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ORIGINAL RESEARCH

Differences in nutrient remobilization characteristics and relationship to senescence and grain nutrient content among rice varieties

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Abstract

During leaf senescence, essential nutrients are remobilized to sink tissues such as developing seeds and grains. Nutritional contents in the grains of crop plants may be influenced by the extent of the nutrient remobilization process, which may be influenced by the leaf senescence programming. To test these hypothetical relationships in rice plants, nutrient remobilization characteristics of three macro-elements—nitrogen (N), phosphorus (P), and potassium (K)—were examined among ten rice genetic backgrounds including nine representative Thai rice varieties and one Indian variety. Greenness colorations and the N, P, and K contents of flag leaves of the field-grown rice plants were quantified at 0, 7, 14, 21, and 28 days after flowering. Rice varieties that exhibited a stay-green trait or high nutrient remobilization efficiency were identified. On average, the N, P, and K remobilization efficiencies were 50%, 27%, and 22%, respectively, suggesting a poor remobilization process in rice compared to other crop plants. No significant relationship ($P < 0.05$) was found between the nutrient remobilization rates or efficiencies and the leaf greenness reduction efficiencies among the rice varieties. Furthermore, no significant relationship ($P < 0.05$) was found between the N, P, and K contents in mature rice grains and the nutrient remobilization rates and efficiencies, or the initial nutrient content stored in flag leaves. Further studies using a larger number and broader range of rice varieties and examining other characteristics of the leaf senescence and nutrient remobilization processes may be needed to verify this lack of association.

Keywords Senescence · Nutrient remobilization · Stay green · Biofortification

Introduction

Rice is a staple source of carbohydrate for over 3.5 billion of the world's population (Zhao et al. 2020). In addition, rice can provide protein, vitamins, and minerals if consumed as brown or partially milled rice (Butardo and Sreenivasulu 2016; Kennedy et al. 2003; Fukagawa and Ziska 2019). The intake of micronutrients from the whole rice consumption is particularly beneficial for children and adults who live under poverty and/or have limited access to fresh food and vegetables. There is also a growing global trend among health-conscious consumers of consuming brown or minimally processed rice (Saleh et al. 2019). Thus, nutritional fortification of rice grains through genetic improvement and cultivation practices is urgently needed (Saha and Roy 2020; Lee et al. 2019).

Several genetic and physiological parameters are known to affect nutritional contents of seeds and grains of various

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plant species (e.g., see Reuscher et al. 2016; Mahender et al. 2016; Birla et al. 2017; Julia et al. 2016; Mari et al. 2020; Karmann et al. 2018). The sources of nutrients deposited in seeds or grains are attributed to direct post-anthesis uptake from soil and remobilization of pre-anthesis nutrient storage in different plant tissues, with the relative amounts depending on the plant species, growth conditions, and types of nutrients (Zhou et al. 2018; Masclaux-Daubresse et al. 2010). As previously demonstrated, the remobilization efficiency of predeposited nutrients is linked to senescence (Sinclair and de Wit 1975; Uauy et al. 2006). Senescence programming in plants is controlled by hormones and environmental factors including nutrient availability (Guiboileau et al. 2010), and the process is believed to increase plant survival during stress conditions and seed vigor during germination (Schippers et al. 2015). During senescence, the self-digestion process termed autophagy releases nutrients from macromolecules and organelles before the nutrients are translocated to develop tissues such as young leaves, stems, and/or maturing seeds (Liu et al. 2008; Avila-Ospina et al. 2014; Chen et al. 2019). In *Arabidopsis thaliana*, 80% of nitrogen (N) and over 50% of iron (Fe), copper (Cu) and zinc (Zn) are removed from senescing leaves (Himelblau and Amasino 2001). An *A. thaliana* mutant that is impaired in autophagy showed reduced efficiency in the remobilization of essential metals such as Fe and Zn and accumulated less Fe in the seeds (Pottier et al. 2019). Nutrient remobilization has also been observed during leaf senescence in other crop plant species including sugarcane, barley, and wheat, and in tree species (Gregersen et al. 2008; Distelfeld et al. 2014; Martins de Souza et al. 2016; Maillard et al. 2015). Remobilization efficiency of macronutrients, such as N, phosphorus (P), and potassium (K) is usually high, whereas micronutrients are typically more difficult to translocate (White 2012; Have et al. 2017; Maillard et al. 2015; Hill 1980). The actual remobilization efficiency and remobilization rate of the nutrients depend on the plant species and growth conditions (Maillard et al. 2015; Billard et al. 2016; Etienne et al. 2018; Parveen et al. 2018). For instance, comparing eighteen accessions of *A. thaliana*, Masclaux-Daubresse et al. (2010) observed sixfold differences for N remobilization between the lowest and highest performing accessions.

In rice, the nutrient remobilization from senescing rice leaves during the post-anthesis stages and their contribution to the rice grain nutrition have been examined quite extensively for N, but to a limited extent for the other elements. In a newly developing rice leaf, approximately half of N accumulated comes from remobilized N from internal organs. In comparison, 70–90% of N in rice grain is contributed from the N pool deposited in the vegetative tissues prior to the reproductive stage, whereas the remaining depends on the post-anthesis soil uptake (Yoneyama et al. 2016; Mae 2010). The major forms of amino acids released from senescing leaves are glutamine and asparagine. A rice mutant impaired in the ferredoxin-dependent glutamate synthase, which has been suggested to participate in the N reassimilation during the N remobilization process, showed premature leaf senescence and reduced seed setting (Zeng et al. 2017). Even though P is considered a mobile element, only 20% of P in rice grains is attributable to the remobilized P from vegetative tissues and a large amount of grain P has to be supported by the post-anthesis uptake from the soil (Julia et al. 2016). Similarly, rice plants are not naturally known to be an efficient remobilizer of Fe and Zn. The major sources of Fe and Zn deposited in rice grains are attributed to the direct root uptake, with minor contributions from the stem and senescing leaves when the rice plants are under low Zn availability (Sperotto 2013). Future efforts to increase the remobilization efficiency of these valuable nutrients, particularly in the commercial rice cultivars, should help toward the nutrient fortification of rice grains and cut down on fertilizer requirements. To achieve this, more studies are needed to understand trait diversity among the rice lines/varieties and identify efficient remobilizers as well as the genes involved.

In this study, we examined the nutrient remobilization and senescence progression in ten representative rice varieties at different stages after flowering. The N, P, and K contents in flag leaves were used to estimate the nutrient remobilization rates and efficiencies based on the linear regression model. We also investigated for any relationships between the nutrient remobilization characteristics and the rate of senescence progression as shown by the leaf greenness reduction. Furthermore, the correlations between the nutrient remobilization characteristics and the grain nutrient contents were examined.

Materials and methods

Plant materials

Seeds of the rice varieties (Table 1) were kindly provided by the Division of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives, Thailand. The rice plants from each variety were sowed in 6 × 26 rows at 25 cm distance during the rainy season of August 2016, in an irrigated, lowland paddy field at the Suphan Buri Rice Research Center, Suphan Buri province, Thailand (14° 28′ 44″ N, 100° 5′ 10″ E). A 16-20-0 (N–P–K) fertilizer was applied at approximately 30–40 kg ha^{-1} when the seedlings were 20 days old, followed by 46-0-0 urea fertilization at approximately 15–20 kg ha^{-1} at 40 days after sowing and 8–15 kg ha^{-1} at 60 days after sowing. Samples of flag leaves from the main culm of the rice plants in the inner rows were collected at 0, 7, 14, 21 and 28 days after flowering (DAF). The flowering stage, 0 DAF, was defined

Table 1 Greenness reduction efficiecy in flag leaves of rice varieties used in the study

Varieties	GS. No.[a]	Origin	Greenness reduction efficiency (%)[b]
Bora Dhan	2333	India	40.8
Hah Ruang	19,859	Thailand	4.5
Hawm Chonlasit	n/a	Thailand	21.2
Khao Gaw Diaw 35	n/a	Thailand	−9.5
Khao Tah-mon	3679	Thailand	21.2
Mae Lahd	15,969	Thailand	32.6
Sai Yud	19,853	Thailand	46.8
Suphan Buri 1	n/a	Thailand	44.1
Suphan Buri 60	16,240	Thailand	28.7
Suphan Buri 90	19,869	Thailand	24.7
Mean			25.5
SD			17.6

[a]Thailand's rice germplasm database, the National Rice Seed Storage Laboratory for Genetic Resources, Pathum Thani Rice Research Center, Rice Department, Thailand (http://122.154.30.177/)

[b]Estimated from a linear regression model

as the time of approximately 50% anther emergence. Three leaves were combined as one replicate, and three replicates from each rice variety were used in the nutrient analysis. The leaf samples were dried in an oven at 65 °C until a constant dry weight was obtained. Then, the dried leaves were cut into small pieces.

Grains were harvested from each rice plant at 28 DAF. For the nutrient analysis, the whole grain samples, with husk, from three replicate plants were used. The grains were dried in a 65 °C oven until completely dry and then ground in a blender into fine powder.

Analysis of N content

The total N content was determined using the Kjeldahl method. A weighted amount of approximately 0.2 g dried samples was digested in 3 ml H_2SO_4 in a Kjeldahl flask and heated over a flame. After the sample was clear and had cooled down, 10 ml of distilled water was added. Following the addition of 10 ml NaOH, 40% (*w*/*v*), the ammonia vapor was distilled into 10 ml boric acid, 4% (*w*/*v*), containing a pH indicator and titrated against 0.1 M HCl.

Analysis of P content

The total P content was determined according to Johnson and Ulrich (1959) based on the Vanado–Molybdate method. Approximately 0.4 g dried leaf or 0.2 g grain powder samples were digested in 10 ml HNO_3-H_2SO_4-$HClO_4$ (9: 4: 1) acid mixture. After boiling for at least 2 h until the samples became clear, 1 ml of the digested samples was mixed with 2 ml of 2 N HNO_3 and diluted to 8 ml with distilled water. The samples or the standards were mixed with 1 ml of the molybdate–vanadate reagent and then diluted to 10 ml with distilled water. The yellow molybdate phosphoric acid was measured using a GENESYS 10S UV–Vis Spectrophotometer at 440 nm wavelength.

Analysis of K content

Approximately, 0.4 g dried leaf or 0.2 g grain powder samples were digested in 10 ml HNO_3-H_2SO_4-$HClO_4$ (9: 4: 1) acid mixture according to Johnson and Ulrich (1959). The K concentrations in the digested samples and the standards were measured using a microwave plasma-atomic emission spectrometer (4100 MP-AES, Agilent Technologies).

Leaf greenness analysis

Greenness coloration of rice leaves was measured using a chlorophyll meter (SPAD-502, Minolta, Japan) at the middle portion of a flag leaf. The means of three rice plants were used to calculate the greenness reduction efficiency.

Data and statistical analysis

The data were fitted to a linear regression model using Excel, version 16.16.17 (191,208) software (Microsoft, USA). Based on the linear modeling, rates of nutrient remobilization were estimated using the slope of the linear models. Efficiencies of greenness reduction and nutrient remobilization were calculated using the estimated initial (0 DAF) and estimated final (28 DAF) values according to the linear regression models, as follows:

$$\text{Greenness reduction efficiency (\%)} = \frac{\text{Estimated initial greenness} - \text{Estimated final greenness}}{\text{Estimated initial greenness}} \times 100,$$

$$\text{Nutrient remobilzation efficiency (\%)} = \frac{\text{Estimated initial content} - \text{Estimated final content}}{\text{Estimated initial content}} \times 100.$$

Correlations between parameters were examined based on the Pearson's correlation analysis using SPSS Version 22 (SPSS Inc., USA).

Results

Senescence characteristics of rice varieties

Reduction in leaf greenness is a common indication of senescence. To assess the reduction of leaf greenness, the greenness levels of flag leaves from 0 to 28 DAF were recorded and fitted to a linear regression model (Fig. 1). The greenness reduction rates and efficiencies according to the linear models were calculated. Because some of the rice varieties exhibited patterns of the leaf greenness reduction that did not fit a linear model, only the green reduction efficiencies, not the rates, are presented in Table 1. Among the ten rice varieties compared, Khao Gaw Diaw 35 did not show any reduction in leaf greenness at 28 DAF. In contrast, Bora Dhan, Sai Yud, and Suphan Buri 1 showed more than 40% greenness reduction efficiencies. These data demonstrate that the rice plants grown under the field condition used in the experiment were able to undergo the post-anthesis leaf senescence and the rice genetic backgrounds used were able to display differences in the leaf senescence patterns.

N, P, and K contents in flag leaves of rice varieties

To assess the levels of nutrient remobilization of the rice varieties, the N, P, and K contents in flag leaves were measured at different stages after flowering. The N contents (Fig. 2) were found to progressively decline from 0 to 28 DAF. In contrast, the amounts of P (Fig. 3) and K (Fig. 4) decreased much less. To estimate the nutrient remobilization rates and nutrient remobilization efficiency of N, P, and K through the course of flower development, the N, P, and K contents of the flag leaves were fitted using a linear regression model. Based on the models, the nutrient remobilization rates and efficiencies were calculated (Table 2). On average, N, P, and K remobilization efficiencies in the flag leaves of the ten rice varieties were 50%, 27%, and 22% respectively. These data indicate that N was more efficiently remobilized from the leaves in comparison to P and K, and the rice varieties differed in their nutrient remobilization characteristics.

Relationship between nutrient remobilization and leaf greenness

To test the hypothesis that rice varieties that are more active in leaf senescence are more efficient in nutrient remobilization, we examined the correlations between these parameters. The results showed no significant correlation between the leaf greenness reduction efficiency and the nutrient remobilization rates or efficiencies of N, P, and K among the rice varieties (Fig. 5).

Nutrient content in grains and relationship with leaf nutrient remobilization characteristics

The nutrient contents in grains at the harvest stage (28 DAF) were measured. On average, the grain N, P, and K contents were 20.6, 11.0 and 6.6 g kg^{-1}, respectively (Table 2). Differences in the grain nutrient contents among the rice varieties could be observed. To test the hypothesis that rice varieties with high nutrient remobilization rate or efficiency can accumulate more nutrients in the grains, we examined the correlations between these parameters. Because rice leaves with a higher initial nutrient content may hypothetically provide a larger sink for the grain nutrient deposition, we also examined the correlation between the grain nutrients with the initial N, P, and K content in the flag leaves. However, the results showed no significant positive or negative correlations between these parameters (Table 3). Therefore, we found no evidence to support these hypotheses.

Discussion

In this study, we measured the concentrations of N, P, and K in flag leaves and grains of ten representative rice varieties at different post-anthesis stages to assess the levels of nutrient remobilization during the leaf senescence process and investigated the relationships with the level of nutrients deposited in the grains and the stay-green characteristics of the rice varieties. Five of the rice varieties used were traditional Thai lowland cultivars for which limited information is available on their origins namely Hah Ruang, Khao Gaw Diaw 35, Khao Tah-mon, Mae Lahd, and Sai Yud. Hawm Chonlasit is a modern lowland rice variety that was developed through cross-breeding between IR57514-PMI-5-B-1-2 and the Thai jasmine rice Khao Dawk Mali 105 and subjected to marker-assisted selection for the *SUB1* gene that confers a submergence tolerance trait (Department of Agriculture 2020).

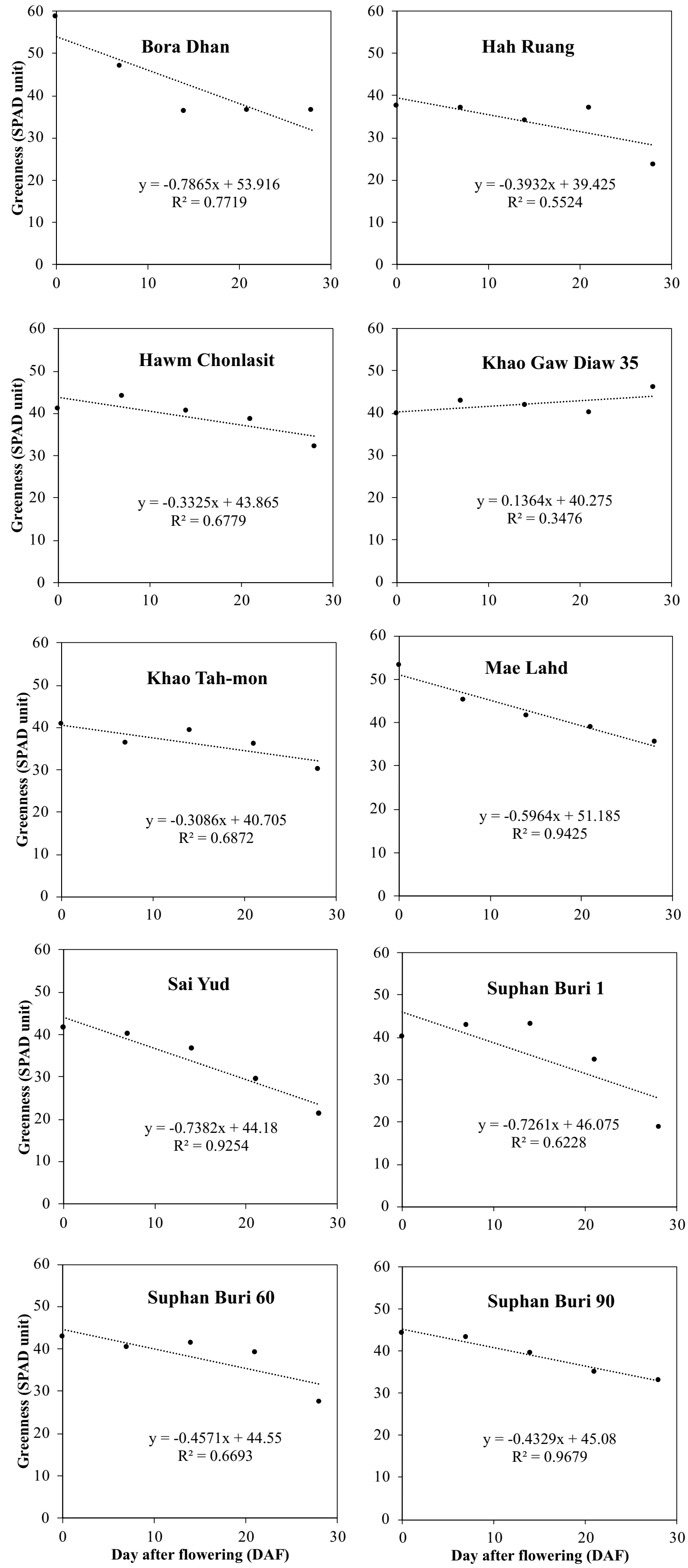
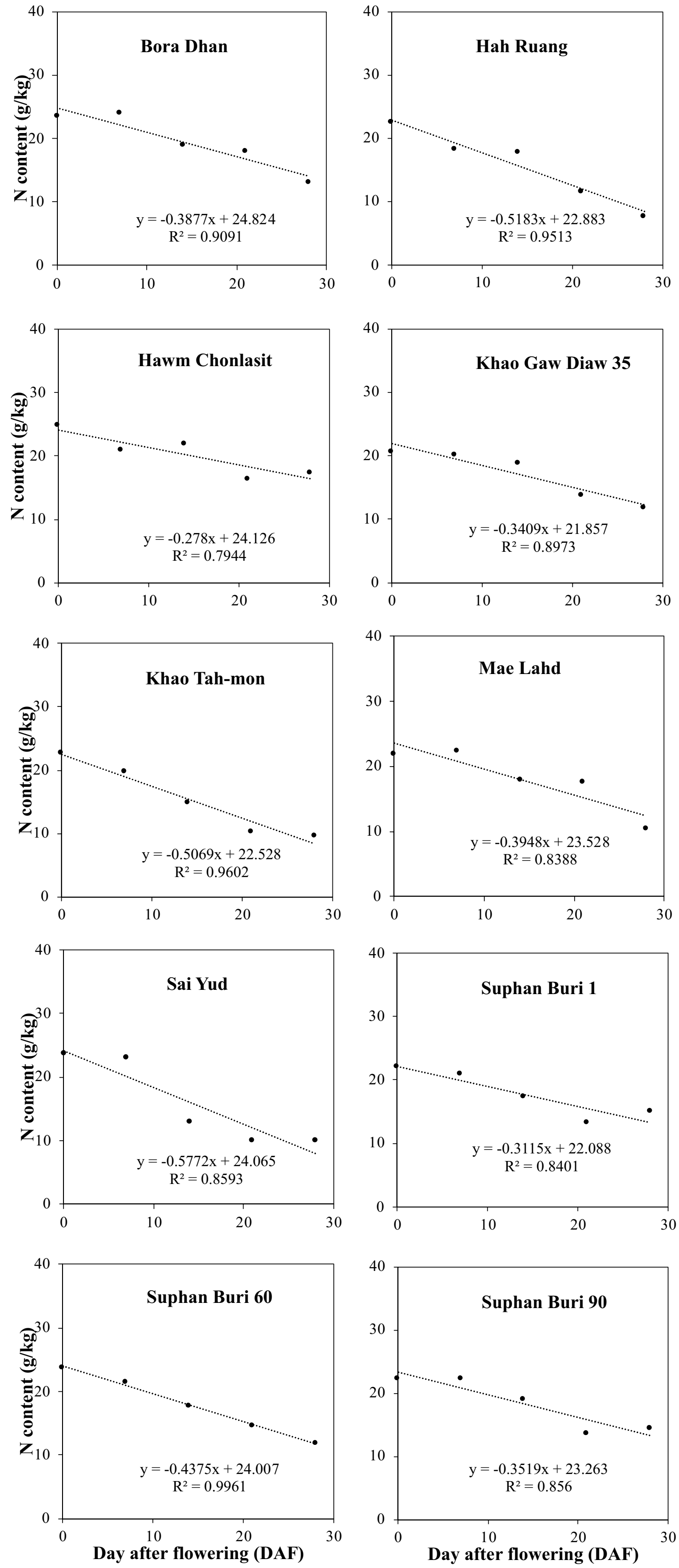


Fig. 1 Linear regression modeling of greenness colorations in flag leaves of rice varieties at various days after flowering

Fig. 2 Linear regression modeling of N contents removed from flag leaves of rice varieties at various days after flowering



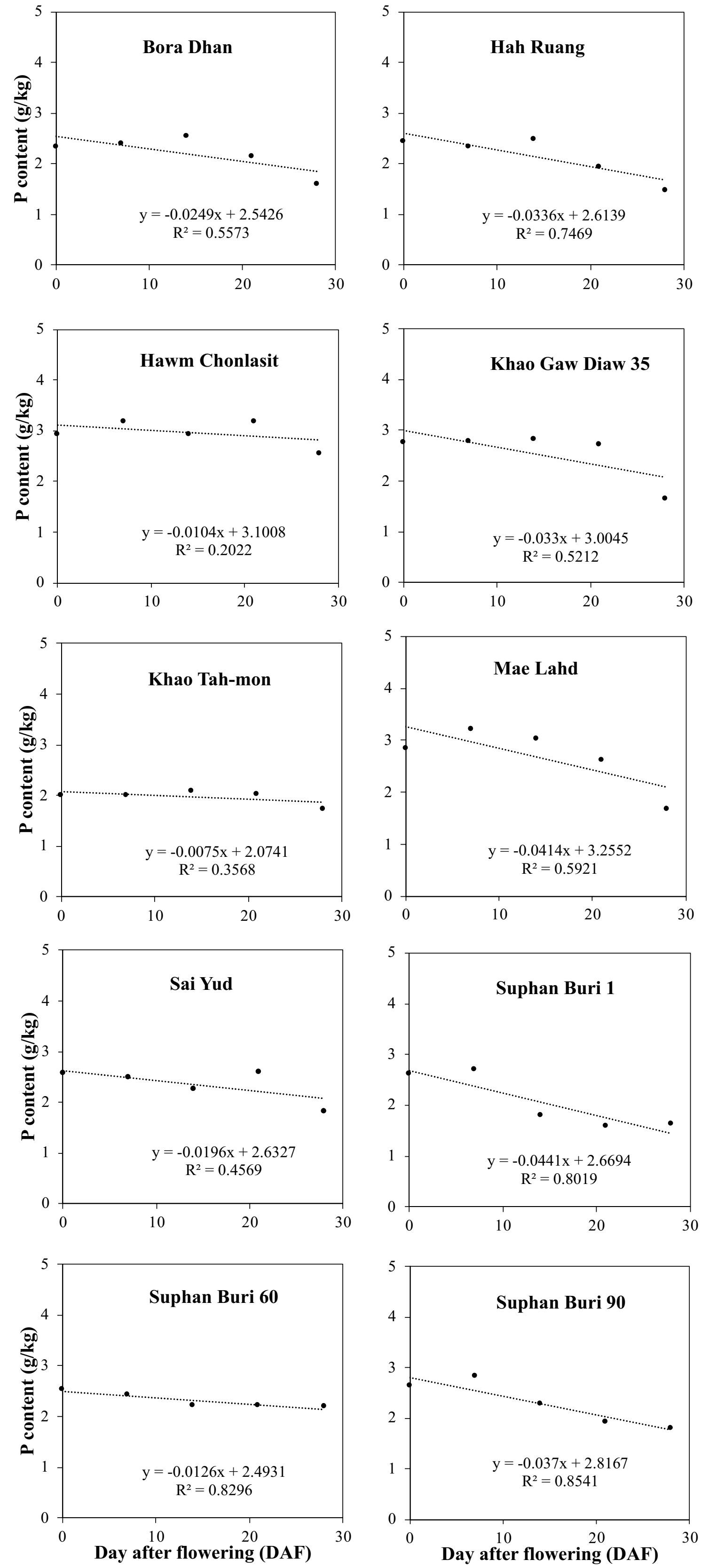


Fig. 3 Linear regression modeling of P contents removed from flag leaves of rice varieties at various days after flowering

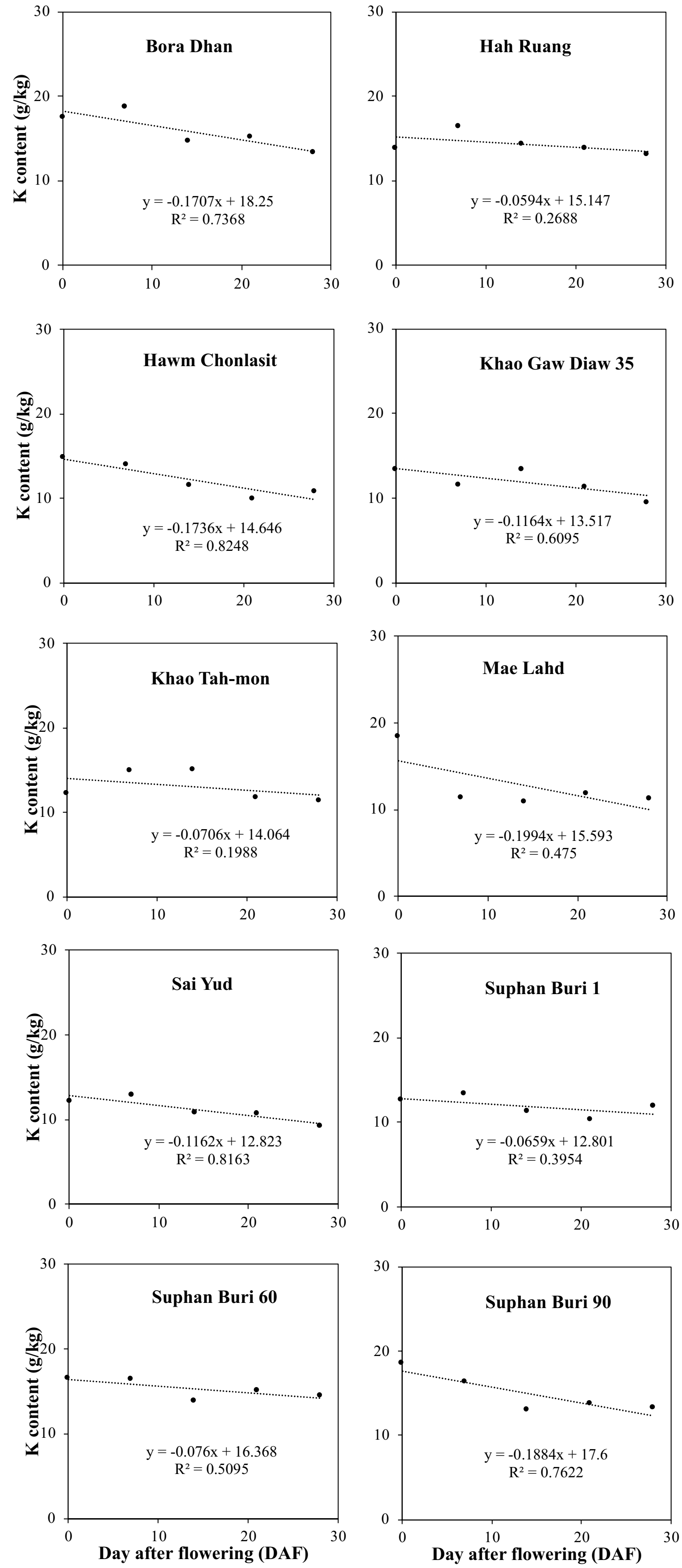


Fig. 4 Linear regression modeling of K removed from flag leaves of rice varieties at various days after flowering

Table 2 Rate of nutrient remobilization and nutrient remobilization efficiecy in flag leaves and nutrient content in grains of rice varieties

Varieties	Rate of nutrient remobilization (g kg^{-1} d.w. d^{-1})[a]			Nutrient remobilization efficiency (%)[a]			Nutrient content (g kg^{-1})		
	N	P	K	N	P	K	N	P	K
Bora Dhan	0.388	0.025	0.171	43.7	27.4	26.2	19.4	11.6	8.2
Hah Ruang	0.518	0.034	0.031	63.4	36	5.7	18	10.2	5.5
Hawm Chonlasit	0.278	0.01	0.174	32.3	9.4	33.2	18.3	9	7.3
Khao Gaw Diaw 35	0.341	0.033	0.116	43.7	30.8	24.1	20.9	11.7	7.9
Khao Tah-mon	0.507	0.008	0.071	63	10.1	14.1	27.2	11.6	6.5
Mae Lahd	0.395	0.041	0.199	47	35.6	35.8	19.3	11.3	6
Sai Yud	0.577	0.02	0.116	67.2	20.8	25.4	18.6	11.6	6.2
Suphan Buri 1	0.312	0.044	0.066	46.8	46.3	14.4	20	13.2	6
Suphan Buri 60	0.438	0.013	0.076	51	14.2	13	20.2	9.5	6.6
Suphan Buri 90	0.352	0.037	0.188	42.4	36.8	30	24.6	10	5.5
Mean	0.411	0.027	0.121	50.1	26.7	22.2	20.6	11	6.6
SD	0.098	0.013	0.059	11.1	12.6	9.9	2.3	1.3	0.9

[a]Estimated from a linear regression model

Suphan Buri 1, Suphan Buri 60, Suphan Buri 90 (Division of Rice Research and Development 2016) are high-yield, lowland modern rice varieties that were developed by the Rice Department, Thailand. Bora Dhan is a traditional rice variety that originated from the northeastern region of India (Sathish et al. 2020). The rice varieties were chosen predominantly based on the preliminary study that compared the senescence characteristics among Thai rice cultivars. The rice varieties were selected to cover a broad range of genetic backgrounds and senescence characteristics. In addition, we intended to include both the traditional and modern varieties. This is firstly because the modern varieties have been bred for higher grain yields which may subjectively affect the senescence and nutrient remobilization characteristics, and secondly because of their economic importance. Bora Dhan, an Indian rice variety, was included to assess the extent of disparity between the Thai rice and a variety from a diverged origin.

The rice varieties in this study were grown at the same time under irrigated, lowland conditions at the field experimental station, Suphan Buri Rice Research Center. Because the rice varieties flowered at different times depending on their photoperiod sensitivity, the irrigation water level was maintained throughout the grain development period. Even though we showed that the senescence process occurred during the grain development, it remained unclear whether the senescence process would have progressed to a greater extent had the water been removed from the field following the flowering stage as is typically practiced in the lowland rice cultivation. We indeed observed that the rice leaves remained slightly more green than usual, suggesting that the prolonged irrigation condition might partially impede the senescence progression. Nevertheless, the rice varieties in our study exhibited distinct senescence progression rates and patterns. As noted previously, in order to quantitatively compare the rice varieties, we calculated the leaf greenness reduction rates and efficiencies as the indicators of senescence progression. However, as shown in Fig. 1, the reduction of leaf greenness in some of the rice varieties did not strictly follow a linear model. Since the declines in leaf greenness were not necessarily linear, we decided not to present the senescence characteristics as the rates of greenness reduction. Thus, we only showed the leaf greenness reduction efficiencies. In addition, it should be noted that the measurement of leaf greenness might not accurately reflect the senescence progression in the rice varieties that exhibit a cosmetic stay-green trait (Thomas and Howarth 2000). Cosmetic stay-green plant varieties are usually deficient in their breakdown of chlorophyll pigments and thus remain green despite the decline in photosynthesis ability. Our results showed that Khao Gaw Diaw 35 and Hah Ruang showed little or no reduction in the leaf greenness at 28 DAF, thus these varieties may be considered to exhibit a stay-green trait. Further studies are needed to verify whether they are functional stay-green varieties. So far, a small number of traditional rice varieties or landraces with a stay-green trait have been reported (Fu et al. 2009; Hoang and Kobata 2009; Sperotto 2013; Shokri et al. 2009; Chen et al. 2008). Even though the beneficial effects of the stay-green trait on grain yield or stress tolerance have been demonstrated in several crop plants, the associations between these traits in rice remain to be examined using a larger number of rice varieties. If proven to be beneficial, more stay-green rice varieties with different geographic origins and genetic backgrounds, such as those identified in this study, would be useful for crop improvement through cross-breeding with the commercial rice varieties.

Table 3 Relationship between nutrient contents in grain and leaf nutrient remobilization characteristics

Nutrient	Coefficient of correlation (*P* value)		
	Initial content at 0 DAF	Remobilization rate	Remobilization efficiency
N	−0.280 (0.437)	0.061 (0.867)	0.122 (0.736)
P	−0.501 (0.143)	−0.203 (0.584)	−0.180 (0.620)
K	0.105 (0.774)	0.080 (0.826)	−0.092 (0.803)

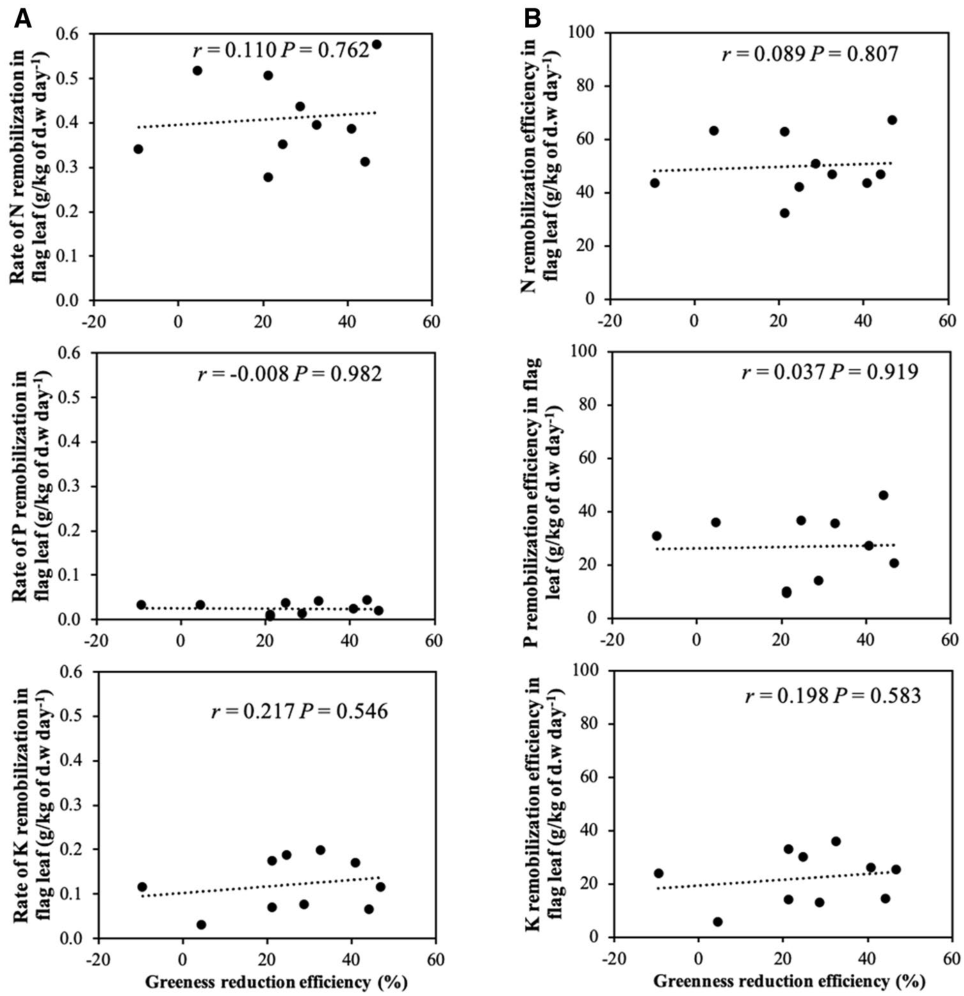


Fig. 5 Correlation between greenness reduction efficiency with **A** rate of nutrient remobilization and **B** nutrient remobilization efficiencies of flag leaves among the ten rice varieties

At present, the nutrient remobilization characteristics from senescing rice leaves during the post-anthesis stages have been examined to some extent (Yoneyama et al. 2016; Sperotto et al. 2012; Jeong et al. 2017; Wu et al. 2010), but little has been done to compare the profiles among a large number of rice varieties. We presented here the nutrient remobilization characteristics in terms of remobilization rates and efficiencies of N, P, and K from flag leaves of the ten rice varieties at 0, 7, 21, 28 DAF. The rates and efficiencies were estimated using linear regression modeling. As mentioned previously, we attempted to fit the data in a linear model because we aimed for the quantitative comparison between the rice varieties. The rate of nutrient remobilization reflects the amount of nutrient that disappeared from the leaf per dry weight per day. In contrast, the efficiency of nutrient remobilization was calculated as the amount of the nutrient removed at 28 DAF in proportion to the amount of each nutrient initially available at 0 DAF. The latter calculation factored in the initial amounts of nutrients deposited in the leaves which substantially differed among the rice varieties. Thus, although the rates and efficiencies often reflect each other, they may offer different views in some rice varieties. In the case of N, the linear regression model fitted well ($R^2 \geq 0.9$) in five of the ten rice varieties. The linear models fitted more poorly when applied to the P and K remobilization. The poor fitness could partially be explained by the delayed onset of P and K remobilization which began sometime after the anthesis stage, as shown by the data. In the cases of the poor fitness to a linear model, the rates of nutrient remobilization should be interpreted with caution and the nutrient remobilization efficiencies may be more useful for comparing the rice varieties.

We found that N was remobilized with the highest rate and efficiency compared to P and K. High N remobilization efficiency has been shown in other plant species, as N is generally a mobile element (Have et al. 2017). In wheat and barley, more than 80% of N is remobilized from senescing leaves (Maillard et al. 2015). The N remobilization in rice, approximately 50% as shown in our study, is therefore much lower than wheat and barley, but still greater than maize, in which only 40% of N is remobilized (Maillard et al. 2015). Our finding that the average P remobilization rate and efficiency were low concurred with the conclusion of Julia et al. (2016). The authors found that the primary source for P for the panicle partitioning in the rice variety IR64 was attributed to the exogenous P uptake through the roots, whereas the P remobilization from vegetative tissues contributed only 20% of the panicle P content. Nevertheless, in their study, more than half of the P content in flag leaves of field-grown rice was reduced at 30 DAF which was beyond the final time point of our data collection. In addition, the difference between ours and that of Julia et al. (2016) may be attributed to the genetic background of the rice varieties and the growth condition. For example, among the rice varieties tested in our study, the P remobilization efficiencies ranged from the highest at 46.3% for Suphan Buri 1 to the lowest at 9.4% for Hawm Chonlasit. The P remobilization efficiencies in our study were more similar to the 20.7% P remobilization from the rice leaves grown under an average N condition at 35 DAF compared to 7 DAF reported by Wang et al. (2018). In the study by Wang et al. (2018), the average K remobilization efficiency from rice leaves was 10.2% which was in the range observed in our study. These findings together suggest that rice plants are a poor nutrient remobilizer. According to Maillard et al. (2015), in which wheat and barley were classified in the first group of efficient nutrient remobilizers and plant species, such as *Brassica napus*, *Pisum sativum*, and *Quercus robur* belong to the second group of intermediate remobilization efficiency, rice may be grouped together with maize in the third group with the lowest efficiency. As rice grains are one of the most important staple crops, immediate attention is needed to improve their nutrient remobilization efficiency.

It could be hypothesized that the delayed or slow leaf senescence process allows complete transport of nutrients out of the rice leaves; on the contrary, it could be possible that a more active senescence progression results in rapid self-degradation leading to a more efficient release of nutrients of the plant cells. In either case, our results showed no significant correlation between the leaf greenness reduction efficiency and the nutrient remobilization efficiency among the rice varieties tested. The insignificant correlation may be caused by the small number of varieties included in this study, or by other unknown interfering parameters. It remains possible that the stay-green trait based on the leaf greenness measurement is not a good indication of the functional stay-green trait, as discussed above. The linear regression of greenness reduction efficiency used in the analysis which did not fit well with the leaf greenness profiles of many rice varieties should also be reinvestigated using a different model and/or parameter.

Because there had only been a few lines of evidence demonstrating the significance in contribution of the nutrient remobilization from rice leaves to the nutrient accumulation in rice grains, we tested the hypothesis that rice varieties with high nutrient remobilization efficiency showed a high nutrient grain content. The results showed no significant positive correlation between the nutrient remobilization efficiency/rate of flag leaves and the grain nutrient efficiency for N, P, and K. Because it was also possible that the grain nutrient contents were influenced by the amounts of nutrients initially stored in the rice leaves before senescence, we additionally examined the correlation with this parameter. However, we did not find a significant correlation, except a negative correlation between the initial N content and the grain N content—which did not support our hypothesis. It should be noted that this study did not factor in the amount of leaves available per plant—for example, a rice variety with a high nutrient remobilization rate but a small number of leaves may not be able to contribute significantly to the nutrient accumulation in developing grains. Similarly, the number of seeds per plant was not quantified—thus, the size of the sink tissue was not known. Nevertheless, the lack of association between these parameters could be explained by our and others' previous findings that rice leaves were not highly efficient in the nutrient remobilization, thus the senescing leaves might not be a major source of nutrients for the grain deposition. The conclusion was also supported by a study that showed flag or second leaf removal did not alter Fe and Zn concentrations or contents in the rice's mature seeds (Sperotto et al. 2009). In addition, Yilmaz et al. (2017) showed that the grain Zn concentration did not correlate with Zn uptake and its translocation in wheat cultivars. Further investigation may be needed using different techniques and under different growth conditions. An investigation using closely related rice genetic backgrounds with contrasting nutrient remobilization rates/efficiencies may also yield a clearer result. A genetic improvement to increase essential nutrient contents in the rice grains should be highly beneficial to consumers around the world.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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