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RESPONSE OF PROMISING RICE CSSL IAS66 AND ITS PARENTS UNDER DIFFERENT NITROGEN LEVELS

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ABSTRACT

Pot experiments were conducted on rice to estimate the relationship among dry weight, translocation of non-structural carbohydrates (NSC), and grain yield in IAS66, a chromosome segment substitution line (CSSL) derived from a cross between the *indica* cultivar IR24 and the *japonica* cultivar Asominori, in comparison with its parental cultivars (IR24 and Asominori) under non-nitrogen (N0), normal nitrogen (N1), and high nitrogen (N2) conditions in the spring season 2017 in a greenhouse at the Faculty of Agronomy, Vietnam National University of Agriculture, Vietnam. Increasing the nitrogen level increased the photosynthetic rate in terms of the CO_2 exchange rate (CER) and leaf area, which lead to both greater dry weight and NSC transportation from culms and leaves sheaths (stem) to panicles in all genotypes. However, the rate of increase in dry weight and NSC in the panicles at the maturing stage observed in IAS66 was higher than that in its parental cultivars. As nitrogen increased from the N1 to N2 levels, the grain yield significantly increased because the number of panicles per plant increased, but the other yield components including the number of spikelets per panicle, grain-filling, and 1000-grain weight were not significantly different. Grain yield of IAS66 was 11.4% higher than that of the recurrent parent IR24 at the same high nitrogen levels and the rate of grain yield increase in IAS66 was significant higher than that of its parental cultivars.

Key words: dry weight, non-structural carbohydrates, grain yield, nitrogen use efficiency

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INTRODUCTION

Rice is the staple food grain for more than half of the world's population. It was estimated that the world will have to produce 40% more rice per year, equivalent to 852 million tons, by 2035, indicating that the yield potential of rice will need to increase from 10 to 12.3 tons per hectare to satisfy the growing demand without affecting the resource base adversely (Khush 2013). Crop yield mainly depends on both the formation and translocation of carbohydrates before flowering, and the partitioning of the accumulated assimilates during the grain filling period (Yoshinaga et al. 2013). Additionally, the photosynthesis activity of sources (leaves) and storage ability of the sinks (grains) impacts carbohydrate remobilization from the stem and also affects the final grain weight. Katsura et al. (2007) suggested that the grain yield of rice was affected primarily by large amount of biomass accumulation before heading stage, while Takai et al. (2006) and Tang et al. (2008) reported that grain yield is mainly determined by carbohydrates supplied from photosynthesis after heading and partly by reserve carbohydrates in the culm and leaf-sheaths accumulated before heading stage. Therefore, further studies are required to clarify whether the roles of the reserve carbohydrates and biomass accumulation are different among the genotypes.

Nitrogen plays an important role in increasing photo-assimilates during the ripening period, contributing to the size of the sink by decreasing the number of degenerated spikelets and enhancing the rate of grain filling. Increasing nitrogen application has been shown to increase nitrogen content in leaves which leads to an enhanced photosynthetic capacity and increased dry weight (Mae 1997; Nagata et al. 2001). Thus, it was concluded that the supply level of nitrogen greatly regulates dry weight production and non-structural carbohydrate (NSC) transportation in rice genotypes.

Recently, chromosome segment substitution lines (CSSLs) that cover the whole genome have been constructed in rice and contain target genes that aid molecular marker techniques (Kubo et al. 2002; Ebitani et al. 2005). CSSLs can be used as a good source of genetic material for breeding programs based on the performance of interested traits from donor chromosome segments. Kubo et al. (2002) developed 70 CSSLs in a cross IR24 x Asominori with IR24 genetic background called IAS. Among these lines, IAS66 was shown to be a promising rice line with good phenotypic traits such as having green leaves, thicken roots and long panicle (Nguyen et al. 2017). Therefore, this study was conducted to examine the dry weight (DW), transportation of NSC, and relationship among DW and NSC on the grain yield of IAS66 compared to its parental genotypes under three nitrogen levels. Several physiological characteristics and yield components were identified to learn more about the cause of higher DW and grain yield in IAS66.

MATERIALS AND METHODS

Plant materials. IAS66, a chromosome segment substitution line developed from a cross between IR24 and Asominori at BC_2F_3 (Kubo et al. 2002), was used in this study along with its two parental genotypes. The maternal cultivar, IR24, belongs to the *indica* subspecies, and the paternal cultivar, Asominori (ASO), belongs to the *japonica* subspecies.

Experimental design. The green house experiment was arranged in Randomized Complete Block Design (RCBD). Each genotype was replicated four times and exposed to three nitrogen application levels.

Plant growth and fertilizer application. The pot-experiment was conducted during the spring season from February to June in 2017 in a greenhouse at the Faculty of Agronomy, Vietnam National University of Agriculture, Vietnam. IAS66, IR24, and ASO were sown on different dates (January 17 for IR24, January 22 for IAS66, and January 30 for ASO) in order to synchronize heading times. Thirty-day-old seedlings from wet-bed nurseries were transplanted with one seedling per hill into each pot. All crop management practices were in accordance with standard cultural practices (IRRI, 2002). The plants were grown in the pots and supplied with chemical fertilizer at the treatment rates of non-nitrogen (0 g N pot^{-1}), normal nitrogen (1 g N pot^{-1}), and high nitrogen levels (2 g N pot^{-1}). The amount of fertilizer applied is shown in Table 1. The nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) used in the present study were urea (46% N), super phosphate (16% P_2O_5), and potassium chloride (60% K_2O), respectively. 'Song Gianh' micro-organic fertilizer (relative humidity 30%, effective phosphorus 1.5%, organic matter 15% and effective microorganism $1x10^6$CFU g^{-1}) was applied at a rate of 10 g pot^{-1} and a basal dressing of all the chemical fertilizers was applied to the alluvial soil in the 0.03m^2 pots containing 5.5 kg of soil one day before transplanting. Additional nitrogen was applied during the active tillering and panicle initiation stages (PI). Water, weeds, insects, and diseases were controlled as necessary to avoid yield loss.

Table 1. Rate of fertilizer applications

Nitrogen levels	Nitrogen (g N pot^{-1})				Phosphorus (g P_2O_5 pot^{-1})				Potassium (g K_2O pot^{-1})			
	Basal	**Tillering**	**PI**	**Total**	**Basal**	**Tillering**	**PI**	**Total**	**Basal**	**Tillering**	**PI**	**Total**
N0	0	0	0	0	0.5	0	0	0.5	0.1	0.25	0.15	0.5
N1	0.2	0.5	0.3	1.0	0.5	0	0	0.5	0.1	0.25	0.15	0.5
N2	0.4	1.0	0.6	2.0	0.5	0	0	0.5	0.1	0.25	0.15	0.5

PI: panicle initiation stage

Sampling and Measurements

Physiological characteristics. Photosynthetic rates were measured in terms of carbon dioxide exchange rates (CER) at tillering, heading, and dough-ripening stage (14 days after heading (DAH)) by the use of a LICOR-6400 portable photosynthesis system (USA) at the CO_2 concentration of 360-370 ppm, light intensity of 1500 µmol m^{-2} s^{-1}, and varied relative humidity. Four plants of each rice genotype (i.e. IAS66, IR24, and ASO) were randomly selected for the measurement and each plant was considered as one replication. The flag leaf of the main culm of each rice plant was used to measure the CER between 9:00 and 13:00 hours on sunny days. After the CER measurement, the green leaves were cut to measure leaf area (LA) using a LICOR-3100 Area Meter. Plant samples were divided into: leaves, culms plus leaf sheaths, and panicles, then oven-dried at 80^0C for at least 72 hours to determine dry weight (DW). The dried flag leaves were powdered and then analyzed for the flag leaf nitrogen content (FLNC) by the Kejldalh method.

$$\text{Biomass nitrogen use efficiency (BNUE)} = \frac{Dry\ weight}{FLNC} \quad (\text{BNUE - g mg}^{-1}\text{N})$$

The non-structural carbohydrate content (NSC) of stems and leaf sheaths, and the panicles at both the heading and maturing stages were measured using the gravimetric method (Ohnishi and Horie 1999).

Yield components and grain yield. At the ripening stage, 4 plants in each treatment were selected to determine the yield components and grain yield by measuring the number of panicles, number of grains per panicle, percentage of filled-grain, and 1000-grain weight. Grain yield was measured after threshing, cleaning, and drying.

The formulas used for calculating the nutrient use efficiency are as follows (Fageria *et al.*, 2010):

$$\text{Agronomic nitrogen use efficiency (ANUE)} = \frac{\text{Grain yield at N1 or N2} - \text{grain yield at N0}}{\text{quantity of nitrogen applied}} \ (\text{ANUE - g g}^{-1})$$

Statistical analyses. Analysis of variance (ANOVA) was performed using 'Minitab 16' (Meyer and Krueger 2004), according to (RCBD) to assess genotypes differences, nitrogen levels and genotypes × nitrogen levels interactions. The significance of mean values was analyzed using Tukey's test (0.05).

RESULTS AND DISCUSSION

Photosynthesis, leaf area, and dry matter accumulation. As shown in Figure 1, the photosynthetic rate, in terms of CO_2 exchange rate (CER), increased significantly under high nitrogen conditions in IAS66 and the recurrent parent IR24 at all stages, but the rate of increase was significantly higher in the CSSL than in IR24. In the N0 level, there were no significant differences in CER between IAS66 and its parental cultivars at the tillering, heading, or dough-ripening stages. At the tillering stage, the CER of ASO in the N1 level was higher than that of IAS66 and IR24, but when nitrogen increased to the N2 conditions, the CER of IAS66 was as high as that of ASO with values of 25.0 and 25.9 µmol CO_2 m^{-2} leaf s^{-1}, respectively. At the heading stage, the CER of the ASO at N1 level still had similar trends to that of tillering stage, the highest value being 28.3 µmol CO_2 m^{-2}leaf s^{-1}, but no significant differences were observed between Asominori and IAS66 at N2 level with CER values of 27.0 and 25.9 µmol CO_2 m^{-2} leaf s^{-1}, respectively (Fig. 1). However, the rate of increase in CER in IAS66 from the N0 to the N2 level was significantly higher than that in its parental cultivars. At the dough-ripening stage, the paternal parent, ASO maintained a high photosynthetic rate at N1 levels, whereas the photosynthetic rate of IAS66 was not significantly different to that of the IR24 cultivar. There was no significant difference in CER between N2 and N1 level of all genotypes at all stages.

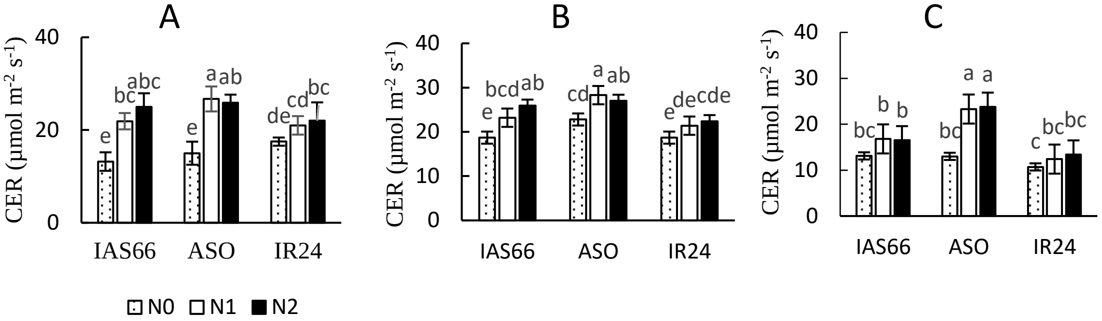

Fig. 1. CO_2 exchange rate (CER) of the promising line IAS66 and its parental cultivars grown under different nitrogen conditions at the tillering stage (A), heading stage (B), and dough-ripening stage (C). Vertical bars show the standard error of four replications. Mean values followed by the same letter are not significantly different at each stage ($P \leq 0.05$) by Tukey's test.

Leaf area (LA) per plant significantly increased when nitrogen increased from N0 to the N2 conditions in IAS66 and both parental cultivars (Fig. 2). The rate of increase in IAS66 was significantly higher than that of its parental cultivars at the tillering and heading stages. The observed values of LA of IAS66 were 16.5 and 34.4% higher than that of IR24 and ASO, respectively at the heading stage. However, at the dough-ripening stage, there were no significant differences in LA of IAS66 compared to its parent under all nitrogen levels.

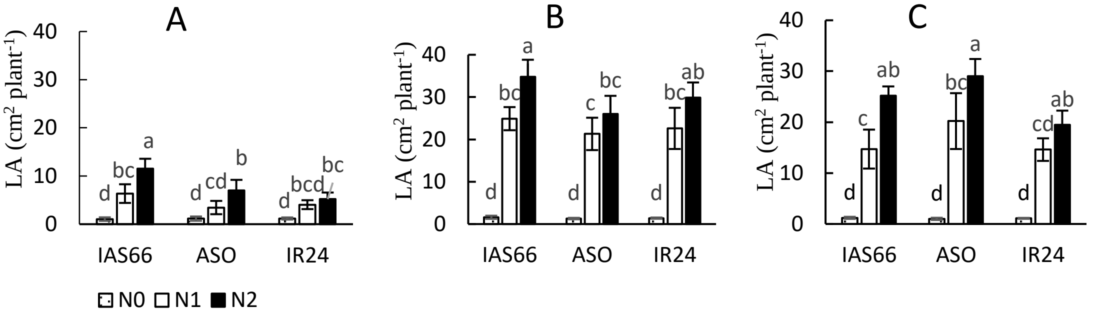

Fig. 2. Leaf area (LA) of the promising line IAS66 and its parent cultivars grown under different nitrogen conditions at the tillering stage (A), heading stage (B), and dough-ripening stage (C).

Aboveground dry weight also increased throughout the growth stages as N levels increased in IAS66 and both parental cultivars (Fig. 3). In the N0 treatment, there were no significant differences in dry weight among genotypes. Increasing nitrogen to the N1 conditions, dry weight of IAS66 also increased to 45.6 g plant^{-1}, and increased again to 56.5 g plant^{-1} in the heading and maturing stages, but no significant differences were measured in comparison to IR24 at the ripening stage. Under the N2 conditions, the dry weight of IAS66 was significantly higher than that of its parent at all stages and also 13 and 23% greater than that in the N1 conditions in both the heading and maturing stages, respectively (Fig. 3). The rate of increase was higher in IAS66 than that in its parental cultivars.

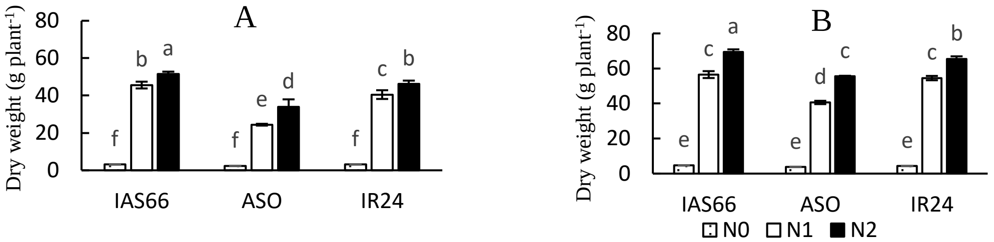

Fig. 3: Dry weight of the promising line IAS66 and its parental cultivars grown under different nitrogen conditions at the heading stage (A) and maturing stage (B).

Non-structural carbohydrate (NSC) mobilization. The total carbohydrates in grain are derived from both the accumulated carbohydrates in the culm and leaf sheaths before heading, and the photosynthetic products during grain filling (Nakano et al. 2017). In general, accumulated NSC in the culms and leaf sheaths (stem) increased from the tillering to heading stage, and then decreased during the ripening period (Yoshida 1981). In contrast to the NSC in the stems, NSC in the panicles of all genotypes showed an increasing trend with increased nitrogen application, from the heading to mature stages. There was a large decrease (8.9g hill^{-1}) of accumulated NSC in the stems of IAS66 from the heading to mature stages under N1 conditions (Table 2). However, as the nitrogen level increased, the amount of NSC reserved in the stems of the IAS66 and IR24 genotypes decreased. Under the N2 condition, the NSC of IAS66 panicles had the highest average (31.5 g hill^{-1}) at the mature stage, but was not significantly different to that of IR24 (28.8 g hill^{-1}). High nitrogen application led to a significant increase in the amount of NSC reserved in the panicles from the heading to mature stages, however the percentage of increase of NSC in the panicles at the mature stage of IAS66 was 39.4 and 9.4% higher than in ASO and IR24, respectively.

Table 2. Non-structural carbohydrates (g hill^{-1}) in stems and panicles of the promising line IAS66 and its parent cultivars grown at different nitrogen conditions

Genotype	Nitrogen	Stems			Panicles		
		Heading	Mature	Decrease	Heading	Mature	Increase
IAS66	N0	0.7^{d}	0.3^{d}	0.4^{d}	0.1^{bc}	2.0^{f}	1.9^{e}
	N1	12.4^{a}	3.5^{ab}	8.9^{a}	1.7^{a}	27.0^{bc}	25.3^{ab}
	N2	9.9^{b}	3.0^{bc}	6.9^{b}	2.3^{a}	31.5^{a}	29.2^{a}
	Average	7.7^{A}	2.3^{B}	5.4^{A}	1.4^{A}	20.2^{A}	18.8^{A}
ASO	N0	0.6^{d}	0.3^{d}	0.2^{d}	0.1^{c}	1.8^{f}	1.6^{e}
	N1	4.1^{c}	3.3^{bc}	0.8^{d}	2.0^{a}	18.2^{e}	16.2^{d}
	N2	5.7^{c}	4.3^{a}	1.4^{cd}	1.4^{ab}	22.6^{d}	21.1^{c}
	Average	3.5^{C}	2.6^{A}	0.8^{C}	1.2^{A}	14.2^{B}	13.0^{B}
IR24	N0	0.7^{d}	0.2^{d}	0.5^{d}	0.1^{bc}	1.8^{f}	1.7^{e}
	N1	9.3^{b}	3.2^{bc}	6.1^{b}	1.8^{a}	25.5^{cd}	23.7^{bc}
	N2	6.1^{c}	2.6^{c}	3.5^{c}	2.4^{a}	28.8^{ab}	26.6^{ab}
	Average	5.4^{B}	2.0^{B}	3.4^{B}	1.4^{A}	19.1^{A}	17.6^{A}

Values within a stage for each treatment followed by the same uppercase letter and lowercase letter are not significantly different at the 0.05 probability level by Tukey's test.
Decrease refers to the amount of NSC decrease in the stems = NSC at heading – NSC at mature
Increase refers to the amount of NSC increase in the panicles = NSC at mature – NSC at heading

Table 3. Yield and yield components of the promising line IAS66 and its parental cultivars under different nitrogen conditions

Genotype	Nitrogen	Number of panicles hill^{-1}	Number of spikelets panicle^{-1}	Percentage of filled-grain (%)	1000-grain weight (g)	Grain yield (g hill^{-1})
IAS66	N0	2.0^{f}	81^{d}	84.6^{c}	25.3^{b}	2.9^{f}
	N1	17.3^{d}	155^{b}	93.3^{ab}	26.3^{a}	40.1^{c}
	N2	21.0^{bc}	153^{b}	94.2^{ab}	26.3^{a}	47.9^{a}
	Average	13.4^{B}	130^{B}	90.7^{B}	26.0^{B}	30.3^{A}
ASO	N0	2.5^{f}	34^{e}	95.7^{a}	26.5^{a}	2.4^{f}
	N1	22.0^{b}	91^{cd}	92.6^{ab}	27.0^{a}	25.1^{e}
	N2	31.0^{a}	96^{cd}	94.1^{ab}	27.0^{a}	32.8^{d}
	Average	18.5^{A}	74^{C}	94.1^{A}	26.8^{A}	20.1^{C}
IR24	N0	1.5^{f}	111^{c}	83.3^{c}	23.5^{c}	2.8^{f}
	N1	13.2^{e}	245^{a}	90.8^{b}	23.9^{c}	37.3^{c}
	N2	17.5^{cd}	240^{a}	90.7^{b}	24.0^{c}	43.0^{b}
	Average	10.7^{C}	199^{A}	88.3^{C}	23.8^{C}	27.7^{B}

Values within a stage for each treatment followed by the same uppercase letter or lowercase letter are not significantly different at the 0.05 probability level by Tukey's test.

Yield components and grain yield. Nitrogen levels had a significant effect on the number of panicles per hill of IAS66 and both the parental cultivars. The highest number of panicles hill^{-1} was in the ASO cultivar subjected to the high nitrogen treatment, followed by IAS66 and IR24 cultivars, respectively (Table 3).

Increasing the nitrogen application level from the N1 to N2 conditions did not affect the number of spikelets per panicle, the percentage of filled-grain and the 1000-grain weight. The number of spikelets per panicle in IR24 was much higher than that of IAS66 with about 240 spikelets per panicle at both nitrogen levels. However, the percentage of filled-grain of IAS66 was higher than in IR24 with 93.3% and 94.2% at the N1 and N2 levels, respectively. The 1000-grain weight of IAS66 was significantly different than that of IR24, and this could be induced by a segment of chromosome derived from the ASO cultivar.

Large variations among genotypes were observed in the grain yield as nitrogen levels increased. Grain yield was not significantly different among all cultivars under the N0 condition, except for ASO under the N1 conditions. However, there was a significant difference in grain yield of IAS66 compared to its parental cultivars under the N2 conditions. The increase in grain yield from N0 to N2 conditions was significantly difference with 45, 30.4 and 40.2 g hill^{-1} in IAS66, Asominori, and IR24, respectively. In addition, the rate of increase in grain yield for IAS66 was 20 and 32% higher than that of IR24 and ASO, respectively.

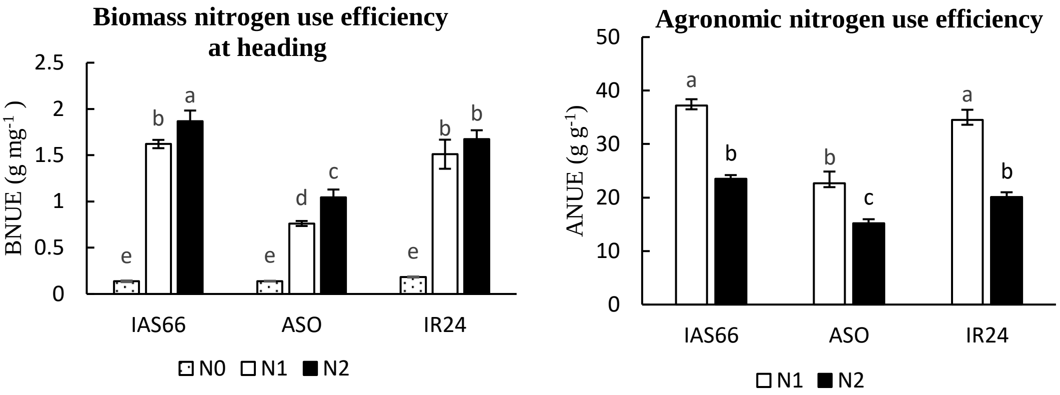

Fig. 4. Biomass nitrogen use efficiency **(**BNUE) and agronomic nitrogen use efficiency (ANUE) of the promising line IAS66 and its parental cultivars grown under different nitrogen conditions.

Nitrogen use efficiency (NUE). One of the strategies to improve yields is to choose genotypes with high nitrogen use efficiencies (NUE) that can produce higher economic yields under limited conditions. The biomass nitrogen use efficiency (BNUE) of IAS66 at heading stage was significantly higher than in its parents under high nitrogen conditions (N2) (Fig. 4). The rate of increase in BNUE in the IAS66 from the N0 to N2 conditions was 15 and 92% higher than in IR24 and ASO, respectively. In contrast to BNUE, the agronomic nitrogen use efficiency (ANUE) of all-genotypes decreased when increasing nitrogen from N1 to N2 conditions. The ANUE of IAS66 was higher than that of its parental cultivars, but it was not significantly different to IR24 in the N1 level. These results are in agreement with previously published data demonstrating that under high N concentrations, both absorption nitrogen use efficiency (aNUE) and agronomical nitrogen use efficiency (ANUE) decrease gradually (Nguyen et al. 2014). This indicates the plants are able to acquire adequate nutrition from the normal nitrogen applications.

The main objective of this study was to examine the ability of production of dry weight and translocation carbohydrates of promising chromosome segment substitution line (CSSL) in comparison to its parental cultivars. The different nitrogen application levels were provided to evaluate the response of the genotypes over growth stages. Under the N1 condition, the NSC had the highest values in the culm and leaf sheaths (stems) at the heading stage. Increasing the nitrogen levels to the N2 condition resulted in a decrease in the amount of NSC reserved in stems. The high nitrogen conditions increased the number of spikelets m^{-2} but did not increase the amount of reserved NSC (Table 3 and Table 2), resulting in lower amounts of reserved NSC compared with the larger sink size in the high nitrogen application conditions (Nagata et al. 2001). However, in the high nitrogen treatment, the NSC reserved in the panicles at the maturing stage was higher than that under normal nitrogen conditions. Sources of the carbohydrates can be separated into two components: those assimilated during the ripening period and reserved in the culms and leaf sheaths before heading stage. Many previous studies indicated that a higher grain yield of rice was due to reserve carbohydrates in the culms and leaf-sheaths accumulated before heading (Takai et al. 2006). Other studies stated that dry matter production after heading is more important than the reserved carbohydrates before heading because the fraction of carbohydrates from assimilates after heading is usually larger than that those reserved before heading (Nagata et al. 2001). The results of the present study showed that the carbohydrates from the assimilates of IAS66 after the heading stage had the highest values under the N2 conditions. There was a close relationship between the increase of NSC in panicles at the ripening stage and grain yield with $r = 0.99^{**}$ in all genotypes (Fig. 5A). Additionally, high rate of decrease of NSC in the stems of IAS66 could lead to a higher grain-filling ability (Li et al. 2012, Zheng et al. 2010). Higher NSC accumulation under the high nitrogen conditions promoted the translocation of labeled ^{13}C from the culms and leaf sheaths to the grains, and increased sucrose synthase enzyme activity in the grains enhanced the duration of higher activity

(Zheng et al. 2010). However, the rate of increase of NSC in the panicles of IAS66 was higher than that of its parental cultivars.

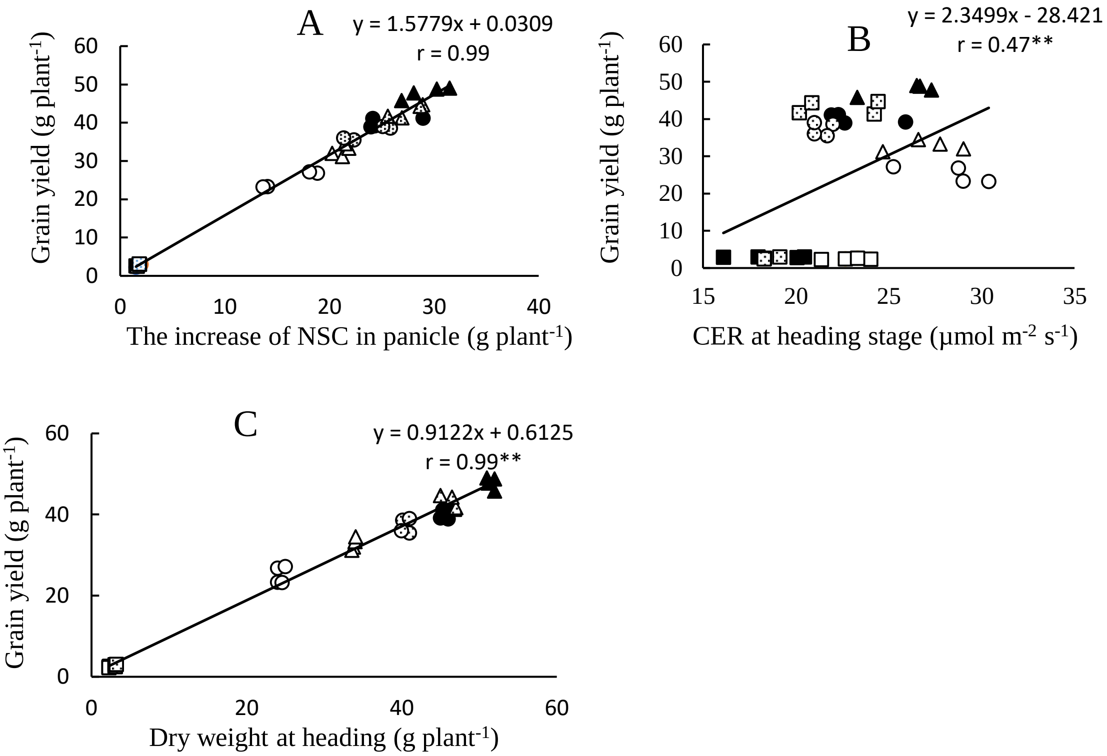

Fig. 5. Correlation between the increase of NSC in the panicles from heading to maturing stage with grain yield (A), CER at heading stage with grain yield (B) and dry weight at the heading stage with grain yield (C) in IAS66 (closed symbol), Asominori (open symbol), and IR24 (meshed symbol) rice cultivars grown under the N0 (square symbol), N1 (circle symbol), and N2 (triangle symbol) conditions.

ns: not significant; *, ** significant difference at $P<0.05$, 0.01, respectively.

The higher grain yield of IAS66 relative to the parent should be the result of higher contributions of biomass at the heading and mature stages. Nakano et al. (2017) showed evidence that larger dry weights might be attributed to higher source activity. In our study, the dry weight production of IAS66 had the highest values at the heading and mature stages under the N2 conditions due to higher leaf areas (Fig. 3) and CER at heading (Fig. 2). These results agree with Nakano and co-workers (2017) who indicated that high-yielding cultivars originating from a cross between *indica* and *japonica* sub-species have a higher sink capacity than *indica* high-yielding cultivars, and a higher grain yield of F_1 hybrid rice was attained by greater DM production at heading (Pham et al. 2003). There was a significant correlation between CER at the heading stage and grain yield of IAS66 and its parent cultivars with r = 0.47** (Fig. 5B). A positive correlation between dry weight and grain yield at the heading stage was also observed with r = 0.99** (Fig. 5C). These results suggest the importance of maintaining dry weight and that the translocation of NSC could be contributing to the high grain yield of IAS66 during the mature stage under high nitrogen conditions.

CONCLUSION

A high level of nitrogen application is a critical factor in IAS66 for greater increase in grain yield compared to the parents. Increasing the nitrogen level resulted in an increase in the rate of dry matter production and number of panicles per plant compared to its parental cultivars. IAS66 under high nitrogen condition had higher CER values before heading, a higher dry weight, and higher levels of carbohydrates from assimilates after the heading stage in the panicles, which consequently contributed to the higher grain yield of the promising CSSL IAS66 relative to its parental cultivars.

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REFERENCES CITED

Ebitani, T., Y. Takeuchi., Y. Nonoue., T. Yamamoto., K. Takeuchi, and M. Yano. 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar ‘Kasalath’in a genetic background of *japonica* elite cultivar ‘Koshihikari’. Breeding Science. 55: 65-73.

Fageria, N., O. De Morais, and A. Dos Santos. 2010. Nitrogen use efficiency in upland rice genotypes. Journal of Plant Nutrition. 33: 1696-711.

IRRI, 2002. Standard evaluation system for rice. International Rice Research Insitute, Manila, Philippines. pp 22-32.

Katsura, K., S. Maeda., T. Horie, and T. Shiraiwa. 2007. Analysis of yield attributes and crop physiological traits of Liangyoupeijiu, a hybrid rice recently bred in China. Field Crops Research. 103: 170-177.

Khush, G. S. 2013. Strategies for increasing the yield potential of cereals: case of rice as an example. Plant Breeding. 132: 433-436.

Kubo, T., Y. Aida., K. Nakamura., H. Tsunematsu., K. Doi, and A. Yoshimura. 2002. Reciprocal chromosome segment substitution series derived from *japonica* and *indica* Cross of rice (*Oryza sativa* L.). Breeding science. 52: 319-325.

Li, H., L. Liu., Z. Wang., J. Yang, and J. Zhang. 2012. Agronomic and physiological performance of high-yielding wheat and rice in the lower reaches of Yangtze River of China. Field Crops Research. 133: 119-129.

Mae, T. 1997. Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. Plant and soil. 196: 201-210.

Meyer, R and D. Krueger. 2004. A Minitab guide to statistics. Third edition, Pearson, 2004. pp 448.

Nagata, K., S. Yoshinaga., J. I. Takanashi, and T. Terao. 2001. Effects of dry matter production, translocation of nonstructural carbohydrates and nitrogen application on grain filling in rice cultivar Takanari, a cultivar bearing a large number of spikelets. Plant Production Science. 4: 173-183.

Nakano, H., S. Yoshigana, and T. Takai. 2017. Quantitative trait loci for large sink capacity enhance rice grain yield under free-air CO_2 enrichment conditions. Scientific Reports. 7: 18-27.

Nguyen H. H., V. H. Nguyen, and V.C. Pham. 2017. Morphology and anatomy characteristics of rice line DCG66 harboring chromosome segment from *japonica* Asominori in *indica* IR24 genetic background. Vietnam J. Agri. Sci. 1 (15): 20-26.

Nguyen, H.T.T., V. C. Pham, and P. Bertin. 2014. The effect of nitrogen concentration on nitrogen use efficiency and related parameters in cultivated rices (*Oryza sativa* L. subsp. *indica* and *japonic*a and *O. glaberrima* Steud.) in hydroponics. Euphytica. 198: 137-151.

Ohnishi, M and T. Horie. 1999. A proxy analysis of nonstructural carbohydrate in rice plant by using the gravimetric method. Jpn J. Crop Sci, 68:126-136.

Pham V.C., S. Murayama, and Y. Kawwamitsu. 2003. Heterosis for photosynthesis, dry matter production and grain yield in F1 hybrid line (*Oryza sativa* L.) from thermo-sensitive genic male sterile line cultivaed at different soil nitrogen levels. Enviroment Control in Biology, 41 (4): 335-345.

Tang T. H., T. Araki. V.C. Pham., T. Mochizuki., A. Yoshimura, and F. Kubota. 2008. Effect of nitrogen supply restriction on photosynthetic characters and dry matter production in Vietlai20, a Vietnamese hybrid rice cultivar, during grain filling stage. Trop. Agri. Develop. 52: 111-118.

Yoshinaga, S., T. Takai., Y. Arai-Sanoh., T. Ishimaru, and M. Kondo. 2013. Varietal differences in sink production and grain-filling ability in recently developed high-yielding rice (*Oryza sativa* L.) varieties in Japan. Field Crops Research. 150: 74-82.

Zheng, Y-M., Y-F. Ding., Z-H. Liu, and S-H. Wang. 2010. Effects of panicle nitrogen fertilization on non-structural carbohydrate and grain filling in *indica* rice. Agricultural Sciences in China. 9: 1630-1640.