

The Role of Assimilate Dynamic in Rice Grain Filling Under Two Nitrogen Conditions

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Abstract

The assimilate obtained during the grain filling phase is sourced from photosynthesis and Non Structural Carbohydrate (NSC) reserves produced during the vegetative phase in the vegetative organs. Assimilate from different sources plays different roles in the grain filling process. This study aims to investigate varietal difference on assimilate distribution, nitrogen response and yield performance. The experiment was carried out in the rice fields of Petapahan Village, Kampar Regency, Riau. The experimental design used was a split-plot in randomized complete block design (RCBD) with 2 factors and 3 groups. The first factor, as the main plot, was the condition of N. Specifically without N (NoN) (0 kg ha⁻¹ urea) and treatment with normal N (NN) (250 kg ha⁻¹ urea). The second factor, as a sub-plot, was rice variety, specifically Hipa 18, IPB 3S, Ciherang, and Way Apo Buru. Hipa 18 was a variety having the largest reserves of NSC. A positive correlation was found between plant characters observed in this research, they are stem and leaf dry weight, total carbohydrates in stems (proximate analysis), photosynthetic rate, and leaf nitrogen content (Kjeldahl method). As well as other agronomic characters such as leaf area index and yield components. The highest dry weight of stems and leaves of Hipa 18 was at the heading phase, 22.6 g. While the highest carbohydrate content of the stems of the variety was 7.93 g in the same phase. This large NSC reserve supported the largest sinks as well at Hipa 18. However, the source capacity of the varieties used was found to be lower than the sink capacity. It was also known that nitrogen affected the condition of all plant characters observed in this study. Therefore optimal nitrogen fertilization is needed to get a maximum yield.

Keywords: Dry weight, Yield, Carbohydrate, Non structural carbohydrate, Variety

Introduction

The problem of conversion and land use competition has changed the pattern of increasing rice yields, which no longer relies on expanding agricultural land on but through increasing productivity. It is possible to increase rice productivity by carrying out genetic and environmental engineering. But we cannot do both without understanding the physiological characteristics of the plant. Therefore, things related to its physiological properties must be understood first. One of them is the characteristics of grain filling as grains are the main economic product of the rice plant.

Longer duration is an important factor in effective grain filling [1]. Based on this, the grain filling phase is very important for varieties cultivated in Indonesia as a tropical country. In addition to the shorter duration, high temperatures cause the decline of light use efficiency [2]. This results in lower yields (up to more than 50 %) in rice crops in the tropics compared to subtropics [2]. The grain filling phase is closely related to the relationship between the source and sink of rice plants [3]. In plant physiology, organs producing organic molecules are called sources, while organs that store and use them are called sinks. A simple example of an organ acting as a source is a leaf, and an organ acting as a sink is a grain [4]. The capacity of the source and sink in each plant varies depending on the character of each. Low sink strength is the cause of low grain filling in rice [5]. The more synchronous the relationship between the 2, the more effective the production of a plant will be [3].

The assimilate needed in the grain filling process comes from photosynthesis after heading (50 % flowering) and remobilization of assimilate from plant vegetative parts called Non-Structural Carbohydrate (NSC) that have been stored before heading. High photosynthetic rate after heading is very important in

determining crop yield. Photosynthate after heading contributes 74 % to the yield, while the rest comes from NSC remobilization [6]. NSC metabolism is the basis for plant growth and development, even bringing different strategies to deal with environmental stress [7]. High NSC accumulation in the vegetative part does not always indicate high translocation in grain filling. Besides the genetic factor, the contribution of assimilate and photosynthetic remobilization after heading is determined by environmental factors including N status [8].

Nitrogen (N) is a very important element for plants. Its presence greatly affects the growth and yield of plants. The use of appropriate nitrogen fertilizers, both in bare-dry cultivation and flooding cultivation, leads to high quantity and quality of the grain [9]. In addition, fertilizers can also increase NSC reserves stored in stems [10]. Added that N fertilization may reduce the percentage of empty grain [11].

Genetics, environment, and their interactions are 3 factors affecting the yield performance of plants [12]. As genetically different, rice varieties showed different physical appearance as well as the yield potential [13]. Therefore, it is necessary to understand the cause of the difference. What needs to be investigated is whether there are differences in the source and sink characteristics of each rice variety and what is the role of nitrogen in these differences. This study aims to investigate varietal difference on assimilate distribution, nitrogen response and yield performance. The novelty of this research is that the pattern of carbohydrates in the stem during grain filling can be seen clearly, then the source and sink capacities of each variety can be known. Meanwhile, the research gap in this study is that the factors that influence the translocation of assimilate to seeds are not yet known.

Materials and methods

Experimental site and design

The research was conducted in the rice fields in Petapahan Village, Kampar Regency, Riau Province, Indonesia. Soil analysis was carried out using soil samples taken with a depth of 0 - 30 cm. The results of the analysis showed that the land used was very acidic (pH 4.29), with high C-Organic (3.16 %), moderate nitrogen (0.21 %), very high phosphate (188.8 mg/100 g), very low potassium (5 mg/100 g), very low calcium and magnesium (0.43 and 0.14 cmol/kg) and moderate cation exchange capacity (20.13 cmol/kg). Field experiments were carried out from September to December 2020. During the experiment, average temperature was 26.88 °C with humidity of 83.71 %, and rainfall of 242.15 mm/month.

The experimental design used was a Split Plot in a Randomized Complete Block Design with 2 factors and 3 groups (total 24 experimental units), planting space was 4×4 m² in each experimental plot. The first factor as the main plot was nitrogen (N) treatment, which consisted of treatment without application of nitrogen fertilizer (NoN) (0 kg ha⁻¹ urea) and application of normal nitrogen fertilizer (NN) (250 kg ha⁻¹ urea), determined based on the regulation of the Indonesian minister of agriculture regarding recommendations for N, P, and K fertilization in site-specific lowland rice. In urea fertilization, NN treatment was given in 3 stages, with quantities of 75, 100 and 75 kg ha⁻¹ urea at 10, 30 and 45 day after planting (DAP). The second factor as a sub-plot was variety, namely Hipa 18, IPB 3S, Ciherang, and Way Apo Buru. The 4 varieties were selected to represent the types of rice which are hybrid rice (Hipa 18), new high yielding varieties (Ciherang and Way Apo Buru), and new types of rice (IPB 3S).

Table 1 Agronomic characteristics of each varieties.

Varieties	Growth period (days)	Plant height (cm)	Weight of 1,000 grains (g)	Average yield (ton ha ⁻¹ MDG)	Potensial yield (ton ha ⁻¹ MDG)
Hipa 18	113	103.5	24.9	7.8	10.3
IPB 3S	112	118	28	7	11.2
Ciherang	116 to 125	91 to 106	27 to 28	5 to 7	7
Way Apo Buru	115	105 to 113	27 to 28	5 to 8	8

Source: Indonesian agency for agricultural research and development. MDG = Milled Dry Grain

Establishment and management

Basic fertilization (fertilizer applied at the beginning of planting) was not used in this study. Fertilization was done after sowing. Grains that had been sown for 15 - 18 days were then planted with a 2:1 *jajar legowo* system with planting space of $25 \times 12.5 \times 50$ cm³, with details: 25 cm was the distance between rows, 12.5 was the distance in rows, and 50 cm was the distance between 2 rows of plants. Three grains were planted in each hole. P fertilization was given in the amount of 80 kg TSP ha⁻¹ at 10 DAP, while K was given in 2 stages, namely 50 kg KCl ha⁻¹ at 10 DAP and 50 kg KCl ha⁻¹ at 30 DAP. Mounds are made in each NN treatment plot to avoid nitrogen drift. The water level must be maintained 3 - 5 cm since the middle of tiller formation until 1 week before harvest. Weeding was done manually or mechanically at 21 and 42 DAP, while pest and disease control was done by spraying pesticides. Harvesting was done when the plant reached physiologically mature, indicated by 90 - 95 % of the grain had turned yellow or the moisture content of the grain ranged 22 - 27 %.

Plant growth and yield component measurements

Observations were made in 4 general phases, namely panicle initiation (PI), heading (H), heading + 20 days (H20), and harvesting (Hv). The variables observed were plant height at harvest (PH-Hv) (cm), leaf and stem dry weight (LSDW) (g), N content of leaves (NC) (%), leaf area index (LAI), photosynthesis (Pn) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), total carbohydrate of stems (TCS) (g), source and sink sizes. LSDW and NC (using the Kjeldahl method [43]) were observed in all phases, while Pn was observed in all phases except Hv using the LI-6400XT portable photosynthesis system. LAI and TCS (using proximat analysis) were observed in all phases except PI phase. Observations of yield components carried out after harvest were the number of panicles (NOP), weight of 100 grains (g), number of grain per plant (NOG), weight of grain per plant (WOG) (g), and percentage of empty grain per plant (EG) (%).

Statistical analysis

Data analysis was performed using R software 4.0.0. ANOVA test was performed with a level of 5 %. If there was a different effect between treatments, then the test was continued with DMRT (Duncan Multiple Range Test) at a 95 % confidence level. In addition, a correlation test between several observations was also carried out. Correlation analysis was carried out with the Pearson multiple correlation test.

Results and discussion

Dry matter of leaves and stems and total carbohydrate of stems

The results of the study showed that the total carbohydrate content of stems in the NN treatment was greater than in the NoN (**Figure 1(c)**), so the same thing happened to the dry weight of stems and leaves (**Figure 1(a)**) in each phase. Dry matter refers to all organic matter produced through photosynthetic activity and protein metabolism [14] that will be stored in the form of NSC in vegetative organs. Most of these NSCs are stored in the stem and will be remobilized to grains after entering the grain filling phase [7]. The dynamics of dry weight of stems, leaves, and total carbohydrates of stems can represent changes in NSC that occur in each phase. In this case, nitrogen plays a role in increasing NSC stored in the stem [10]. The high content of NSC in the stems will increase the content of zeatin and zeatin riboside and reduce the rate of evolution of ethylene in grains. This will cause the sink strength to increase in the inferior grains [10]. A strong sink will increase grain accumulation and yield [15]. Meanwhile, a low sink will reduce the absorption of NSC from the stem [5].

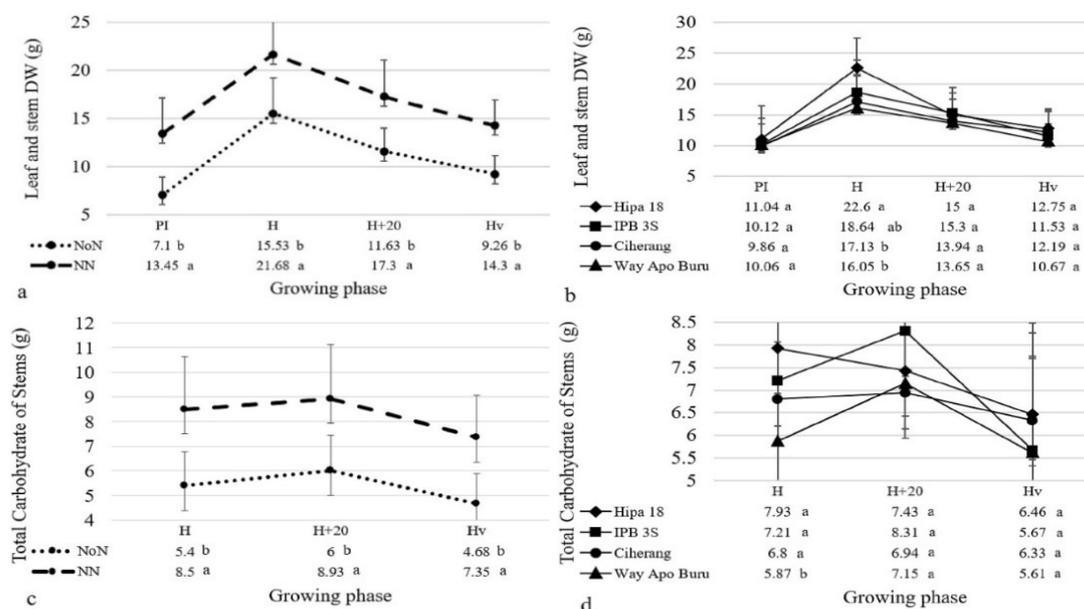


Figure 1 Dry weight of stems and leaves graph under N treatment (a) and 4 different varieties (b), Total stem carbohydrate graph under N treatment (c) and 4 different varieties (d). Numbers followed by the same letter in the same column are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, DW = dry weight, PI = panicle initiation, H = heading, H + 20 = heading + 20 days, Hv = harvest.

The increase in dry weight of stems and leaves (**Figures 1(a) - 1(b)**), and total carbohydrates in stems (**Figures 1(c) - 1(d)**) indicates an increase in stored assimilate. While the decrease indicates the remobilization of assimilate from stems and leaves to grains and the use of assimilate to build organ structures and carry out life activities, such as respiration [18]. The dynamic pattern of stem and leaf dry weight of Hipa 18 was slightly different from that of IPB 3S, Ciherang, and Way Apo Buru (**Figure 1(b)**). Hipa 18 experienced the highest increase in stem and leaf dry weight at the panicle initiation stage to heading. This shows that the Hipa 18 variety had the highest dry matter production rate compared to all the tested varieties in that phase. However, this variety actually had the highest amount of dry matter reduction in the heading to heading + 20 days phase. This indicates that the amount of assimilate remobilized from stems and leaves to grains was the highest compared to other varieties. This could be due to the large sink size in the Hipa 18 variety (**Figure 3**), so that assimilate remobilization played a greater role in helping photosynthesis during the grain filling phase in this variety.

Similar to the dry weight of stems and leaves, Hipa 18 also had a pattern of total carbohydrate dynamics in the stem that was different from the other 3 varieties. This indicates the different dynamics of NSC in these varieties. **Figure 1(d)** shows that total carbohydrate accumulation still occurred in IPB 3S, Ciherang, and Way Apoburu up to 20 days after heading. However, this was not the case with Hipa 18. After the heading phase, Hipa 18 continued to experience a decrease in total carbohydrates in stems until harvest time. This indicates that the intensity of NSC remobilization in the Hipa 18 variety was greater than the other 3 varieties. Allegedly, the other 3 varieties had more than sufficient amount of assimilate, which was used for filling grains at heading to heading + 20 days from photosynthesis. In fact, the 3 varieties experienced an excess of assimilate, so that they could still be stored in the stem and caused an increase in the NSC content. This is related to the large sink capacity per plant (number of grains per plant x average grain weight) in Hipa 18. Therefore, dry matter production from photosynthesis could not meet the starch demand by grains. In this case, NSC plays a role in covering the deficiency of assimilate resulting from the photosynthesis process [19]. The more rapid remobilization of NSCs contributes to the high grain filling [20].

Remobilization of NSCs in cereal crops during grain filling is modulated by water and nitrogen [16]. Adequate nitrogen fertilization increases NSC reserves in stems, NSC content per seed, strength and sink filling rate [42]. Remobilization of assimilate from vegetative organs to grains requires initiation of plant senescence. Aging initiation can be delayed due to the application of excessive nitrogen fertilizer, so that

plants maintain the character of stay green for too long. Finally, many NSCs are not remobilized and are left in the vegetative organs. In this case, water stress in the grain filling phase can induce faster aging and shorten the grain filling period but increase assimilate remobilization from vegetative organs to grains [17].

Leaf area index, leaf nitrogen content, and photosynthesis

NN treatment had a significant effect on LAI (**Figure 2(a)**), leaf nitrogen content (**Tables 2 and 3**), and photosynthetic rate (**Table 4**). Nitrogen plays a role in increasing production by controlling LAI and the amount of N per unit leaf area [24], LAI and optimal leaf N concentration will induce maximum photosynthetic process, so that the assimilate produced will also be maximized. The element N is mentioned as a determinant of the photosynthetic ability of a single leaf in a plant [25]. Under N deficiency condition, plants will experience a decrease in the rate of photosynthesis, because under this conditions, the stomatal conductance of mesophyll cells and bundle sheath cells will decrease and will affect the intercellular CO₂ concentration. In addition, the electron transport rate is inhibited; the lost light energy increases; and the activity of photosynthetic enzymes will be reduced, which has an impact on decreasing the rate of carboxylation [26]. Added that high nitrogen uptake at heading is an important determinant of flag leaf photosynthesis rate [27]. It is also associated with a slower aging process and higher grain yields. Optimal nitrogen levels can maintain the greenness of the leaves in plants, so that aging can be slowed down. N deficiency can induce plant aging and degradation of N components [26].

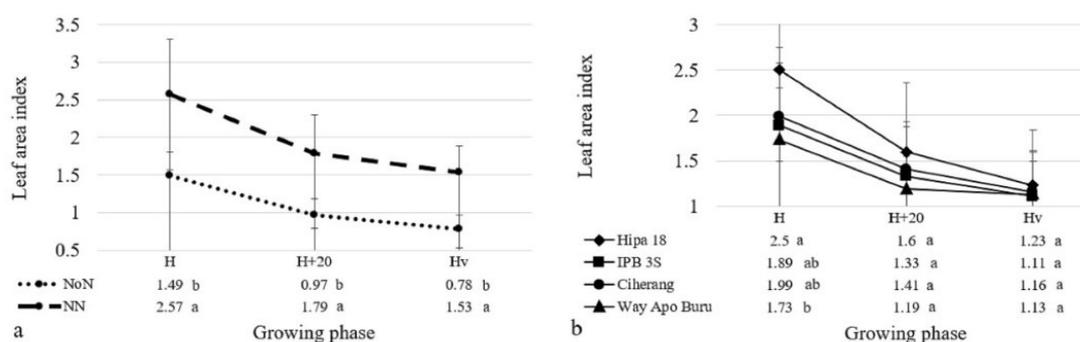


Figure 2 Leaf area index under N treatment (a) and 4 different varieties (b). Numbers followed by the same letter in the same column are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, H = heading, H + 20 = heading + 20 days, Hv = harvest.

Leaf area index is an agronomic character of plants that can indicate growth conditions and forecast yields, so that leaf area index plays an important role in increasing plant production [21]. Leaf area index has a correlation on leaf nitrogen content and photosynthesis. The rate of photosynthesis per unit area and leaf surface area that can receive light are the determinants of the total assimilate that will be used for growth and storage [22]. This is because the leaf is an organ that produces energy, the more surface area of the leaf that receives light, the more assimilate that can be produced for grain filling [23]. [24], Even used the leaf area index as the basis for determining the critical nitrogen curve in wheat which was needed to diagnose the N nutrient status of the plant, so that the N fertilizer requirement to be applied was known.

Tables 2 and 3 shows the decrease in leaf nitrogen content from the panicle initiation phase to harvest. This indicates that the mechanism of N remobilization from old leaves to young leaves during the vegetative phase or from leaf to grain during the reproductive phase played a role in continuing plant life [28]. The majority of the remobilized N is in the form of amino acids, obtained from degradation or reformation of macromolecules [29]. One of the effects of N remobilization is chlorophyll degradation, which has implications for leaf yellowing due to a decrease in chlorophyll content. The continuous remobilization of assimilate and N makes the leaf structure weak. Thus, the leaves eventually fall by themselves during the aging phase. As a result, the decline in LAI occurred continuously until harvest (**Figure 2**). The largest decrease in LAI occurred in the Hipa 18 variety, indicating a decrease in the number of leaves during the plant entering the aging phase. This is consistent with the magnitude of NSC and N remobilization in the Hipa 18 variety.

Table 2 Interaction between N treatment and variety on leaf N content during panicle initiation and heading.

Varieties/N treats	NC (%) PI				NC (%) H			
	NoN	SD	NN	SD	NoN	SD	NN	SD
Hipa 18	2.18 ^c	0.04	2.98 ^a	0.09	1.48 ^c	0.06	1.71 ^{cd}	0.08
IPB 3S	2.24 ^c	0.09	2.78 ^a	0.07	1.60 ^{de}	0.17	1.83 ^{bc}	0.11
Ciherang	2.18 ^c	0.18	2.97 ^a	0.18	1.56 ^{de}	0.07	2.31 ^a	0.10
Way Apo Buru	2.18 ^c	0.17	2.56 ^b	0.11	1.53 ^{de}	0.14	1.91 ^b	0.09

Numbers followed by the same letter in the same phase are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, NC = nitrogen leaf content, PI = panicle initiation, H = heading, SD = standart deviation.

Table 3 Leaf N content under N treatment and varieties at heading + 20 days and harvest.

Varieties/N condition	H20		Hv	
	NC (%)	SD	NC (%)	SD
N treats				
NoN	1.18 ^a	0.15	1.02 ^b	0.14
NN	1.31 ^a	0.13	1.14 ^a	0.12
Varieties				
Hipa 18	1.35 ^a	0.06	1.20 ^a	0.13
IPB 3S	1.08 ^c	0.11	0.95 ^c	0.13
Ciherang	1.33 ^a	0.15	1.10 ^b	0.10
Way Apo Buru	1.22 ^b	0.10	1.07 ^b	0.06

Numbers followed by the same letter in the same column are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, NC = nitrogen leaf content, H20 = heading + 20 days, Hv = harvest, SD = Standart deviation.

The interesting thing found in this study was the interaction that occurred between the N treatment factors and varieties on the N content of the leaves at the time of panicle initiation and heading. **Table 2** shows that the 4 varieties had a percentage of N content that was not significantly different in the NoN treatment at panicle initiation and heading. However, when given NN treatment, the variety with the highest leaf N content at panicle initiation was the Hipa 18 variety, which was significantly different from Way Apo Buru. However, at the time of heading, Hipa 18 became the variety with the lowest leaf N content, which was significantly different from Ciherang and Way Apo Buru (**Table 3**). The low N content of Hipa 18 leaves in heading might occur because the nitrogen usage in this variety was higher than other varieties. Thus, although the highest percentage value occurred at panicle initiation, the lowest N content percentage value occurred in the heading phase. This illustrates that there was a possibility of a higher N requirement in the Hipa 18 variety for panicle formation and development, so that the decrease in leaf N content from panicle initiation to heading was quite large. This phenomenon was seen in the highest number of grains per plant from Hipa 18 (**Table 5**). Meanwhile, NC of Ciherang at heading + 20 days (**Table 3**) was not significantly different from the Hipa 18 variety but significantly different from IPB 3S and Way Apo Buru. In contrast to the reduction of NC at the panicle initiation to heading, Ciherang variety actually had the largest decrease from the heading phase + 20 days of harvest. According to [30], genotypic factors and nitrogen elements affect panicle character performance. Sufficient N levels can affect the diversity of panicle characters, such as the number of panicles, panicle length, and number of primary branches.

In contrast to LAI and NC, the rate of single leaf photosynthesis did not show any significant difference in the 4 varieties. This may be due to the narrow genetic variation that regulates the photosynthesis process in the 4 varieties. Several genes playing a role in photosynthesis have not been

identified, so that the development of varieties rarely relies on increasing the photosynthetic ability of single leaves [31], especially in Indonesia. Variations in photosynthesis may occur at the canopy level, because in this phase there is an integration between leaf area index and canopy structure [32]. Increased production through genetic manipulation of photosynthesis may take longer and is carried out collectively to address all the limiting factors of photosynthesis, including the frequently neglected capacity of the phloem [33].

Table 4 Single leaf photosynthesis rate of rice plants under N treatment and varieties at different growth stages.

N treats and Varieties	PI		H		H + 20	
	Pn	SD	Pn	SD	Pn	SD
N treats						
NoN	28.15 ^a	4.59	28.09 ^b	2.77	25.79 ^b	2.54
NN	32.47 ^a	3.75	31.58 ^a	2.04	29.99 ^a	3.00
Varieties						
Hipa 18	30.64 ^a	5.05	29.69 ^a	2.47	29.41 ^a	2.84
IPB 3S	30.24 ^a	4.12	30.13 ^a	3.90	28.23 ^a	3.97
Ciherang	30.99 ^a	5.76	28.50 ^a	3.03	27.42 ^a	3.38
Way Apo Buru	29.37 ^a	3.69	31.03 ^a	1.64	26.49 ^a	3.10

Numbers followed by the same letter in the same column are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, PI = panicle initiation, H = heading, H + 20 = heading + 20 days, Pn = photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), SD = standart deviation.

Source-sink

Figure 3 shows that the sink capacity is higher than the source capacity, both in the single factor treatment of N and varieties. This indicates that the source capacity of the rice plant used in this experiment was not able to meet its own sink capacity. This affected grain filling process not optimal. However, nitrogen was able to provide significantly higher values for the source and sink capacities of rice plants. Meanwhile the Hipa 18 variety had the highest sink potential compared to other varieties tested in this study. However, the source capacity of the Hipa 18 variety was not able to meet the needs of the sink, so the percentage of empty grain in the Hipa 18 variety increased. In contrast to Hipa 18, the Way Apo Buru variety actually had the smallest difference between the source and sink capacity values. This means that the source capacity of this variety was almost able to produce assimilate that was suitable for sink (grain) needs. According to [15], the main metabolic factors involved in the strength of the inter-cultivar sink are the activity of sucrose synthase, uridine diphosphoglucose pyrophosphorylase, and protein phosphorylase.

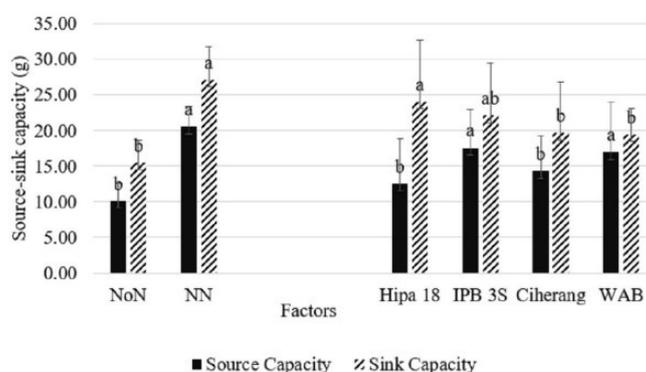


Figure 3 Source and sink capacity of rice plants under N treatment and 4 different varieties. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea.

According to [33], source-sink regulation is closely related to the NSC content and the dry matter produced. In the results of this study, application of 250 kg ha⁻¹ of urea fertilizer was able to have a significantly different effect on the accumulation of dry matter and total carbohydrate content (including NSC) (**Figure 1**). This will affect the capacity of the source and sink in general. The low sink strength in the early stages of grain filling must be compensated for by the high degree of translocation of NSCs in the stem. Thus, remobilization of NSCs to grain is as important as storage capacity of NSCs before heading to grain filling. N application must still be adapted to rice varieties with different panicle types. Excess N can reduce the structural carbohydrate content in the stem. This results in reduced physical strength of the trunk and the risk of collapsing [3].

Source capacity indicates the ability of a plant to provide the assimilate required by the sink. Meanwhile, the sink capacity shows the maximum yield potential that a plant can achieve. Discussing the relationship between the 2 means also discussing the remobilization of assimilate to grains. According to [35], in addition to leaves and leaf sheaths that play a role in photosynthesis and stems that store assimilate reserves before the anthesis phase, it turns out that the contribution of inflorescence organs such as panicles in producing assimilate through the photosynthesis process is quite taken into account in the grain filling phase. The contribution of each organ depends on the genetic differences carried by each variety and the growing environment. Even severe heat stress can turn stems into carbohydrate absorbers during grain filling [36].

Yield component

Table 5 shows that the NN treatment gave significantly higher yields on the observed yield components, in addition to the percentage of empty grain. Meanwhile the difference in grain size is also caused by differences in the characteristics carried by each variety. This is evidenced by the finding that Hipa 18 had the highest value in almost all yield components, except for the weight of 100 grains. This indicates that there were differences in grain characters between varieties that were not tested in this study. However, through data on 100 grains, in general we could find out that Hipa 18 grain was the smallest among the varieties used. [39] Found that there are a number of genes that regulate grain width and weight. [40], Added that allelic variations of genes regulating grain length and weight could cause yield differences between cultivars. These genes have also been reported to play a role in auxin and cytokinin biosynthesis, so that they play a role in cell growth through biosynthesis and hormonal signaling [41].

Table 5 Rice yields components under N treatments and different varieties.

N treats and Varieties	Number of panicle/plant	SD	Number of grains/plant	SD	Weight of 100 grains	SD	% Empty grain/plant	SD	Weight of grains/plant	SD
N treats										
NoN	10.86 ^b	1.85	658.92 ^b	139.66	2.37 ^b	0.25	24.46 ^a	10.30	11.55 ^b	3.87
NN	14.47 ^a	1.81	1,042.06 ^a	202.47	2.62 ^a	0.14	16.49 ^a	5.39	21.22 ^a	3.21
Varieties										
Hipa 18	13.61 ^a	2.33	1,037.22 ^a	289.15	2.27 ^b	0.24	29.71 ^a	11.33	19.27 ^a	5.76
IPB 3S	11.00 ^b	2.52	869.67 ^b	240.24	2.50 ^a	0.2	17.86 ^b	4.60	16.45 ^{ab}	5.79
Ciherang	12.28 ^{ab}	2.56	759.56 ^b	262.22	2.57 ^a	0.09	14.21 ^b	4.67	15.19 ^{ab}	7.03
WAB	13.78 ^a	1.99	735.50 ^b	99.19	2.63 ^a	0.23	20.10 ^b	5.91	14.64 ^b	4.63

Numbers followed by the same letter in the same column are not significantly different using DMRT with $\alpha = 5\%$. NoN = 0 kg ha⁻¹ urea, NN = 250 kg ha⁻¹ urea, WAB = way apo buru, SD = standart deviaton.

Sufficient N element for plant growth and development will lead to maximum yields. According to [37], nitrogen is able to give a significant effect on the accumulation and physicochemical properties of starch. Nitrogen application promotes starch accumulation between parts of the endosperm, which ultimately affects the agronomic properties of rice. Added that nitrogen content significantly affected the surface of rice starch [38]. This indicates that nitrogen given in optimum amounts can produce good

physicochemical properties and optimal starch accumulation in the grain, so that these characteristics will provide maximum yield with good quality

Correlation

Table 6 shows that plant characters such as stem and leaf dry weight, leaf area index, photosynthetic rate, leaf N content, and stem carbohydrate content had a positive correlation on yield components, such as the number and weight of grain per plant. Photosynthate is the largest assimilate contributor to plant yields. The optimum rate of photosynthesis is supported by the optimum leaf N content and large leaf area index as well because the absorption area of solar radiation becomes larger. This causes a positive correlation between the rate of photosynthesis with leaf area index and leaf N content. Leaf nitrogen content is very important, especially at the panicle initiation stage because nitrogen content can significantly increase starch accumulation in grains [37].

Table 6 Correlation coefficient of vegetative characters and yield components of several rice varieties under N treatment.

	PH-Hv	LSDW-H	LSDW-Hv	NC-PI	NC-H	LAI-H	Pn-PI	Pn-H	TCS-H	TCS-H20	NOG	WOG
LSDW-H	0.57**											
LSDW-Hv	0.49**	0.5**										
NC-PI	0.65**	0.58**	0.76**									
NC-H	0.26*	0.24	0.54**	0.72**								
LAI-H	0.54**	0.54**	0.47**	0.72**	0.45**							
Pn-PI	0.44**	0.46**	0.48**	0.63**	0.46**	0.39**						
Pn-H	0.5**	0.45**	0.46**	0.61**	0.45**	0.45**	0.51**					
TCS-H	0.67**	0.93**	0.58**	0.64**	0.42**	0.43**	0.54**	0.43**				
TCS-H20	0.43**	0.34*	0.45**	0.51**	0.43**	0.42**	0.42**	0.45**	0.4**			
NOG	0.71**	0.66**	0.62**	0.84**	0.43**	0.8**	0.52**	0.62**	0.6**	0.47**		
WOG	0.73**	0.67**	0.65**	0.8**	0.49**	0.69**	0.54**	0.57**	0.7**	0.5**	0.87**	
100 grains	0.19	0.08	0.39**	0.44**	0.5**	0.23	0.36*	0.26	0.2	0.29*	0.26	0.36*

* = significant at $\alpha = 5\%$, ** = significant at $\alpha = 1\%$. PH = plant height, LSDW = leaf and stem dry weight, NC = leaf nitrogen content, LAI = leaf area index, Pn = photosynthesis, TCS = total carbohydrate of stems, NOG = number of grains, WOG = weight of grains, 100_grains = weight of 100 grains, PI = panicle initiation, H = heading, H20 = heading+20 days, Hv = harvest.

Optimal photosynthate during the vegetative phase will increase the NSC content stored in the stem and increase the dry weight of stems and leaves which are the assimilate source for plants when entering the grain filling phase. Therefore, there is a positive correlation between the rate of photosynthesis and dry weight of stems, leaves and stem carbohydrate content. Optimal photosynthate during the reproductive phase will increase the starch content in the grains, resulting an increase in grain weight per plant.

NSCs play a role in filling the assimilate deficiency, especially when the photosynthate is insufficient for grain filling, especially under conditions of low solar radiation [19]. Therefore, the carbohydrate content of stems and leaf N content at the time of heading would greatly affect the weight and amount of grain per plant. NSCs in stems in the pre-heading phase not only functioned as assimilate providers, but also played an important role in increasing sink strength in the early grain filling stage, because they could accelerate endosperm development and the grain filling process. With high NSC reserves stored in stem pre-anthesis, zeatin and zeatin riboside content increased and ethylene evolution rate decreased significantly in inferior grains, so that sink strength and grain filling rate were increased [42].

Conclusions

Assimilate reserves stored in the form of NSC play an important role in the grain filling phase. The high NSC reserves in the pre anthesis had an effect on the high source capacity. This NSC reserve had a positive correlation with several observed plant characters such as LAI, NC, LSDW, and Pn. Hipa 18 was the variety that had the largest NSC reserves as measured by the dry weight of stems and leaves and the carbohydrate content of the stems. This large NSC reserve supported the largest sinks as well as Hipa 18 as measured by NOG. However, the source capacity of the varieties used was found to be lower than the sink capacity. Therefore, it necessary to improve the character of the plant through breeding programs, genetic engineering or the environment to increase the size of the source or increase the absorption of assimilate in the sink, so that maximum yield is obtained. Then it was also known that nitrogen affected the condition of all plant characters observed in this study, this indicates that nitrogen had an important role in the regulation of source-sink. Therefore optimal nitrogen fertilization is needed to get a maximum yield.

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