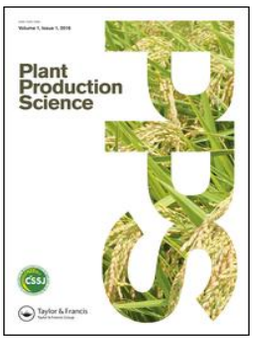

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Suppression of starch accumulation in 'sugar leaves' of rice affects plant productivity under field conditions

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ABSTRACT

While many plants accumulate the majority of their photoassmilates as starch during the daytime, some plants accumulate sucrose. Although the existence of these high-sucrose leaves, called 'sugar leaves', has long been known, the physiological characteristics of sugar leaves compared to 'starch leaves' remain unclear. In this study, the physiological roles of starch accumulation in rice, which has typical sugar leaves, were investigated using a mutant with suppressed leaf-starch biosynthesis. When grown under controlled conditions with light intensity of 400 μmol m^{-2} s^{-1}, the initial growth of the mutant was similar to that of the wild-type plant, even with a 6-h-light/18-h-dark photoperiod in which carbon resources for growth are required during the night. This finding indicates that rice does not rely on leaf starch as a carbon resource during the night. By contrast, under field conditions, the grain yields of the mutant were significantly lower than those of the wild type only when the plants were exposed to full sunlight during the ripening period. These results may indicate that starch accumulation in sugar leaves plays an important role in maintaining a high source capacity under sufficient light conditions rather than as a carbon resource for the plant's growth at night.

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Crop Physiology

Introduction

In many plants, the majority of photoassimilates are stored in the leaves as starch during the day. Starch is degraded and converted to sugar, transported from source to sink, and utilized as a carbon resource for continuous metabolism and growth at night (Stitt & Zeeman, 2012). Therefore, adequate daytime accumulation of starch and its optimized linear degradation at night are thought to be indispensable for stable growth (Graf et al., 2010; Mugford et al., 2014; Yazdanbakhsh et al., 2011). This is supported by the fact that, in *Arabidopsis thaliana*, the growth rates of mutants defective in starch biosynthesis in the leaf, due to the loss-of-function of either the gene encoding phosphoglucomutase (PGM; EC 5.4.2.2) or the small subunit of ADP-glucose pyrophosphorylase (AGP; EC 2.7.7.27), are reduced compared to wild-type plants in 12-h-light/12-h-dark conditions but not in continuous light conditions (Caspar et al., 1985; Lin et al., 1988). A similar reduction in growth rate was observed in an *A. thaliana* mutant defective in starch degradation due to loss-of-function of glucan, water dikinase (GWD; EC 2.7.9.4) (Caspar et al., 1991). Similar to *A. thaliana*, a PGM mutant in *Nicotiana sylvestris* shows a reduced growth rate in comparison to the wild type in 12-h-light/12-h-dark and 7-h-light/17-h-dark conditions but not in 16-h-light/8-h-dark or continuous light conditions (Hanson & McHale, 1988; Huber & Hanson, 1992). However, in *Lotus japonicus*, a PGM mutation do not reduce growth even in the 12-h-light/12-h-dark condition, while a GWD mutation does in both the 16-h-light/8-h-dark and 12-h-light/12-h-dark conditions (Vriet et al., 2010). These observations indicate that the importance of starch accumulation and degradation varies among plant species.

In rice (*Oryza sativa*), loss-of-function of the gene encoding the large subunit of AGP leads to a defect in starch biosynthesis in the leaf, but does not affect vegetative growth or grain yield under 12-h-light/12-h-dark controlled conditions (Rösti et al., 2007). Our previous study of a GWD mutant revealed that a defect in starch degradation does not affect vegetative growth in 14-h-light/10-h-dark (controlled) and natural light (field) conditions, but reduces grain yield by 20–30% compared to wild-type in

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field conditions (Hirose et al., 2013). In maize (Zea mays), neither the loss-of-function of the small subunit of AGP nor RNAi-mediated suppression of GWD gene affect plant growth in 12-h-light/12-h-dark or natural light conditions, in spite of suppressed or elevated starch accumulation in leaves, respectively (Slewinski et al., 2008; Weise et al., 2012). Based on these results, it appears that grasses do not rely so heavily as *A. thaliana* and *N. sylvestris* on leaf starch as a carbon resource during the night.

These apparent differences in the roles of starch accumulation in the leaf may be explained by differences in the sucrose/starch ratio in the leaves; the ratio is much higher in rice than the dicot plants mentioned above. Although it has been known for decades that the former are 'sugar leaves' and the latter are 'starch leaves' (Stitt et al., 1987), the difference in leaf photosynthesis characteristics, source capacity, and/or plant productivity between sugar leaves and starch leaves remains unclear, mainly due to our lack of knowledge of sugar leaves in comparison with starch leaves, such as those in *A. thaliana*. To clarify the primary role of starch accumulation in sugar leaves, we analyzed the growth characteristics of a rice mutant suppressed in leaf-starch biosynthesis due to loss-of-function of *OsAGPL3* (synonymous with *OsAPL1*; Os05g0580000), a gene encoding the large subunit of AGP (Rösti et al., 2007; Okamura et al., 2015). Furthermore, we examined the agronomic importance of leaf starch in rice based on three years of field experiments with the mutants.

Materials and methods

Plant materials and growth conditions

The *Tos17* insertion mutant line (NG7528), the same line used in the previous study (Okamura et al., 2015), was developed from rice (*O. sativa* var. japonica cv. Nipponbare) at the National Institute of Agrobiological Sciences, Ibaraki, Japan. The mutant line was identified in the Rice *Tos17* Insertion Mutant Database (http://tos.nias.affrc.go.jp/; Miyao et al., 2003). We compared the homozygous *OsAGPL3* mutant lines, referred to as *agpl3*, with the homozygous wild-type lines, referred to as WT, segregated from the same *Tos17* insertion line.

For the evaluation of initial growth under various photoperiods, rice plants were grown in a growth chamber (27/23 °C, photosynthetic photon flux density (PPFD) 400 μmol m^{-2} s^{-1}). Seeds were sterilized in a 2.5% (v/v) sodium hypochlorite solution for 20 min, followed by soaked in water at 30 °C for 3 days. Then, uniformly germinated seeds were selected and sown on plastic seedling cases filled with nursery soil for rice. For the field experiment, rice plants were grown during the summer months from 2011 to 2013 (Supplementary Table S1) in an experimental paddy field at the Institute for Sustainable Agro-ecosystem Services (ISAS), The University of Tokyo (35°44′N, 139°32′E, altitude: 58 m), Tokyo, Japan. One-month-old seedlings, grown in a greenhouse, were transplanted into the paddy field in late May. The plant density was 22.2 hills m^{-2} (hill spacing: 0.30 m × 0.15 m) with one seedling per hill. Compound fertilizer for paddy fields ($N:P_2O5:K_2O$ = 12:16:18) was applied at 50 g m^{-2} as a basal dressing. The plot size was larger than 3.15 m^2 with two or three replicates. In addition to the control plot with standard management, a shading treatment and thinning treatment plot were set up in 2011–2013 and 2013, respectively. In the shading plot, rice plants were covered with 35% shade cloth from full heading to harvest stage; in the thinning plot, half of the plants were thinned at the full-heading stage and the plant density was reduced to 11.1 hills m^{-2} (hill spacing: 0.30 m × 0.30 m) to increase the light income that each plant received.

AGP activity assay and determination of carbohydrate content

AGP activity assays and determination of carbohydrate content were conducted as described previously in Okamura et al. (2013, 2016), respectively.

Analysis of growth characteristics and yield components

Plant height was measured from the soil surface to the highest leaf tip. To measure dry weight, the aerial portions of plants were harvested and divided into leaf blades and stems (i.e. leaf sheaths and culms). These parts were subsequently dried at 80 °C for a minimum of one week. To measure unhulled grain yield, panicles were harvested at maturity and air-dried for at least one week. The ripened grains were selected by sinking unhulled grains in a salt solution with a density of 1.06 g mL^{-1}, thereafter counted by hands and weighed on air-dried basis.

Measurement of photosynthetic CO_2 assimilation rate

Photosynthetic gas exchanges of the flag leaves of the main stems in a paddy field were measured from 1000 to 1200 h using a CIRAS-3 Photosynthesis Systems (PP systems, Amesbury, MA, USA), which allows environmental conditions inside a chamber clamped a leaf to be precisely controlled. Air temperature in the chamber was set at 30 °C, CO_2 concentration was 390 mL L^{-1} and photosynthetically active radiation was 1500 μmol m^{-2} s^{-1}.

Results

Growth characteristics and carbohydrate contents under various photoperiods

Rösti et al. (2007) reported that the knockout mutant of *OsAGPL3* has lower leaf-starch content than wild-type rice, but shows no difference in growth or grain yield under

12-h-light/12-h-dark conditions. However, if starch in rice leaves is used as a carbon resource during the night, as has been shown for *A. thaliana*, a reduction in leaf starch might affect the growth of rice plants under shorter photoperiods (Graf et al., 2010; Mugford et al., 2014; Yazdanbakhsh et al., 2011). To test this hypothesis, we examined the *OsAGPL3* mutant, *agpl3*, and wild type (WT), segregated from the same *Tos17* insertion line, under different photoperiods (i.e. 14-h-light/10-h-dark, 10-h-light/14-h-dark, 6-h-light/18-h-dark) with the PPFD of 400 µmol m^{-2} s^{-1}.

As shown in Figure 1, there was no significant difference in plant height, leaf number, stem number, or shoot dry weight between WT and *agpl3* under any of the photoperiods tested. The starch contents at the end of the light period (EL) were much lower in *agpl3* than WT, regardless of the photoperiod, corresponding with previous reports (Okamura et al., 2015; Rösti et al., 2007; Figure 2(a)). The sucrose content at EL was similar between WT and *agpl3* under the 6-h-light/18-h-dark photoperiod, but significantly higher in *agpl3* than WT under the longer photoperiods and this difference was greater under the 14-h-light/10-h-dark than the 10-h-light/14-h-dark condition (Figure 2(b)). There was no difference in the leaf NSC (i.e. sum of starch and soluble sugars) content at EL except in 6L/18D (Figure 2(c)).

Growth characteristics before heading under field conditions

In the flag leaf at heading stage, AGP activity and starch content were much lower in *agpl3* while the sucrose contents were higher (Figure 3(a)). In the leaf sheath (the second leaf from the flag leaf) at heading stage, there was no significant difference in starch content, while AGP activity was lower in *agpl3* (Figure 3(b)). In both the culm (the second internode from the neck internode) at the heading stage and in the endosperm 10 days after heading, there were no significant differences in AGP activity or carbohydrate contents (Figure 3(c) and (d)). Plant height and stem number of *agpl3* were similar to those of WT, except that plant height at 42 and 52 days after transplanting was slightly higher in *agpl3* (Figure 4).

The effect of shading and thinning treatment on grain filling

In the field experiments, we also measured shoot dry weight, grain yield and stem NSC content of WT and *agpl3* under two different ripening condition across three years to evaluate the effects of different light conditions on the productivity of *agpl3*. While there was no difference between the two lines in shoot dry weights at heading stage, those at harvest and grain yield tended to be lower

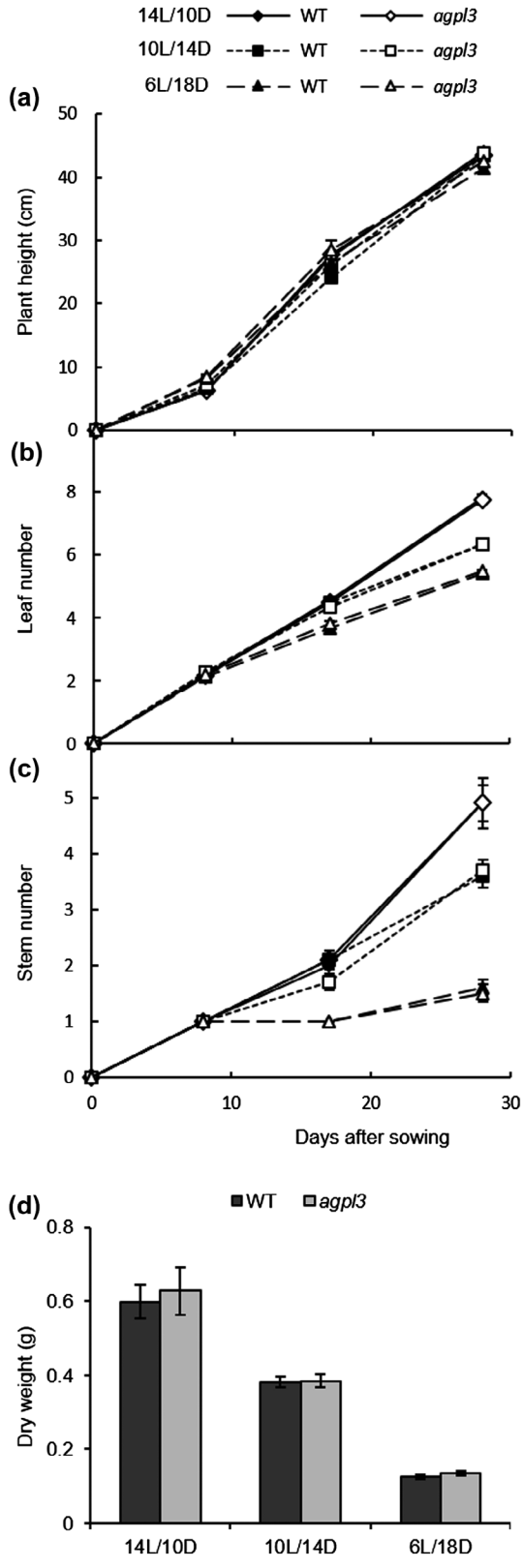

Figure 1. Initial growth of *agpl3* in various photoperiods. (a) Plant height. (b) Leaf number. (c) Stem number. (d) Dry weight of aerial portion 28 days after sowing. '14L/10D', '10L/14D' and '6L/18D' mean photoperiod of 14-h-light/10-h-dark, 10-h-light/14-h-dark and 6-h-light/18-h-dark, respectively. Values are means ± SE (n = 10). There was no significant difference between WT and *agpl3* ($p < 0.05$), by t-test in all measured components.

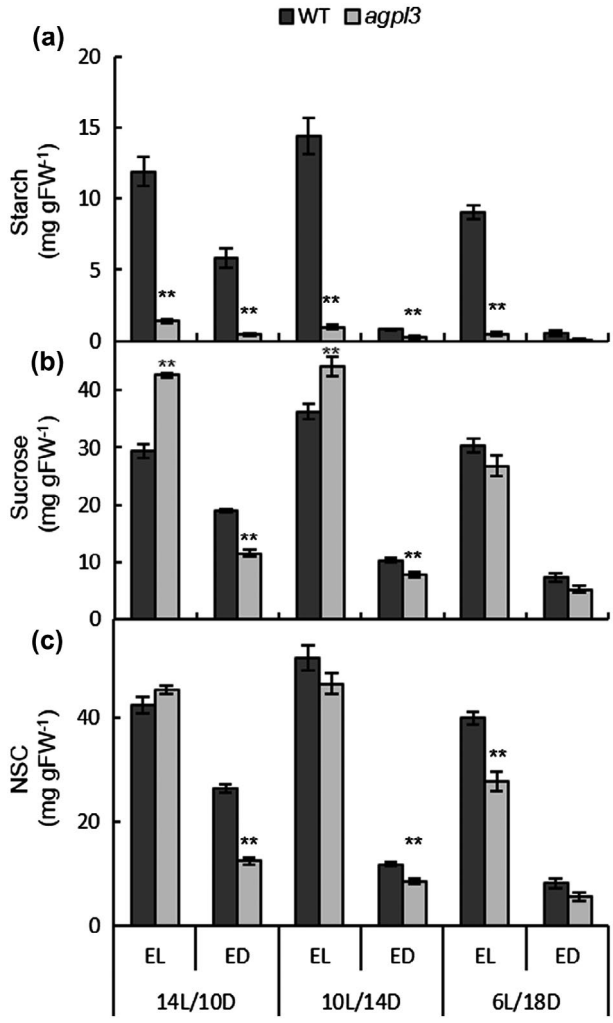

Figure 2. Carbohydrate contents in the leaf blade of *agpl3* in various photoperiods. (a) Starch content. (b) Sucrose content. (c) NSC content. Leaves were harvested from the uppermost expanding leaves at 28 days after sowing. '14L/10D', '10L/14D' and '6L/18D' mean photoperiod of 14-h-light/10-h-dark, 10-h-light/14-h-dark and 6-h-light/18-h-dark respectively. 'EL' is the end of light period, and 'ED' is the end of dark period. Values are means ± SE ($n = 10$). '**' represents significant difference between WT and *agpl3* at $p < 0.01$, by *t*-test.

in *agpl3* than WT across all three years in the control plots (Table 1, also see Supplementary Table S2). In the shading plots, however, the differences in shoot dry weight at harvest and grain yield were not observed in all three years examined. Stem NSC content at harvest of *agpl3* also tended to be lower in the control plot but not in the shading plot (Table 2). Although significant difference between lines by analysis of variance (ANOVA) were not found for grain yield and stem NSC content at harvest, significant interactions between treatment and line were found, suggesting these traits were lower in *agpl3* than in WT only under the control plots.

To evaluate the effects of increasing light more than control plots, we arranged the thinning plots only in 2013. In the thinning plots, the differences between lines in shoot dry weight at harvest, grain yield and stem NSC content at harvest were greater than those in the control plot in that year (Tables 3 and 4, also see Supplementary Table S3). A significant interaction between treatment and line were found for grain yield.

Photosynthetic CO_2 assimilation rate during grain filling

As shown in Figure 5, photosynthetic CO_2 assimilation rate and stomatal conductance under ambient CO_2 concentration (390 μmol mol^{-1}) and sufficient light (PPFD 1500 μmol m^{-2} s^{-1}) of *agpl3* were similar to those of WT from heading to harvest regardless of treatments.

Discussion

Leaf-starch of rice does not play an important role as a transient carbon storage

In some plants with 'starch leaves,' such as *A. thaliana* or *N. sylvestris*, mutants defective in starch biosynthesis or degradation have reduced growth rates compared to the wild type only under shorter photoperiods (Caspar et al., 1985, 1991; Hanson & McHale, 1988; Huber & Hanson, 1992; Lin et al., 1988; Stitt & Zeeman, 2012). These phenotypes were explained by a shortage in the transient storage of carbon during the day, hindering normal growth during longer night periods. In rice, however, the *agpl3* mutant defective in leaf-starch biosynthesis did not show a reduced growth rate compared to wild type, even under extremely long night photoperiods (Figure 1). One possible explanation for this observation is that rice, as a sugar-leaf plant, depends largely on sucrose, rather than starch, for transient storage of carbon resources in leaves. In support of this hypothesis, the nocturnal decrease of starch in *agpl3* leaves was very small compared to that of sucrose, and the NSC content in leaves at the end of light period, which reflects transient carbon storage, showed no difference between *agpl3* and WT under both long and short photoperiod (14-h-light/10-h-dark and 10-h-light/14-h-dark) due to the increased sucrose content (Figure 2(c)). Even under an extremely long night photoperiods (6-h-light/18-h-dark), *agpl3* showed only a 31% reduction in NSC compared to WT. Under these conditions, where growth was very restricted even in WT, the carbohydrate (mainly sucrose) accumulation of *agpl3* would be enough to maintain growth rates comparable to WT.

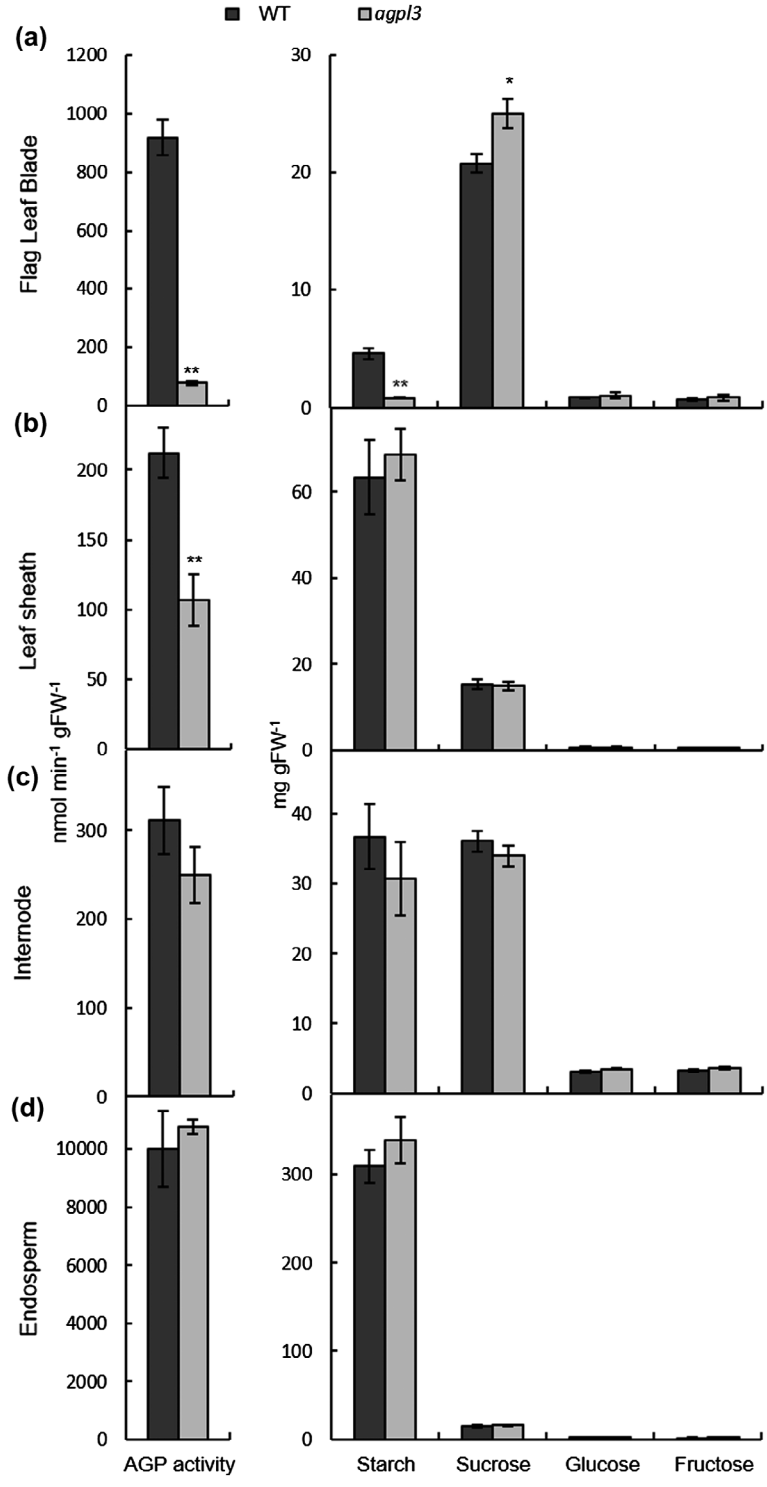

Figure 3. Carbohydrate content of various tissues of *agpl3* from 1600 to 1800 h. (a) Flag leaf blade at heading stage. (b) Second leaf sheath from the flag leaf at heading stage. (c) Second internode from the neck internode at the heading stage. (d) Endosperm 10 days after heading of the main stems, weighing 15 ± 2 mg per endosperm. The data from 2012 are shown as similar results were obtained in the other years. Values are means ± SE ($n = 4$). '**' and '*' represent significant difference between WT and *agpl3* at $p < 0.01$ or $p < 0.05$, respectively, by *t*-test.

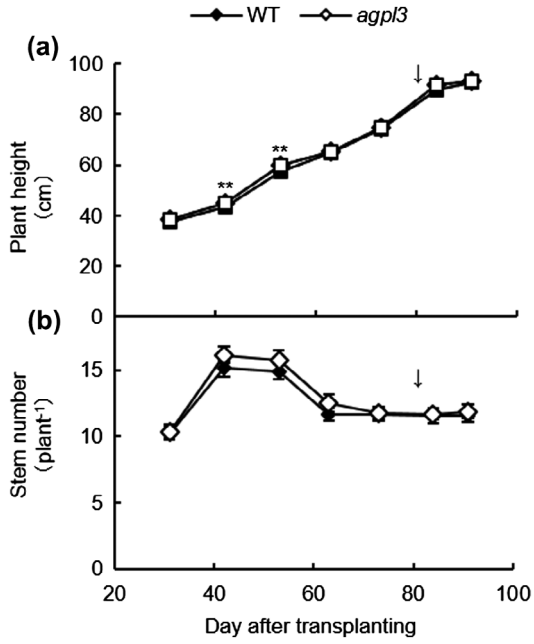

Figure 4. Vegetative growth of *agpl3*. (a) Plant height. (b) Stem number. The data from 2012 are shown as similar results were obtained in the other years. The arrow indicates the heading period. Values are means ± SE ($n \geq 8$). '**' represents significant difference between WT and *agpl3* at $p < 0.01$, by *t*-test.

Leaf-starch accumulation is indispensable for normal grain filling under full-sunlight field conditions

Although there was no difference between *agpl3* and WT in shoot dry weight at heading, shoot dry weight at harvest tended to be lower in *agpl3* and grain yield was lower in the untreated, control plot (Table 1). These reductions in the dry matter production and yield of *agpl3* were greater in the thinning treatment plot where light income for each individual plant was increased (Table 3), but not seen in the shading treatment plot where light income was decreased by 35% (Table 1). In the field experiments presented here, the cumulative solar radiation during the ripening stage (from August to September) was much higher than during the vegetative stage (from June to July) (data not shown). Thus, in the control and thinning plots, the high solar radiation might reduce the source capacity of *agpl3* leaves that support normal biomass production and grain filling during the ripening stage, due to the deficiencies in starch accumulation.

Grain filling of rice is affected not only by the capacity of the source (leaves) but also by activity in the sink, such as starch biosynthesis in endosperm. Although it is

Table 1. Shoot dry weight and grain yield of *agpl3* in control and shading plots.

Treatment	Year	Line	Shoot dry weight (gDW plant^{-1})			Grain yield (g plant^{-1})
			Heading	Middle	Harvest	
Control	2011	WT	29.6 ± 1.3	45.3 ± 3.8	48.9 ± 2.6	24.2 ± 1.2
		agpl3	31.3 ± 1.2	44.7 ± 2.7	43.7 ± 2.0	21.5 ± 0.7
		agpl3/WT	1.06	0.99	0.89	0.89
	2012	WT	29.6 1± 1.7	48.1 ± 1.8	53.4 ± 3.1	24.8 ± 1.3
		agpl3	29.0 ± 0.9	50.0 ± 2.3	50.1 ± 3.1	21.5 ± 1.1
		agpl3/WT	0.98	1.04	0.94	0.87
	2013	WT	31.0 ± 2.0	42.8 ± 1.9	42.5 ± 2.0	20.4 ± 0.9
		agpl3	29.6 ± 1.4	41.5 ± 2.3	41.4 ± 2.2	19.3 ± 0.8
		agpl3/WT	0.95	0.97	0.97	0.94
	mean	WT	30.1	45.4	48.3	23.1
		agpl3	30.0	45.4	45.1	20.8
		agpl3/WT	1.00	1.00	0.93	0.90
Shading	2011	WT	31.7 ± 2.0	36.0 ± 4.2	47.3 ± 5.2	19.0 ± 2.1
		agpl3	28.9 ± 2.0	36.7 ± 3.7	44.3 ± 3.4	19.0 ± 0.9
		agpl3/WT	0.91	1.02	0.94	1.00
	2012	WT	29.4 ± 1.9	44.1 ± 3.3	44.0 ± 2.7	18.5 ± 1.1
		agpl3	26.7 ± 1.5	37.4 ± 1.7	47.4 ± 3.0	18.9 ± 0.7
		agpl3/WT	0.91	0.85	1.08	1.03
	2013	WT	31.8 ± 1.3	39.0 ± 2.2	37.9 ± 2.5	17.8 ± 0.8
		agpl3	28.2 ± 1.8	37.4 ± 2.7	37.4 ± 2.4	18.7 ± 0.8
		agpl3/WT	0.89	0.96	1.00	1.05
	mean	WT	30.97	39.70	43.07	18.4
		agpl3	27.93	37.17	43.20	18.9
		agpl3/WT	0.90	0.94	1.00	1.03
ANOVA	Treatment		n.s.	*	**	**
	Year		n.s.	**	**	*
	Line		n.s.	n.s.	n.s.	n.s.
	Treatment × Year		n.s.	n.s.	**	n.s.
	Treatment × Line		n.s.	n.s.	n.s.	*
	Year × Line		n.s.	n.s.	n.s.	n.s.

Note: Heading: at heading stage, Middle: three weeks after heading, Harvest: at harvest stage. Values are means ± SE ($n \geq 6$). '**' and '*' represent significance at $p < 0.01$ or $p < 0.05$, respectively.

Table 2. Stem NSC content (g plant^{-1}) of *agpl3* in control and shading plots.

Treatment	Year	Line	Heading	Middle	Harvest
Control	2011	WT	2.89 ± 0.09	0.51 ± 0.15	3.53 ± 0.10
		agpl3	2.86 ± 0.23	0.40 ± 0.13	1.60 ± 0.18
		agpl3/WT	0.99	0.78	0.45
	2012	WT	3.01 ± 0.28	1.46 ± 0.67	4.88 ± 0.43
		agpl3	3.37 ± 0.27	1.90 ± 0.37	3.61 ± 0.60
		agpl3/WT	1.12	1.30	0.74
	2013	WT	3.33 ± 0.13	1.97 ± 0.26	2.24 ± 0.31
		agpl3	3.15 ± 0.19	2.62 ± 0.25	2.58 ± 0.25
		agpl3/WT	0.95	1.33	1.15
	mean	WT	3.08	1.31	3.55
		agpl3	3.13	1.64	2.60
		agpl3/WT	1.02	1.25	0.73
Shading	2011	WT	3.65 ± 0.30	0.07 ± 0.02	3.01 ± 0.72
		agpl3	3.93 ± 0.11	0.07 ± 0.01	2.92 ± 0.37
		agpl3/WT	1.08	1.00	0.97
	2012	WT	4.07 ± 0.28	0.66 ± 0.38	2.77 ± 0.27
		agpl3	3.21 ± 0.37	0.88 ± 0.53	2.78 ± 0.39
		agpl3/WT	0.79	1.33	1.00
	2013	WT	3.52 ± 0.42	1.15 ± 0.52	1.59 ± 0.07
		agpl3	2.92 ± 0.19	1.02 ± 0.10	1.80 ± 0.42
		agpl3/WT	0.83	0.89	1.13
	mean	WT	3.75	0.63	2.46
		agpl3	3.35	0.66	2.50
		agpl3/WT	0.90	1.07	1.04
ANOVA	Treatment		**	**	*
	Year		n.s.	**	**
	Line		n.s.	n.s.	n.s.
	Treatment × Year		n.s.	n.s.	**
	Treatment × Line		n.s.	n.s.	*
	Year × Line		n.s.	n.s.	n.s.

Note: Heading: at heading stage, Middle: three weeks after heading, Harvest: at harvest stage. Values are means ± SE ($n \geq 3$). '**' and '*' represent significance at $p < 0.01$ or $p < 0.05$, respectively.

Table 3. Shoot dry weight and grain yield of *agpl3* in control and thinning plots.

Treatment	Line	Shoot dry weight (gDW plant^{-1})			Grain yield (g plant^{-1})
		Heading	Middle	Harvest	
Control	WT	31.0 ± 2.0	42.8 ± 1.9	42.5 ± 2.0	20.4 ± 0.9
	agpl3	29.6 ± 1.4	41.5 ± 2.3	41.4 ± 2.2	19.3 ± 0.8
	agpl3/WT	0.95	0.97	0.97	0.94
Thinning	WT	32.4 ± 2.1	47.1 ± 1.8	48.3 ± 3.3	20.4 ± 1.3
	agpl3	32.4 ± 2.1	43.8 ± 1.5	43.4 ± 4.3	14.4 ± 0.6
	agpl3/WT	1.00	0.93	0.90	0.71
ANOVA	Treatment	n.s.	n.s.	n.s.	**
	Line	n.s.	n.s.	*	*
	Treatment × Line	n.s.	n.s.	n.s.	*

Note: Heading: at heading stage, Middle: three weeks after heading, Harvest: at harvest stage. Values are means ± SE ($n = 9$). '**' and '*' represent significance at $p < 0.01$ or $p < 0.05$, respectively.

Table 4. Stem NSC content (g plant^{-1}) of *agpl3* in control and thinning plots.

Treatment	Line	NSC content (g plant^{-1})		
		Heading	Middle	Harvest
Control	WT	3.33 ± 0.13	1.97 ± 0.26	2.24 ± 0.31
	agpl3	3.15 ± 0.19	2.62 ± 0.25	2.58 ± 0.25
	agpl3/WT	0.95	1.33	1.15
Thinning	WT	3.85 ± 0.11	3.53 ± 0.37	3.01 ± 0.41
	agpl3	3.78 ± 0.23	3.23 ± 0.18	2.27 ± 0.40
	agpl3/WT	0.98	0.92	0.75
ANOVA	Treatment	n.s.	n.s.	n.s.
	Line	*	**	n.s.
	Treatment × Line	n.s.	n.s.	n.s.

Note: Heading: at heading stage, Middle: three weeks after heading, Harvest: at harvest stage. Values are means ± SE ($n = 3$). '**' and '*' represent significance at $p < 0.01$ or $p < 0.05$ respectively.

reported that the mRNA level of *OsAGPL3* in endosperm was much lower than that of *OsAGPL2*, the other gene coding large subunit of AGP (Hirose et al., 2006; Ohdan et al., 2005), we cannot ignore the possibility that the loss of *OsAGPL3* affected starch biosynthesis in the endosperm, thereby reducing grain yield. However, AGP activity in the endosperm at 10 days after heading, when the grain filling rate is assumed to be maximum, was similar between *agpl3* and WT (Figure 3(c)). If starch biosynthesis in the endosperm of *agpl3* had been decreased, the surplus carbohydrates should accumulate in the stem. However, stem NSC content at harvest was lower in *agpl3* (Tables 2 and 4), indicating that the decrease in grain yield in *agpl3* was not caused by a reduction of sink activity but by a loss of source capacity.

What, then, was main factor responsible for restricting the source capacity of *agpl3* under sufficient light? One explanation may be a role of starch storage in the feedback control of photosynthesis. The relationship between feedback control of photosynthesis and starch or sucrose

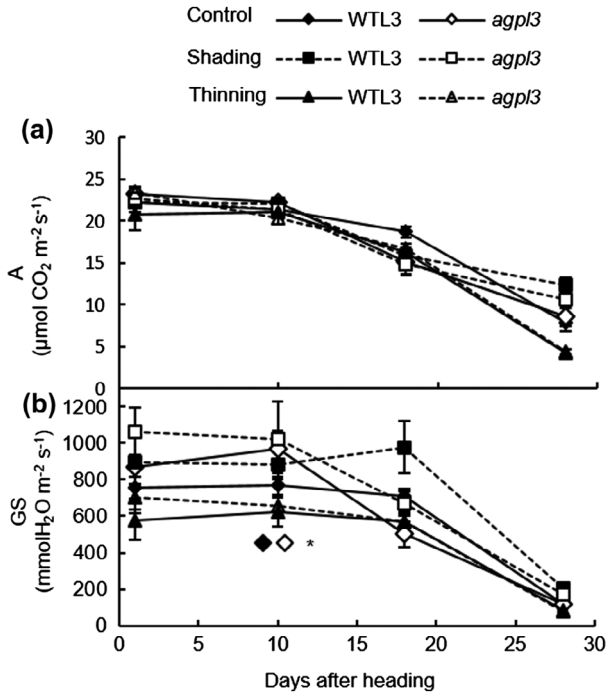

Figure 5. Photosynthetic assimilation rates (A) (a), stomatal conductance (GS) (b) in flag leaves of *agpl3* from 1000 to 1200 h. Values represent means ± s.e. ($n = 6$). Significant difference was only found between WT and *agpl3* in control condition at 10 days after heading at $p < 0.05$, by *t*-test.

biosynthesis is often explained by Pi recycling (Paul & Foyer, 2001). Considering that the leaf NSC of *agpl3* was similar to that of WT (Figure 2), sucrose biosynthesis was activated enough to compensate for the reduction of starch biosynthesis. Based on this, the amount of Pi released by sucrose or starch biosynthesis in the leaves of *agpl3* should be similar to that of WT, and the feedback inhibition of photosynthesis by Pi shortage would be unlikely to occur. For plants, soluble sugars, including sucrose, are not only a carbon resource but also signal molecules regulating expression of various genes involved in photosynthesis (Pego et al., 2000; Rolland et al., 2006). Recently, Kelly et al. (2013) reported that stomatal closure can be regulated by sugar signaling. Considering these insights, it is possible that the higher sugar accumulation in *agpl3* could modify the expression of genes involved in photosynthesis, resulting in feedback inhibition of photosynthesis. However, photosynthetic CO_2 assimilation rate under ambient CO_2 concentration and sufficient light intensity were similar between *agpl3* and WT through ripening stage (Figure 5), as far as we measured from 1000 to 1200 h to avoid midday depression of photosynthesis (Guo et al., 2009; Ishihara & Saitoh, 1987). To clarify whether the higher sucrose concentration in the leaves of *agpl3* causes feedback inhibition of photosynthesis, the diurnal change of photosynthetic rate of *agpl3* need to be measured under sufficient light conditions.

Conclusion

Although further experiments are needed to understand whole picture of the mechanism, our data presented here indicate that leaf starch in rice plays an important role in maintaining a high source capacity under sufficient light conditions, such as full sunlight in paddy fields, rather than as a carbon resource for the plant's growth at night.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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*In Japanese with English summary.