Agricultural Sciences, 2025, 16(10), 1100-1114
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Impact of Nitrogen and Zinc Fertilizers on the Synthesis of Functional Compounds during Seed Development in Various Types of Purple Rice

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How to cite this paper: Utasee, S., Jamjod, S., Veeradittakit, J. and Prom-u-Thai, C. (2025) Impact of Nitrogen and Zinc Fertilizers on the Synthesis of Functional Compounds during Seed Development in Various Types of Purple Rice. *Agricultural Sciences*, **16**, 1100-1114.
https://doi.org/10.4236/as.2025.1610063

Received: September 1, 2025
Accepted: October 8, 2025
Published: October 11, 2025

Abstract

The study evaluated total anthocyanin, phenol, and DPPH activity during seed development in two traditionally improved and one modern improved purple rice varieties under varying nitrogen (N) and zinc (Zn) applications. Two N levels were tested: 120 kg N/ha (N120) and no N application (N0). Three Zn application methods were applied: (1) no Zn fertilizer (Zn0), (2) soil Zn application at 50 kg $ZnSO_4$/ha (ZnS), and (3) foliar Zn application at 0.5% $ZnSO_4$ (800 L/ha) (ZnF), with foliar Zn applied twice at flowering and 10 days after flowering. The seeds were randomly collected at 7, 14, 21, 28, and 35 (fully maturity) days after flowering (DAF). This study observed variations in seed appearance as affected by N and Zn application during development among different purple rice varieties. The darkest pericarp color was found when ZnF was applied with N120. The concentration of grain anthocyanin, phenol, and DPPH activity was synthesized at 7 DAF, peaked at 14 DAF, and gradually declined until maturity at 35 DAF. At early seed developing, applying ZnS under N0 yielded as high total anthocyanin, phenol, and DPPH activity as for applying ZnF under N120. At maturity, applying N120 with ZnF established the highest grain anthocyanin, phenol, and DPPH activity compared with the others. Therefore, grain anthocyanin, phenol, and DPPH activity in purple rice varieties can be enhanced through N and Zn fertilization from early seed development to maturity, especially at 14 DAF, soil Zn application without N (N0 + ZnS) yielded total anthocyanin, phenol, and DPPH activity levels comparable to the high input N120 + ZnF. However, the optimal Zn application method, combined with N, should be carefully considered at each seed developing and rice variety to maximize value for both farmers and consumers.

Keywords

Purple Rice, Pigmented Rice, Functional Compounds, Bioactive Compounds, Fertilization

1. Introduction

Pigmented rice is primarily distinguished by its black, red, and dark purple colors. The color of the rice is determined by the composition and concentration of anthocyanin pigments in different layers, including the aleurone, pericarp, and seed coat [1]. The pigment contains various functional compounds, including peonidin-3-glucoside cyanidin-3-glucoside, *γ*-oryzanol, *γ*-tocotrienol, proanthocyanidin, cinnamic acid, and anthocyanins, act as pro-apoptotic, anti-proliferative, and anti-metastatic agents preventing from chronic disorders related to oxidative stress due to their antioxidant properties [2] [3]. Anthocyanins, in particular, are glucosides of anthocyanidins, which are flavonoid derivatives produced via the phenylpropanoid pathway, predominantly found in foods in the forms of cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin [4] [5]. In pigmented rice, the common anthocyanin type is cyanidin-3-glucoside, while the minor is peonidin-3-glucoside [6]. The concentration and composition of anthocyanins in rice plants are vary depend on the rice variety and developmental stages such as seedling, vegetative, reproductive, and mature stages [7] [8], which the level increases with the progression of developmental stages and gradual grain filling, starting to accumulate in the caryopsis 8 - 14 days after flowering (DAF), for example, the caryopsis starts to turn black at the milk stage, and the highest anthocyanins concentration accumulates in the caryopsis at the maturity stage (35 - 45 DAF) [9].

Grain anthocyanin concentration in pigmented rice varieties is influenced not only by developmental stages but also by fertilizer management, particularly nitrogen (N) and zinc (Zn) applications during crop cultivation. Nitrogen fertilizer application has been reported to increase anthocyanin concentration in the vegetative parts of purple rice [10]. Meanwhile, Zn application can enhance functional compounds in rice grain, increasing phenolic content by 40% and flavonoid content by 71.54% compared to no Zn application [11]. Recent studies have reported that combined N and Zn fertilization, applied either to the soil or as a foliar spray, synergistically enhances grain Zn concentration and anthocyanin levels in purple rice [12] [13]. However, limited information is available on the interaction effects of N and Zn fertilizer applications on the synthesis of functional compounds during seed development in different purple rice varieties. We hypothesize that the optimal application of N rates and methods of Zn fertilizer applications will differentially promote the synthesis of grain functional compounds during seed development in purple rice varieties. Therefore, this present study was to investigate effects of N and Zn fertilizer application on grain functional compounds synthesis

during seed development of purple rice varieties. The information gain from this study would be very useful to promote high functional compounds in purple rice for multi-purpose uses in human health.

2. Materials and Methods

2.1. Plant Culture

The experiment was conducted at research station field, Lanna Rice Research Center, Chiang Mai University, Thailand. The field experiment was arranged in 2 × 3 × 3 factorials in RCBD with three independent replications. The three purple rice varieties (KDK; Kum Doi Saket, KJ-CMU 107; Kum Chao Morchor107, traditional improved rice varieties, and CMU-K4, Kum Chao Morchor K4; a modern improved rice variety) were planted during the wet season (June-November) in 2021. The average temperature during the cropping season was at 28.6˚C, with 76.4% relative humidity and 138.4 mm precipitation during crop cultivation [14]. For seedling preparation, rice seeds were soaked in water for 24 h, and then incubated at ambient temperature for 24 h. The germinated seeds were sown in a prepared seedbed for a month and transplanted into the field at each of the prepared subplots, with a single seedling at 25 × 25 cm spacing between hills. Each variety was planted under two conditions, with applying N at 120 kg N/ha as urea in three equal doses at basal fertilizer before planting, maximum tillering, and flowering stages and without N application (N0). Each growing condition was divided into two methods of Zn fertilizer application, including soil Zn application at 50 kg $ZnSO_4$/ha (ZnS) and foliar application of 0.5% $ZnSO_4$ (ZnF) at the rate of 800 L/ha. Soil N and Zn fertilizers were applied a week before transplanting, while foliar Zn was applied two times at the flowering stage and 10 days after flowering. The field was kept flooded under 0.1 m above the soil surface throughout the rice crop. The water was drained out from the field seven days before harvesting.

2.2. Data Collection and Sample Preparation

During seed development, the plants were marked at the initiation of the fertilization stage. The seed samples were collected randomly at 7, 14, 21, 28, and 35 (maturity) days after flowering (DAF) described by Butsat *et al.* [15]. Rice seeds were manually separated the husk for brown rice (caryopsis). The physical characteristics were recorded (seed size and color shade) then the samples were freeze-dried, ground into powder, and the fine powder was stored in a zip-lock plastic bag before being kept in a freezer at 20˚C in the dark until analysis.

2.3. Chemical Analysis

For anthocyanin analysis, the total anthocyanin content (TAC) was analyzed by the modified procedure of Abdel-Aal and Hucl [16]. About 2.5 g of whole grain samples were extracted with 24 mL of acidified methanol (70% methanol and 30% 1.5 mol/L HCl, v/v) with shaking for 1 h, and then the sample was filtered through a Whatman No. 1 filter paper. The supernatant was collected and added to the

two buffer solutions 0.025 mol/L potassium chloride buffer, pH 1.0, and 0.4 mol/L sodium acetate buffer, pH 4.5. The absorbance of anthocyanin was measured at 520 and 700 nm using a spectrophotometer (Biochrom Libra S22, England). The absorbance of the anthocyanin pigment was expressed as cyanidin-3-glucoside, the main anthocyanin in rice.

$$\text{Anthocyanin} = \left(\text{A} \times \text{MW} \times \text{DF} \times 1000\right) / \left(\varepsilon \times 100\right)$$

Where A = (A520 nm - A700 nm) pH 1.0 - (A520 nm - A700 nm) pH 4.5, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/ mol, DF is the dilution factor, ε is the molar absorbance (26,900 L/mol/cm, and L is the cell path length (1 cm).

For total phenol analysis, the concentration was determined through the reducing power against Folin-Ciocalteu as described by Singleton *et al.* [17]. The extract (100 mL) was mixed with Folin-Ciocalteu reagent (500 mL) before 1.5 mL of sodium carbonate aqueous solution (7.5% w/v) was added. The intensity of the developed blue color was measured at 750 nm using a spectrophotometer. Gallic acid was used for building the standard curve. The concentration of total phenols was expressed as milligram gallic acid equivalent (mg GAE/100 g dw).

For antioxidant capacity analysis, the free radical scavenging activities of bran extracts were determined by using a stable DPPH radical [18]. Exactly 0.1 ml of the extract solution was well mixed with 3.9 ml of methanol and 1.0 ml of DPPH solution. The mixture was kept at ambient temperature for 30 min before measurement of the absorbance at 517 nm.

$$\text{DPPH scavenging capacity } (\%) = \left(\text{AC} - \text{AS}\right) / \text{AC}$$

Where AC is the absorbance of the control and AS is the absorbance of the sample. The DPPH radical-scavenging activity was calculated using a calibration curve with Trolox concentrations ranging from 10 to 62 μg/mL ($R^2 = 0.995$).

2.4. Statistical Analysis

All statistical analyses were carried out by using analysis of variance (ANOVA), followed by LSD comparison tests by using Statistix 10 (analytical software SX). Statistically significant differences were identified at a level of $P < 0.05$. Correlation coefficient analyses were conducted for each set of parameters.

3. Results

3.1. Morphological Characteristics of Developing Seed

Seed morphological characteristics was changed during 7, 14, 21, 28 and 35 DAF among the purple rice varieties grown under N and Zn fertilizer applications. Regardless of the N and Zn fertilizer application (N0Zn0), there were variation of color during seed development in both the paddy rice (with husk) and caryopsis (without husk) among the varieties (**Figure 1(A)**). Pericarp color of the caryopsis at 7 DAF was initiated in green-purple in KDK, light-purple in KJ-CMU 107, and dark purple color in CMU-K4. The overall appearance of the caryopsis color in all

varieties continued to develop from very light purple at the milky stage (14 DAF) to darker purple at the maturity stage (35 DAF). On the other hand, applying N and Zn fertilizer had differently affected the morphological characteristics of caryopsis during development among varieties (Figure 1(B)). It was noticed that applying N120 resulted in a darker pericarp color than those with no N application in all Zn treatments. Additionally, applying ZnF resulted in a darker purple color of the caryopsis color than by ZnS and no Zn application at all N treatments. The darkest purple color was observed when applying together N120 and ZnF. Applying N and Zn fertilizers were not only affected the morphological characteristics of caryopsis during development of purple rice varieties, but also grain functional compounds synthesis such as anthocyanin, phenol an DPPH activity.

3.2. Grain Anthocyanin Concentration

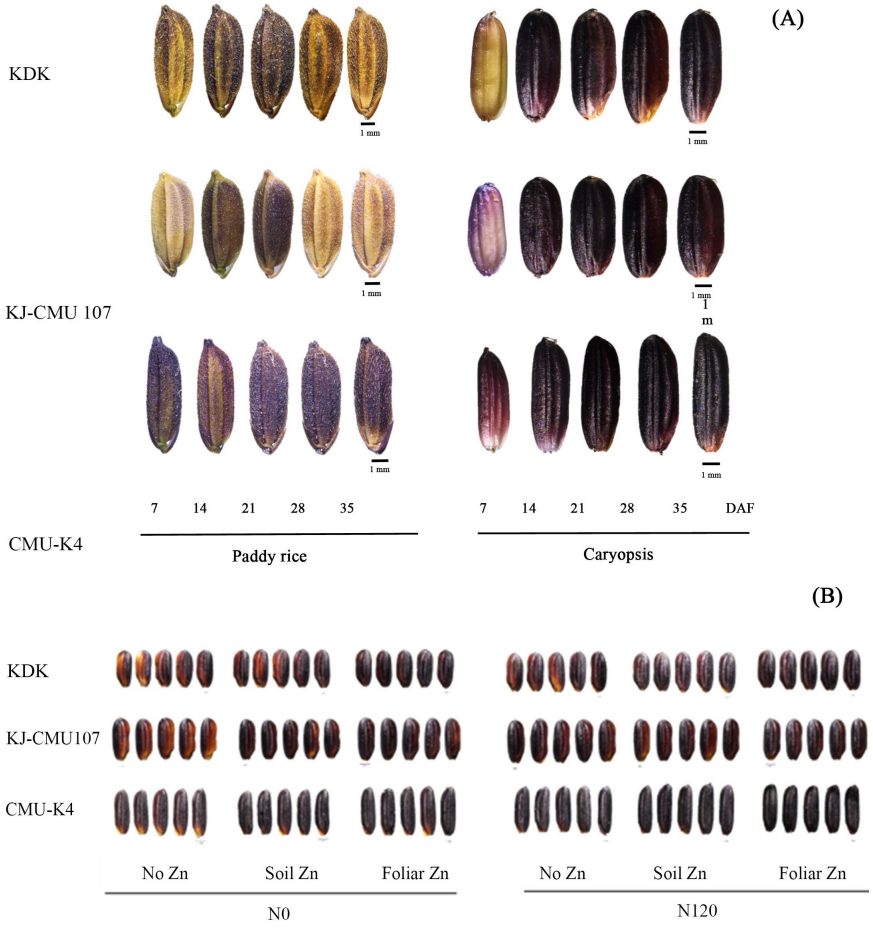

Figure 1. The appearance of paddy rice during 7, 14, 21, 28 and 35 DAF grown under no N and Zn (N0Zn0) application (A) and the maturity caryopsis at 35 DAF grown under two rates of N at 120 kg/ha (N120) and no nitrogen application (N0), and three Zn fertilizer treatments (no Zn, soil Zn and foliar Zn) of KDK, KJ-CMU 107 and CMU-K4 rice varieties (B).

Applying N and Zn had influenced grain anthocyanin concentration during seed development differently among rice varieties ($P < 0.05$) (Figure 2). In KDK,

at 7 DAF, plants grown at N120 without Zn produced slightly higher grain anthocyanin (22.6 mg/100g) than at N0 (21.8 mg/100g). At N0, applying ZnS increased grain anthocyanin at 35.9 mg/100g which was higher than ZnF at 22.1 mg/100g. At N120, it was increased to 28.0 mg/100g by ZnF, but it was decreased by ZnS.

The highest grain anthocyanin was found at 14 DAF in all treatments, particularly under ZnF in both N0 and N120, produced grain anthocyanin average by 31.7 mg/100g. The concentration of grain anthocyanin gradually declined at 21, 28, and 35 DAF, while it remained affected by N and Zn application. At maturity stage (35 DAF), without Zn application plants grown at N120 produced higher grain anthocyanin (31.7 mg/100g) than at N0 (17.4 mg/100g). At N0, applying ZnF increased grain anthocyanin at 69.2 mg/100g which was higher than applying with ZnS at 45.1 mg/100g. At N120, grain anthocyanin was increased to 37.4 and 77.4 mg/100g by ZnS and ZnF, respectively (**Figure 2(A)**).

For KJ-CMU 107, at 7 DAF, grain anthocyanin of plants grown under N0 and N120 without Zn produced 61.9 and 45.1mg/100g, respectively (**Figure 2(B)**). At N0, applying ZnS and ZnF did not result in a significant difference in grain anthocyanin average at 58.4 mg/100g. At N120, applying ZnS decreased grain anthocyanin at 38.1 mg/100g, while ZnF increased grain anthocyanin at 74.8 mg/100g. The highest grain anthocyanin was also observed at 14 DAF for plants grown at N0 and N120 without Zn at 122.3 and 187.1 mg/100g, respectively. At N0, it was increased to 422.9 mg/100g by ZnS, while it was not affected by ZnF. At N120, it was increased to 205.4 and 230.9 mg/100g by ZnS and ZnF, respectively. Similarly, the concentration of grain anthocyanin continued to decline at 21 until 35 DAF at maturity, remaining affected by N and Zn application. At 35 DAF, plants grown at N0 and N120 without Zn produced grain anthocyanin at 29.5 and 57.2 mg/100g, respectively. At N0, applying ZnS and ZnF had no different of grain anthocyanin average for 35.8 mg/100g. At N120, grain anthocyanin decreased at 39.4 mg/100g by ZnS, while increased at 72.4 mg/100g by ZnF.

In CMU-K4, the effect of N and Zn application on grain anthocyanin was ununiform at each seed developing stage compared with the above varieties (**Figure 2(C)**). Plants grown at N0 and N120 without Zn application produced grain anthocyanin at 91.0 and 107.0 mg/100g, respectively. At N0, applying ZnS and ZnF significantly increased the grain anthocyanin to 128.4 and 188.8 mg/100g, respectively. Similarly, at N120, it was increased at 157.1 and 211.8 mg/100g by ZnS and ZnF, respectively. However, the highest grain anthocyanin concentration was also found at 14 DAF in all treatments with the highest grain anthocyanins when plants were grown under N0 and N120 without Zn, produced grain anthocyanin at 83.6 and 140.7 mg/100g, respectively, but the effect was changed regarding on seed development. At N0, grain anthocyanin was significantly increased at 383.7 and 211.7 mg/100g by ZnS and ZnF, respectively, at N120, it was increased to 266.8 and 248.2 mg/100g, by ZnS and ZnF respectively. Similarly, the concentration continued to decline at 21, 28 and 35 DAF, differently affected by N and Zn application. At 35 DAF, plants grown at N0 and N120 without Zn produced grain

anthocyanin at 77.8 and 125.4 mg/100g, respectively. At N0, applying ZnS and ZnF had significantly increased grain anthocyanin at 80.3 and 101.8 mg/100g, respectively Zn0, at N120, applying ZnS and ZnF had significantly increased grain anthocyanin at 133.5 and 194.1 mg/100g, respectively.

3.3. Grain Phenol Concentration

Grain phenol concentration during seed development was affected by N and Zn application differently among rice varieties (**Figure 3**). Overall, grain phenol was the highest in CMU-K4 variety compared with the other two purple rice varieties which were in the similar level. The highest concentration of grain phenol was found at 14 days in all varieties, and the concentration continued to decline at 21, 28 and 35 DAF, differently affected by N and Zn application among purple rice varieties, similar as found in grain anthocyanin concentration.

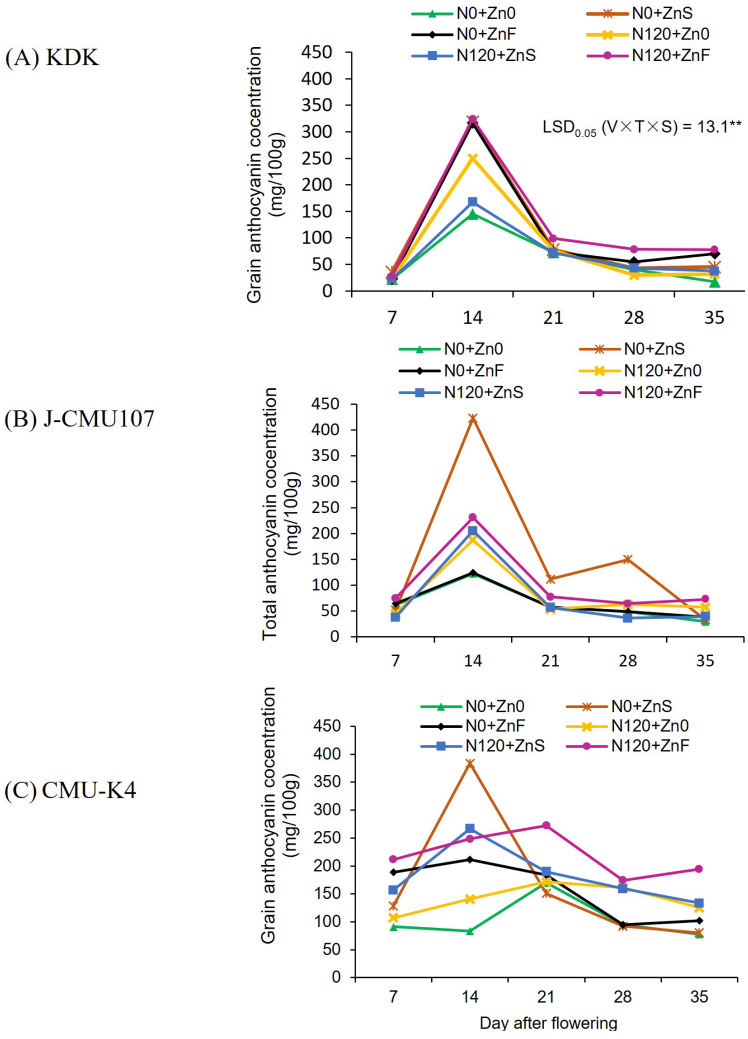

Figure 2. Grain anthocyanin concentration in caryopsis of three purple rice varieties KDK (A), KJ-CMU 107 (B) and CMU-K4 (C) during grain development under two rates of N at 120 kg/ha (N120) and no N application (N0), and three Zn fertilizer treatments (no Zn, soil Zn and foliar Zn).

In KDK, at 7 DAF, plants grown at N0 and N120 without Zn application produced grain phenol concentration at 173.3 and 148.4 mg gallic acid/100 g, respectively. Applying ZnS and ZnF had no difference in grain phenol concentration for an average of 171.2 mg gallic acid/100 g under N0 and 162.2 mg gallic acid/100 g under N120. At 14 DAF, grain phenol concentration in plants grown at N0 and N120 without Zn application produced grain phenol concentration at 389.2 and 466.3 mg gallic acid/100 g, respectively. At N0, it was increased at 496.9 and 525.7 mg gallic acid/100 g by ZnS and ZnF, respectively, while at N120, applying ZnS and ZnF had no different grain phenol concentration for an average of 543.1 mg gallic acid/100 g. The variation effects from N and Zn application were continued to observed at 21, 28 and 35 DAF.

At 35 DAF, plants grown at N0 and N120 without Zn application had an average grain phenol concentration of 228.4 and 245.1 mg gallic acid/100 g, respectively. At N0, applying ZnS and ZnF increased grain phenol concentration at 242.2 and 290.3 mg gallic acid/100 g, respectively, while at N120, applying ZnS and ZnF had no different of grain phenol concentration for an average of 267.6 mg gallic acid/100 g (**Figure 3(A)**).

The KJ-CMU 107, at 14 DAF, plants grown under N0 and N120 without Zn application produced grain phenol concentration at 242.2 and 406.9 mg gallic acid/100 g, respectively. At N0, applying ZnS and ZnF increased grain anthocyanin concentration at 524.2 and 358.4 mg gallic acid/100 g, respectively, while at N120 grain phenol concentration decreased at 307.4 mg gallic acid/100 g by ZnS, but it was increased at 529.6 mg gallic acid/100 g by ZnF. At 35 DAF, plants grown at N0 and N120 without Zn application had grain phenol concentration at 227.8 and 292.8 mg gallic acid/100 g, respectively. At N0, applying ZnF increased phenol concentration at 258.4 mg gallic acid/100 g, while applying ZnS did not affect grain phenol concentration compared to Zn0. At N120 applying ZnF increased grain phenol concentration at 321.8 mg gallic acid/100 g, while applying ZnS did not affect grain phenol concentration compared to Zn0 (**Figure 3(B)**).

For CMU-K4, At 14 DAF, plants grown with N0 and N120 without Zn application produced grain phenol concentration at 535.6 and 567.6 mg gallic acid/100 g, respectively. At N0, applying ZnS increased to 648.9 mg gallic acid/100 g, while applying ZnF did not effect on phenol concentration compared to Zn0. However, at N120, grain phenol concentration decreased to 535.6 mg gallic acid/100g with ZnS, but it was increased to 658.0 mg gallic acid/100 g by ZnF. At 35 DAF, plants grown at N0 and N120 without Zn application had grain phenol concentrations at 377.0 and 383.9 mg gallic acid/100 g, respectively. Under N0, applying ZnS and ZnF decreased grain phenol concentration for an average of 343.3 mg gallic acid/100 g, compared to Zn0, while at N120, applying ZnF increased phenol concentration to 413.9 mg gallic acid/100 g, but did not affect grain phenol concentration for ZnS compared to Zn0 (**Figure 3(C)**).

3.4. Grain DPPH Activity

The responses of purple rice varieties to N and Zn application during seed devel-

opment on DPPH activities were difference from those anthocyanin and phenol compounds (Figure 4). Overall, the highest DPPH activity was found at 7 DAF and continued to decline until 35 DAF at maturity with differently affected by N and Zn applications.

In KDK, at 7 DAF, plants grown grown at N0 and N120 without Zn application produced grain phenol concentration at 4096.7 and 3592.5 mg Trolox/100 g, respectively. At N0, applying ZnS and ZnF decreased grain DPPH activity for an average of 3979 and 3712.8 mg Trolox/100 g, while at N120, applying ZnS and ZnF did not affect grain DPPH activity average at 3,712.9 mg Trolox/100 g, compared to Zn0. At 14 DAF, the highest DPPH activity was found in all treatments. Plants grown at N0 and N120 without Zn application had an grain DPPH activity at 3890.3 and 5181.1 mg Trolox/100 g, respectively. At N0, applying ZnS and ZnF increased grain DPPH activity for an average of 5833.1 mg Trolox/100 g, while at N120 ZnS and ZnF increased grain DPPH activity for an average of 6025.5 mg Trolox/100 g.

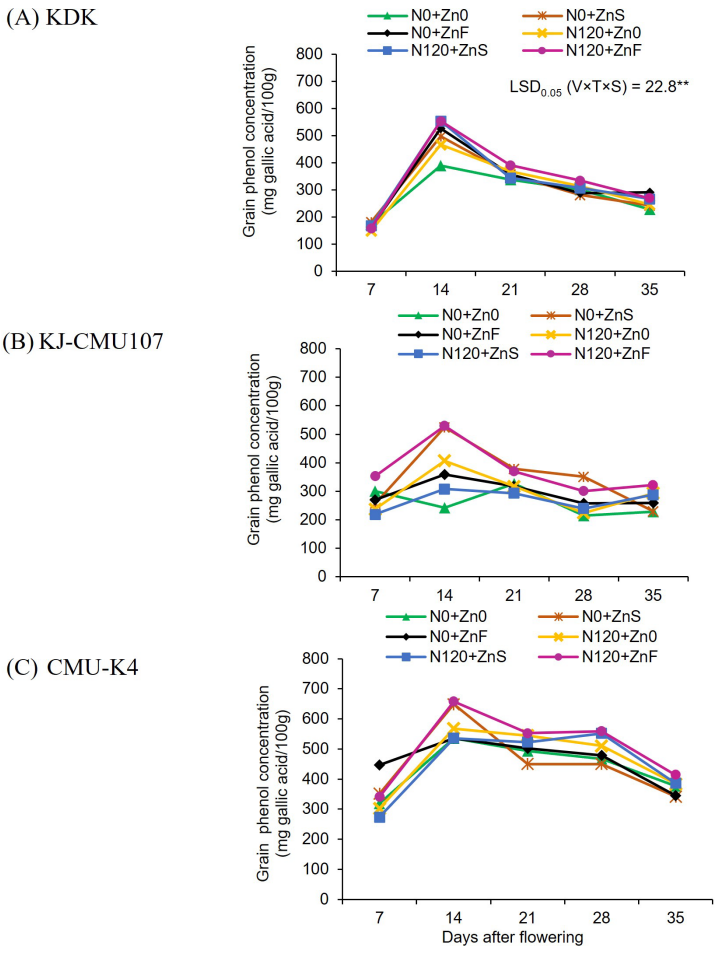

Figure 3. Grain phenol concentration in the caryopsis of three purple rice varieties KDK (A) KJ-CMU 107 (B) and CMU-K4 (C) during grain development under two rates of N at 120 kg/ha (N120) and no nitrogen application (N0), and three Zn fertilizer treatments (no Zn, soil Zn and foliar Zn).

The DPPH activity substantially declined at 21, 28 and 35 DAF, while remained to affected by N and Zn application differently among rice varieties. At 35 DAF, plants grown at N0 and N120 without Zn application had grain DPPH activity at 1183.6 and 1231.6 mg Trolox/100 g, respectively. At N0, applying ZnF increased DPPH activity at 2061.0 mg Trolox/100 g, but no effect was found in ZnS compared with Zn0. Similarly, at N120 the DPPH activity increased at 1980.8 mg Trolox/100g by ZnF, but applying ZnS did not affect grain DPPH activity compared to Zn0 (**Figure 4(A)**).

For KJ-CMU 107, the highest DPPH activity was found at 7 DAF in all treatment of N and Zn applications. At 7 DAF, plants grown at N0 and N120 without Zn application produced grain DPPH activity at 5742.2 and 5120.3 mg Trolox/100 g, respectively. At N0, applying ZnS decreased grain DPPH activity at 5319.5 mg Trolox/100 g, while applying ZnF increased grain DPPH activity to 6651.6 mg Trolox/100 g. At N120, applying ZnF increased grain DPPH activity at 6624.2 Trolox/100 g, but applying ZnS did not affect grain DPPH activity from Zn0. The responses were quite similar at 14, 21 and 28 DAF. At 35 DAF, plants grown at N0 and N120 without Zn application had grain DPPH activity at 786.6 and 1283.3 mg Trolox/100 g, respectively. At N0, applying ZnS and ZnF increased grain DPPH activity for an average of 988.0 mg Trolox/100 g, while at N120 applying ZnS and ZnF did not affect grain DPPH activity compared to Zn0 (**Figure 4(B)**).

For CMU-K4, the highest DPPH activity was also found at 7 DAF in all treatment of N and Zn applications (**Figure 4(C)**). At 7 DAF, plants grown at N0 and N120 without Zn application produced grain DPPH activity at 5880.2 and 5433.9 mg Trolox/100 g, respectively. At N0, applying ZnS increased grain DPPH activity to 6194.3 mg Trolox/100 g, while applying ZnF was not different from Zn0. At N120, applying ZnS increased grain DPPH activity to 5820.5 mg Trolox/100 g, while applying ZnF decreased DPPH activity to 4878.1 mg Trolox/100 g. The DPPH activity was continued to decline at 14, 21, 28 and 35 DAF with variation effects by N and Zn application at each development stage. At 35 DAF, plants grown at N0 and N120 without Zn application had grain DPPH activity at 3130.7 and 3763 mg Trolox/100 g, respectively. At N0, applying ZnS and ZnF decreased grain DPPH activity for an average of 3000.2 mg Trolox/100 g, while at N120, ZnS and ZnF increased grain DPPH activity for an average of 3841.7 mg Trolox/100 g, respectively.

4. Discussion

The well recognition grain quality of purple rice is known by its benefit of functional compounds to human health. This study has shown that the functional compounds initiate to synthesize and accumulate at the beginning of fertilization and the highest concentration was mostly found at 14 days DAF with variation effect from N and Zn application among the purple rice varieties.

Regardless of the effects of N and Zn application, the pigment color of the husk and pericarp continued to change from lighter to darker purple until maturity at

35 DAF, depending on the rice variety (**Figure 1(A)**). This aligns with a previous study that reported anthocyanin accumulation in the litchi pericarp begins with chlorophyll, which is gradually replaced by anthocyanin pigments during fruit development [19]. However, in purple rice, it has been reported that the replacement of chlorophyll by anthocyanins after chlorophyll degradation may lead to yield loss due to a reduction in the photosynthesis rate [20]. It would be interesting to further investigate whether there is a linkage between yield production and anthocyanin pigmentation in purple rice or other pigmented rice varieties as it would be valuable information in breeding program for crop improvement purpose. Moreover, the present study has shown that foliar Zn either with soil or foliar applications under N0 and N120 resulted in a darker purple pericarp color in all varieties, especially at N120 (**Figure 1(B)**). Thus, applying N and Zn fertilizers may enhance the pigmentation of both chlorophyll and anthocyanin compounds, not only in the caryopsis but also in the leaves, potentially improving photosynthesis and productivity.

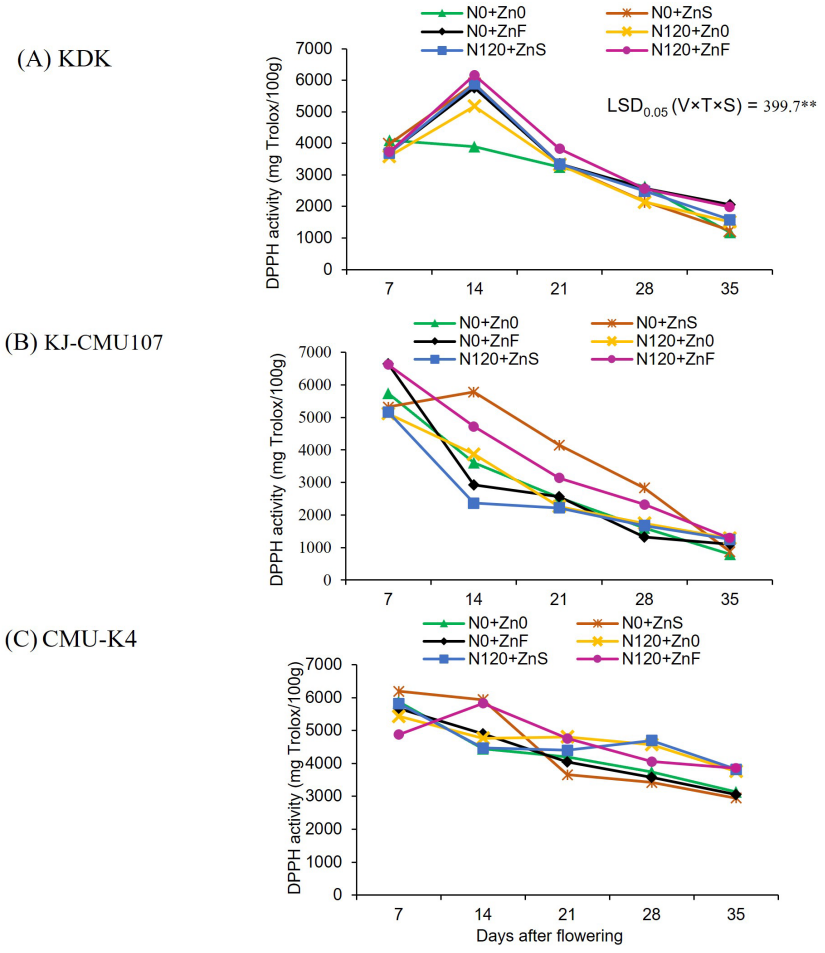

Figure 4. Grain DPPH activity in caryopsis of three purple rice varieties KDK (A) KJ-CMU 107 (B) and CMU-K4 (C) during grain development under two rates of N at 120 kg/ha (N120) and no nitrogen application (N0), and three Zn fertilizer treatments (no Zn, soil Zn and foliar Zn).

The current study has demonstrated that the highest grain total anthocyanin and phenol was peaked at 14 DAF, while it was 7 DAF for DPPH activity and continuing to decline until grain maturity at 35 DAF, which was also similarly indicated by the previous study that the total anthocyanins of the pigmented rice grain was peaked at the dough stage (15 to 21 DAF) and declining at the further seed developmental stage, depending on rice variety [9] [21]. In the molecular level, the upregulation of pigmentation genes was expressed during the first 20 DAF with the highest activity between 7 - 18 DAF, while their peak was attained between 14 - 21 DAF [22]. The gradual changes in anthocyanin concentration correlate with the expression of genes *OsDFR, OsF3H*, *OsAns*, and *OsCHS* that control anthocyanin biosynthesis [23] [24]. The anthocyanin accumulation was mainly occurred inside the pericarp at after 7 DAF, and then inside the testa and aleurone layer after 15 - 30 DAF, and almost does not accumulate in the endosperm cells excluding the aleurone layer cells [25]. This could explain the reason why the concentration of anthocyanin and other functional compounds started to decline after grain fillings >14 DAF due to no accumulation of the compounds in the endosperm. Thus, genetically modifying the structural and regulatory genes involved in functional compound biosynthesis, such as anthocyanins in the endosperm layers during grain filling, could enhance the accumulation of these beneficial compounds in purple rice.

On the other hand, the current study has established that applying N and Zn generally improved all functional compounds synthesis in purple rice grain during seed development until maturity. Previous studies reported that applying N fertilizers can alter the quality of antioxidant compounds, total phenols and flavonoids in many plants [26]-[28]. The highest anthocyanin and antioxidant content in red cabbage and pomegranate were induced when applied N and Zn fertilizer, respectively [29] [30]. For the combination of N and Zn fertilizer, our previous studies reported that it enhanced anthocyanin in purple rice, depending on variety [12] [13]. The present study further established that during seed development, particularly at 14 DAF when the compounds reach peak concentration applying ZnS alone without N potentially increased the compound levels as much as, or even higher than, applying N120 and ZnF in some varieties. However, this effect declined during later stages of seed development. A similar trend was observed in productivity for KDK and KJ-CMU 107, but not in CMU-K4.

5. Conclusion

The results of the present study have proposed a promising method for managing N and Zn fertilizers to increase grain anthocyanin, phenol, and DPPH activity among purple rice varieties for the benefits of farmers and consumers. The variety KDK, KJ-CMU 107, and CMU-K4 significantly increased grain anthocyanin, phenol concentration and DPPH activity under N120 + ZnF compared with ZnS and Zn0 application. The impact of the Zn application method on the synthesis of anthocyanin, phenol, and DPPH activity in rice grain was as in the following:

ZnF > ZnS > Zn0. The total anthocyanin, phenol, and DPPH activity synthesis during seed development among the purple rice varieties was peaked at the milky stage (14 DAF) and continued to decrease until reaching the fully ripe stage (35 DAF) depending on rice variety and fertilization of N and Zn. However, changes of the total anthocyanin, total phenol, and DPPH activity in purple rice grain may continuously occur during long-term storage after harvesting. The requirement of total anthocyanin, phenol, and DPPH activity from rice grain in many purposes e.g., foods, drinks and pharmaceutical products may need to consider the appropriate stage of seed development for the maximum yield of the total anthocyanin, phenol, and DPPH activity. Additionally, the stability of anthocyanin, phenol, and DPPH activity should be considered, as these may change during storage and should be further investigated in future studies. Therefore, enhancing total anthocyanin, phenol, and DPPH activity in purple rice varieties could be managed through N rate and Zn application method, a common practice in rice crops, but the specific rate and method of fertilizer application for each rice variety may need to carefully attention for the maximum benefit of the total anthocyanin, phenol, and DPPH activity.

Funding

This research was also partially supported by the agricultural research development agency (public organization) grant number GSCMU (HRD65050085)/12/2565 and Chiang Mai University.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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