitosan and brassinosteroids on growth and biomass of the ‘RD6’ rice plant. Different letters indicate significant differences according to the Duncan’s test ($p \leq 0.05$). *Significant difference at the 0.05 probability level; ^{ns}nonsignificant difference.

Treatments	Height	Root length	Stem dry weight	Leaf dry weight	Root dry weight
	cm	cm	g	g	g
Water (control)	87.38^{ab}	19.25^{ab}	15.95^{bc}	0.21	6.07^{cd}
Chitosan 2.5 mg L^{-1}	86.00^{ab}	20.25^{ab}	15.97^{bc}	0.26	7.95^{a-d}
Chitosan 5.0 mg L^{-1}	89.75^{ab}	22.13^{ab}	19.54^{abc}	0.27	9.75^{abc}
Chitosan 7.5 mg L^{-1}	92.13^{ab}	22.13^{ab}	24.54^{ab}	0.27	11.60^{a}
Chitosan 10.0 mg L^{-1}	84.00^{ab}	14.50^{c}	11.98^{c}	0.20	5.09^{d}
Brassinosteroids 0.00005 mg L^{-1}	81.13^{b}	18.00^{bc}	18.82^{abc}	0.23	5.89^{cd}
Brassinosteroids 0.0005 mg L^{-1}	81.88^{ab}	19.00^{ab}	18.90^{abc}	0.26	7.44^{bcd}
Brassinosteroids 0.001 mg L^{-1}	81.25^{ab}	19.75^{ab}	21.25^{abc}	0.25	8.16^{a-d}
Brassinosteroids 0.002 mg L^{-1}	96.88^{a}	22.38^{a}	28.00^{a}	0.26	10.65^{ab}
F-test	*	*	*	ns	*
CV, %	8.39	4.84	8.74	7.40	4.71

Impacts of chitosan and brassinosteroids on leaf growth, transpiration and photosynthetic rates

Chitosan and brassinosteroid hormones caused alterations in the growth and dimensions of leaves, including leaf area, leaf length, and leaf width (Table 2). These results align with findings from Ullah et al. (2020), who noted that foliar application of chitosan improves overall leaf area per plant, contributing to an enhanced absolute growth rate. Our data indicated that chitosan concentrations ranging from 5.0 to 7.5 mg L^{-1} effectively increased leaf area and length, whereas lower (2.5 mg L^{-1}) and higher (10.0 mg L^{-1}) concentrations were less effective, suggesting that the optimization of chitosan concentrations is critical for influencing plant phenotype. In contrast to controls and plants treated with water, 7.5, and 10.0 mg L^{-1} chitosan, supplementation with brassinosteroids (0.0005-0.002 mg L^{-1}) significantly altered leaf morphology, with marked increases in leaf area, length, and width. Brassinosteroids are known to be associated with cell elongation and expansion, facilitating plant development and morphogenesis (Nolan et al., 2020). Notably, a higher concentration of brassinosteroids (0.002 mg L^{-1}) appeared to further enhance leaf morphology. In terms of transpiration and photosynthesis rates, chitosan and brassinosteroids did not induce significant changes. There were no notable differences in transpiration rates across all treatment groups. However, a modest increase in photosynthesis rate was observed in rice plants treated with brassinosteroids at concentrations of 0.001 and 0.002 mg L^{-1} (Table 2). Siddiqui et al. (2018) reported that brassinosteroids could stimulate photosynthesis by activating rubisco and modifying expressions of photosynthetic genes.

Table 2. Effects of foliar application of chitosan and brassinosteroids on leaf growth, leaf greenness as measured by SPAD value, and leaf transpiration and photosynthetic rates of the 'RD6' rice plant. Different letters indicate significant differences according to the Duncan's test ($p \leq 0.05$). *, **Significant difference at the 0.05 and 0.01 probability levels respectively; ^{ns}nonsignificant difference.

Treatments	Leaf area	Leaf length	Leaf width	Transpiration rate	Photosynthesis rate
	cm^2	cm	cm	mol H_2O m^{-2} s^{-1}	µmol CO_2 m^{-2} s^{-1}
Water (control)	51.32^{e}	40.33^{e}	2.46^{c}	0.40	66.93^{abc}
Chitosan 2.5 mg L^{-1}	52.28^{e}	49.72^{d}	2.76^{ab}	0.40	66.24^{bc}
Chitosan 5.0 mg L^{-1}	115.85^{b}	85.58^{a}	2.79^{a}	0.46	67.27^{ab}
Chitosan 7.5 mg L^{-1}	119.28^{b}	67.31^{c}	2.66^{ab}	0.45	67.20^{ab}
Chitosan 10.0 mg L^{-1}	52.14^{e}	33.70^{f}	1.55^{d}	0.42	65.62^{c}
Brassinosteroids 0.00005 mg L^{-1}	52.14^{e}	33.70^{f}	1.55^{d}	0.38	67.20^{ab}
Brassinosteroids 0.0005 mg L^{-1}	83.80^{d}	49.72^{d}	2.62^{b}	0.46	67.45^{ab}
Brassinosteroids 0.001 mg L^{-1}	107.54^{c}	74.29^{b}	2.76^{ab}	0.47	67.83^{a}
Brassinosteroids 0.002 mg L^{-1}	129.28^{a}	85.58^{a}	2.79^{a}	0.48	67.96^{a}
F-Test	**	**	**	ns	*
CV, %	3.87	4.88	4.32	8.79	1.60

Alteration of agronomic traits

The exogenous application of chitosan and brassinosteroids caused changes in some agronomic traits, including the tillers hill^{-1}, seed number panicle^{-1}, grain filling, 100 seed weight, 100 seed volume, and grain yield of 'RD6' rice plants (Table 3). Chitosan at concentrations of 2.5 or 10.0 mg L^{-1} resulted in a slight decrease in seed number panicle^{-1} and 100 seed volume. However, when chitosan was applied at 5.0 and 7.5 mg L^{-1}, rice yield increased by 8.49% and 24%, respectively, compared to untreated groups. The effectiveness of chitosan has been associated with increases in crop yield and quality (Chen et al., 2023). Regarding the foliar application of brassinosteroids, it was observed that tillers hill^{-1}, seed number panicle^{-1}, grain filling, 100 seed weight, and 100 seed volume slightly increased, particularly at 0.002 mg L^{-1} brassinosteroids. In plants, brassinosteroids influence many agronomic traits that affect grain yield, such as branching number and grain size (Chen et al., 2024). They stimulate the flow of assimilates from the source to the sink, enhancing seed filling and inducing genes that promote grain development (Wu et al., 2008). This activity likely contributes to improved grain weight and yield. Moreover, spraying brassinosteroids at 0.0005, 0.001, and 0.002 mg L^{-1} on rice plants resulted in marked increases in grain yield to 2107.0, 2159.5, and 3123.8 kg ha^{-1}, respectively. Brassinosteroids enhance CO_2 assimilation, enlarge glucose pools in the flag leaves, and increase glucose conversion to starch in seeds, which aids seed filling and can significantly boost grain yields in crops (Wu et al., 2008). Additionally, it was reported that brassinosteroids enhance plant physiological functions and molecular mechanisms related to agronomic traits such as plant height, leaf angle, tiller number, and panicle morphology, thereby effectively promoting grain yield (Zhang et al., 2014).

Table 3. Effects of foliar application of chitosan and brassinosteroids on agronomic trait including grain yield of the 'RD6' rice plant. Different letters indicate significant differences according to the Duncan's test ($p \leq 0.05$). *, **Significant difference at the 0.05 and 0.01 probability levels respectively.

Treatments	Tiller number hill^{-1}	Seed number panicle^{-1}	Grain Filling	100 Seeds weight	100 Seeds volume	Grain yield
			%	g	mL	kg ha^{-1}
Water (control)	8.50^{ab}	85.42^{a-d}	91.50^{a}	1.95^{ab}	2.93^{ab}	1819.6^{g}
Chitosan 2.5 mg L^{-1}	9.75^{a}	61.42^{cd}	74.58^{ab}	1.91^{b}	2.81^{ab}	1844.4^{f}
Chitosan 5.0 mg L^{-1}	9.13^{ab}	76.58^{a-d}	89.25^{a}	2.03^{ab}	3.10^{a}	1988.9^{e}
Chitosan 7.5 mg L^{-1}	9.13^{ab}	94.08^{abc}	80.83^{ab}	2.02^{ab}	2.91^{ab}	2395.3^{b}
Chitosan 10.0 mg L^{-1}	8.38^{ab}	60.83^{d}	88.75^{a}	1.96^{ab}	2.65^{b}	1635.3^{i}
Brassinosteroids 0.00005 mg L^{-1}	5.75^{c}	71.75^{bcd}	62.58^{b}	1.93^{b}	2.91^{ab}	1710.5^{h}
Brassinosteroids 0.0005 mg L^{-1}	7.50^{b}	93.00^{a-d}	75.50^{ab}	1.98^{ab}	2.90^{ab}	2107.0^{d}
Brassinosteroids 0.001 mg L^{-1}	7.63^{b}	99.50^{ab}	89.50^{a}	2.06^{ab}	2.93^{ab}	2159.4^{c}
Brassinosteroids 0.002 mg L^{-1}	9.63^{a}	104.75^{a}	91.58^{a}	2.12^{a}	3.16^{a}	3123.8^{a}
F-Test	**	*	*	*	*	**
CV, %	3.72	7.13	5.39	5.86	7.33	2.12

Changes in elemental composition in grains of 'RD6' rice

The elemental composition of 'RD6' rice seeds were dramatically changed when plants were exogenously supplied with both chitosan and brassinosteroids in various concentrations (Table 4). Notably, the use of water resulted in the highest accumulation of Fe in seeds (8.30 mg L^{-1}) compared to chitosan and brassinosteroids. The reduced Fe concentrations in the brassinosteroid treatment groups may be attributed to the hormones' effects on decreasing Fe concentrations in roots and shoots, as well as altering Fe uptake and translocation in rice (Wang et al., 2015). Chitosan application did not alter Mg content in rice grains; however, applying 0.00005 mg L^{-1} brassinosteroids slightly increased Mg accumulation to 34.23 mg L^{-1}, while using 0.002 mg L^{-1} brassinosteroids slightly decreased it to 30.30 mg L^{-1}. Potassium content showed a clear advantage when chitosan was applied, significantly increasing compared to the control. The highest K content was found in the group treated with 10.0 mg L^{-1} chitosan (7111.30 mg L^{-1}). This finding aligns with studies on wheat, where foliar supplementation with nanoform chitosan significantly boosted K and P contents in the grains (Abdel-Aziz et al., 2018). For Ca, applications of chitosan above 2.5 mg L^{-1} and any concentration of brassinosteroids led to higher Ca accumulation in rice grains, increasing by 61%-69% and 56%-68% respectively. These results highlight the effectiveness of both chitosan and brassinosteroids in enhancing mineral composition in rice grains. Amerany et al. (2020) noted chitosan's capacity to chelate ions crucial for mineral assimilation, while Kretynin et al. (2021) reported that brassinosteroids improved Ca^{2+} balance in plants due to stimulation by exogenous applications. For Mn, a low concentration of chitosan (2.5 mg L^{-1}) slightly increased Mn content in rice grains, while a high concentration (10.0 mg L^{-1}) caused a slight decrease. A high concentration of brassinosteroid hormones (0.002 mg L^{-1}) also tended to slightly reduce Mn content. Nonetheless, studies have shown that the external application of chitosan and brassinosteroids (24-epibrassinolide) can increase Mn contents in plants (Amerany et al., 2020; dos Santos et al., 2021). For Zn, chitosan application did not significantly change Zn levels compared to the control, whereas brassinosteroids reduced Zn content in rice grains, with a tendency to decrease further as the concentration of brassinosteroids increased. Brassinosteroids have been reported to reduce the translocation of heavy metals, including Zn, into plants and mitigate their toxicity (Xu et al., 2019). Thus, supplementation with exogenous brassinosteroids could decrease Zn accumulation in rice grains.

Table 4. Effects of foliar application of chitosan and brassinosteroids on elemental (nutritional) contents in grain of the ‘RD6’ rice plant. Different letters indicate significant differences according to the Duncan’s test ($p \leq 0.05$). *, **Significant difference at the 0.05 and 0.01 probability levels respectively.

Treatments	Fe	Mg	K	Ca	Mn	Zn
	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Water (control)	8.30^{a}	33.17^{ab}	5803.00^{d}	12.00^{b}	3.59^{ab}	1.20^{ab}
Chitosan 2.5 mg L^{-1}	4.77^{b}	32.07^{ab}	6560.50^{abc}	12.50^{b}	4.11^{a}	1.13^{ab}
Chitosan 5.0 mg L^{-1}	4.41^{bc}	31.95^{ab}	6733.50^{abc}	30.50^{a}	3.58^{ab}	1.15^{ab}
Chitosan 7.5 mg L^{-1}	3.36^{c}	31.18^{ab}	6965.50^{ab}	39.00^{a}	3.70^{ab}	1.30^{a}
Chitosan 10.0 mg L^{-1}	3.84^{bc}	32.00^{ab}	7111.30^{a}	32.25^{a}	3.42^{b}	1.06^{abc}
Brassinosteroids 0.00005 mg L^{-1}	3.37^{c}	34.23^{a}	$6393.20^{a\text{-}d}$	37.00^{a}	3.65^{ab}	0.99^{bc}
Brassinosteroids 0.0005 mg L^{-1}	3.81^{bc}	31.25^{ab}	6369.20^{bcd}	27.00^{ab}	4.09^{a}	0.98^{bc}
Brassinosteroids 0.001 mg L^{-1}	3.36^{c}	31.04^{ab}	6050.50^{cd}	29.75^{a}	3.61^{ab}	0.84^{c}
Brassinosteroids 0.002 mg L^{-1}	3.32^{c}	30.30^{b}	6321.50^{bcd}	36.25^{a}	3.36^{b}	0.78^{c}
F-Test	**	*	*	*	*	*
CV, %	7.42	8.06	7.85	8.96	7.17	8.26

Alteration of aroma compound 2AP and antioxidant capacities in seeds

2-Acetyl-1-pyrroline (2AP) is a volatile organic compound that imparts the characteristic flavour to aromatic rice (Routray and Rayaguru, 2017). According to Table 5, foliar application of chitosan did not enhance the 2AP content in rice seeds, with observed decreases dependent on the chitosan concentration used. Conversely, the application of brassinosteroids through spraying positively impacted the enhancement of 2AP content, with levels increasing alongside the concentration of brassinosteroids used. The elevated 2AP content is likely due to the induction of proline catabolism by brassinosteroids, as the by-products of this process are precursors for 2AP synthesis (Luo et al., 2020). Regarding antioxidant capacities, the application of chitosan and brassinosteroids caused slight variations in total phenolic content (TPC). However, there were no remarkable changes in ferric reducing antioxidant power (FRAP). The highest TPC activity was observed in the group treated with 7.5 mg L^{-1} chitosan, whereas the lowest activity was noted in the group treated with brassinosteroids at 0.00005 mg L^{-1}. Previous studies have reported that both chitosan and brassinosteroids can enhance plant antioxidant capacities (Çoban and Baydar, 2016; Quitadamo et al., 2021). Despite these findings, this study did not observe any significant effects of chitosan or brassinosteroids on FRAP values.

Table 5. Effects of foliar application of chitosan and brassinosteroids on the aroma compound 2-acetyl-1-pyrroline (2AP) content and antioxidant capacities in ‘RD6’ rice grain. Different letters indicate significant differences according to the Duncan’s test ($p \leq 0.05$). *, **Significant difference at the 0.05 and 0.01 probability levels respectively; ^{ns}nonsignificant difference. TCP: Total phenolic content; FRAP: Ferric reducing ability power.

Treatments	2AP	TPC	FRAP
	ng g^{-1}	mg QE 100 g^{-1}	mg QE 100 g^{-1}
Water	0.275^{c}	41.20^{ab}	104.01
Chitosan 2.5 mg L^{-1}	0.190^{e}	44.38^{ab}	98.04
Chitosan 5.0 mg L^{-1}	0.205^{de}	47.87^{ab}	102.07
Chitosan 7.5 mg L^{-1}	0.210^{d}	51.89^{a}	105.00
Chitosan 10.0 mg L^{-1}	0.219^{d}	45.51^{ab}	103.84
Brassinosteroids 0.00005 mg L^{-1}	0.282^{bc}	34.80^{b}	94.67
Brassinosteroids 0.0005 mg L^{-1}	0.292^{b}	46.91^{ab}	103.63
Brassinosteroids 0.001 mg L^{-1}	0.308^{a}	45.72^{ab}	105.59
Brassinosteroids 0.002 mg L^{-1}	0.314^{a}	49.50^{ab}	104.81
F-Test	**	*	ns
CV, %	2.77	4.92	6.42

Economic value of foliar application of chitosan and brassinosteroids on the rice plant 'RD6'

An assessment was conducted to analyse the economic impact of applying chitosan and brassinosteroid hormone on the production of 'RD6' glutinous rice. The evaluation, detailed in Table 6, focused on the costs and returns per unit plot of 'RD6' rice, which were then converted to cost per hectare. When averaging the results of the nine treatments, it was observed that the treatment with brassinosteroids at a concentration of 0.002 mg L^{-1} resulted in the highest gross return (USD 1018.19 ha^{-1}) and benefit-cost ratio (BCR) of 1.99. Conversely, the use of chitosan at 10.0 mg L^{-1} led to the lowest gross return (USD 538.13 ha^{-1}) and a BCR of 1.01. Applying brassinosteroids at 0.002 mg L^{-1} yielded a net return that was 419% higher than the control, making it the most economically advantageous treatment. Specifically, this brassinosteroid treatment provided the highest net return of USD 505.31 ha^{-1}, whereas the chitosan treatment at 10.0 mg L^{-1} recorded the lowest net return at USD 7.00 ha^{-1}. This analysis clearly demonstrated that the application of brassinosteroids at 0.002 mg L^{-1} maximized net profits compared to other treatments.

Table 6. Estimation of cost of production, returns, and benefit cost ratio (BCR) of the 'RD6' rice plant after treatments with exogenous chitosan and brassinosteroids at different concentrations. [1]Paddy price ('RD6') was 11.74 THB kg^{-1} or 0.33 USD kg^{-1} (Office of Agricultural Economics, 2021). 1 USD ≈ 36 THB (Bank of Thailand, 2024).

Treatments	Treatment cost	Total cost	Gross return	Net return	BCR
	USD ha^{-1}	USD ha^{-1}	USD ha^{-1}	USD ha^{-1}	
Water (control)	-	506.63	597.69	91.06	1.18
Chitosan 2.5 mg L^{-1}	7.69	508.06	603.94	95.88	1.19
Chitosan 5.0 mg L^{-1}	15.38	515.75	658.75	143.00	1.28
Chitosan 7.5 mg L^{-1}	23.06	523.44	783.13	259.69	1.50
Chitosan 10.0 mg L^{-1}	30.75	531.13	538.13	7.00	1.01
Brassinosteroids 0.00005 mg L^{-1}	1.56	501.94	560.63	58.69	1.12
Brassinosteroids 0.0005 mg L^{-1}	3.13	503.50	689.31	185.81	1.37
Brassinosteroids 0.001 mg L^{-1}	6.25	506.63	703.38	196.75	1.39
Brassinosteroids 0.002 mg L^{-1}	12.50	512.88	1018.19	505.31	1.99

CONCLUSIONS

To enhance the production of 'RD6' glutinous rice, exogenous spraying of chitosan and brassinosteroid hormones at various concentrations was employed, in comparison to the use of water alone. It was found that foliar application of chitosan was able to increase nutritional value in 'RD6' rice grain, especially K and Ca, though it suppressed 2-acetyl-1-pyrroline (2AP) accumulation. Controversially, foliar application of exogenous brassinosteroids at 0.002 mg L^{-1} significantly improved leaf growth, increased crop productivity, antioxidant capacity, nutritional elements, and elevated levels of 2AP. These enhancements can be attributed to increased growth, development, and overall yield. Furthermore, the application of brassinosteroids at 0.002 mg L^{-1} demonstrated to be a financially sound decision, offering a superior net return and an advantageous benefit-cost ratio. This study demonstrates that foliar application of exogenous brassinosteroids in glutinous rice 'RD6' offers promising benefits not only in promoting rice plant growth, physiological performance and agronomic traits, but also in terms of antioxidant capacity, elemental composition, and aroma compound 2AP contents in rice grains. To enhance the beneficial effects of foliar application of brassinosteroids in tropical rice fields, it is recommended that foliar spraying be performed in early morning to avoid rapid evaporation of the substances during sunrise. It would also be interesting to investigate the molecular mechanism by which exogenous brassinosteroids improve the elemental composition and aroma compound 2AP at different stages of growth in 'RD6' rice.

Author contribution

Conceptualization: P.K. Methodology: P.K., R.W., D.B., B.K., O.S., C.J., P.K., H.B. Software: P.K., H.B. Validation: P.K., H.D. Formal analysis: P.K., H.D. Investigation: P.K., R.W., D.B., B.K., O.S., C.J., P.K., H.B. Resources: P.K., R.W. Data curation: P.K., H.D. Writing-original draft: P.K. Writing-review & editing: P.K., R.W., D.B., B.K., O.S., C.J., P.K., H.B. Supervision: P.K. Project administration: P.K. Funding acquisition: P.K., R.W. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

This research was financially supported by Mahasarakham University, Thailand. We also thank all the anonymous reviewers for their generous time in providing detailed comments and suggestions that helped us to improve the paper.

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