Cytokinin affects the leaf levels of ribulose-1,5-bisphosphate carboxylase/oxygenase and the accumulation and partitioning of nitrogen in paddy rice at the ripening stage

Taiichiro Ookawa, Yukiko Naruoka, Ayumi Sayama and Tadashi Hirasawa

Tokyo University of Agriculture and Technology, www.tuat.ac.jp Email ookawa@cc.tuat.ac.jp

Abstract

The high yield rice cultivar, Akenohoshi achieves a higher rate of photosynthesis during ripening than cv. Nipponbare by maintaining a higher level of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). To determine the causal factors resulting in the differences in the levels of Rubisco in leaves of these two rice cultivars and the role of cytokinins in maintenance of high levels of Rubisco in leaves, the levels of *rbcL* and *rbcS* mRNAs and the nitrogen content of leaves during ripening were investigated, as well as the absorption and partitioning of nitrogen. The results in this study indicate the two probable effects of the cytokinin, 6-benzylaminopurine (BA) on the leaf levels of Rubisco. The first is the direct effect of BA on the induction of Rubico synthesis, and the second is the indirect effect of BA on inducing the effective partitioning of nitrogen to leaves. Cytokinins may account for the differences in the reduction of the leaf levels of Rubisco during senescence between the rice cultivars.

Media summary

Cytokinins affects the level of Rubisco through retaining the levels of *rbcL* and *rbcS* mRNAs and the partitioning of nitrogen to leaves in rice plants.

Key Words

Cytokinin, Partitioning of nirogen, *rbcS* and *rbcL* mRNAs, Rubisco, Senescence.

Introduction

Dry matter production and yield are higher in the rice cultivar, Akenohoshi than in cv. Nipponbare, due in primarily to the smaller decrease in the rate of photosynthesis during the ripening stage in Akenohoshi (Jiang et al., 1988). The rate of photosynthesis and nitrogen content during senescence are both closely correlated with leaf levels of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). During leaf senescence Akenohoshi shows a smaller decrease in levels of Rubisco than Nipponbare**,** and we have previously demonstrated that Akenohoshi maintains larger amounts of nitrogen in leaves during ripening. This may account for the differences in the ability of these two cultivars to maintain high leaf levels of Rubisco (Ookawa et al., 2003). The high leaf nitrogen content in Akenohoshi results from both the greater total accumulation of nitrogen and the greater partitioning of nitrogen to leaves. In addition to the differences in Rubisco and nitrogen levels in the leaves, it was also noted that larger amounts of cytokinins are transported from the roots to the aboveground parts of the plant during the ripening stage by Akenohoshi than by Nipponbare (Soejima et al., 1995). However, it remains to be determined if cytokinin suppresses a decline in Rubisco content. In the following experiments our main aims were therefore (i) to compare the levels of Rubisco, nitrogen and *rbcL* and *rbcS* transcripts between Nipponbare and Akenohoshi, (ii) to analyze the effects of an exogenous cytokinin on levels of Rubisco, nitrogen and *rbcL* and *rbcS* transcripts in leaves of rice during the ripening stage, and (iii) to analyze the effects of an exogenous cytokinin on the nitrogen content of leaves by determining the nitrogen absorption and partitioning of nitrogen to various organs in a whole plant.

Methods

Rice seedlings (*Oryza sativa* L., cvs. Nipponbare and Akenohoshi) were transplanted to Wagner pots (1/2000 a) filled with a soil on May 27. In this study, the plants in Nipponbare were treated with additional nitrogen fertilizer and cytokinin as follows. Ammonium sulfate was applied to some pots on September 1 at a dose of 10 g per pot as additional nitrogen fertilizer (NF). For treatment with cytokinin, 30 ml of a 10^{-4}M solution of 6-benzylaminopurine (BA), containing 0.05% Tween 20 as a surfactant, were sprayed on the entire aboveground parts of each hill at two-day intervals from September 5 onwards. Levels of Rubisco and nitrogen were determined in the same flag leaf of the main stem. The level of Rubisco was determined by single radial immunodiffusion method. Nitrogen was quantitated with a CN analyzer (MT-600, Yanaco Inc., Kyoto, Japan). For quantification of mRNA, the flag leaves on the main culms were collected between 10 a.m. and 11 a.m. on a clear day and immediately frozen in liquid nitrogen. Levels of *rbcL* and *rbcS* mRNAs were determined by Northern blotting analysis.

Results

1. Comparison of levels of Rubisco and rbcL *and* rbcS *mRNAs in leaves during senescence between cultivars, Nipponbare and Akenohoshi*

The changes in levels of Rubisco and *rbcL* and *rbcS* mRNAs in leaves during ripening were compared between the two cultivars. At the heading stage there were no differences between the two cultivars, but after heading, the levels of Rubisco, *rbcL* and *rbcS* mRNAs remained higher in Akenohoshi than in Nipponbare (Fig.1).

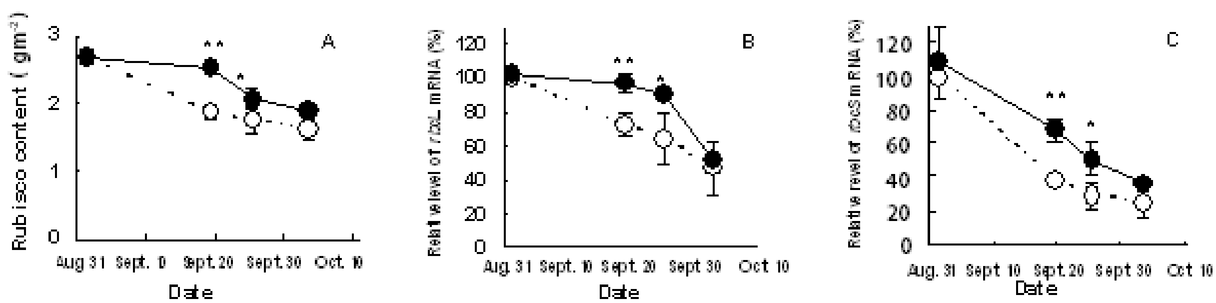

Fig.1. Changes in the Rubisco content (A) and the relative levels of *rbcL* mRNA (B) and *rbcS* mRNA (C) in flag leaves of Nipponbare and Akenohoshi. ○, Nipponbare; ●, Akenohoshi. * and **, Values for the cultivars are significantly different at the 5% and 1% level, respectively.

2. Effects of treatment with 6-benzylaminopurine (BA) and nitrogen fertilizer (NF) on levels of Rubisco and rbcL *and* rbcS *mRNAs in leaves during senescence*

In control of Nipponbare, the Rubisco content in flag leaves decreased with time after heading. Rubisco content remained much higher in the plants treated with NF or BA in Nipponbare than in the controls. The levels of *rbcL* mRNA and *rbcS* mRNA decreased with time in the controls. However, the levels remained high in the plants treated with NF or BA during ripening (Fig. 2).

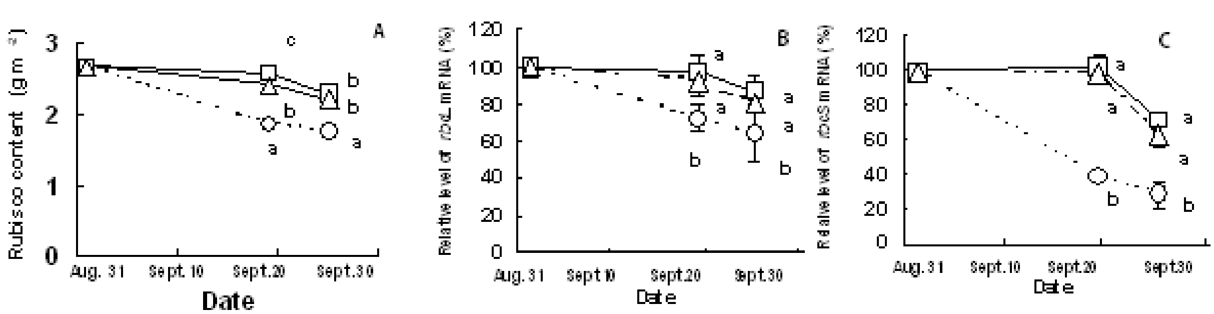

Fig. 2. Effects of 6-benzylaminopurine (BA) and additional nitrogen fertilizer (NF) application on the Rubisco content and the relative levels of *rbcL* mRNA (B) and *rbcS* mRNA (C) in flag leaves of Nipponbare.

○, Control; □, BA; △, NF. Symbols with different letters are significantly different at the 5% level (LSD).

3. Relationships between Rubisco content, levels of *rbcL* and *rbcS* mRNAs and nitrogen content

The relationships between the Rubisco content, the levels of *rbcL* and *rbcS* mRNAs and the nitrogen content were compared among the controls, BA- and NF-treated plants in Nipponbare, including the plants in Akenohoshi. There were close relationships between the Rubisco content and the levels of *rbcL* and *rbcS* mRNAs irrespective of treatments and cultivars (Fig. 3). Over a wide range of nitrogen contents, there was also a tight positive correlation between the Rubisco content and the nitrogen content (Fig. 4). There was also a close correlation between the nitrogen content and the levels of both *rbcL* and *rbcS* mRNA (Fig. 5).

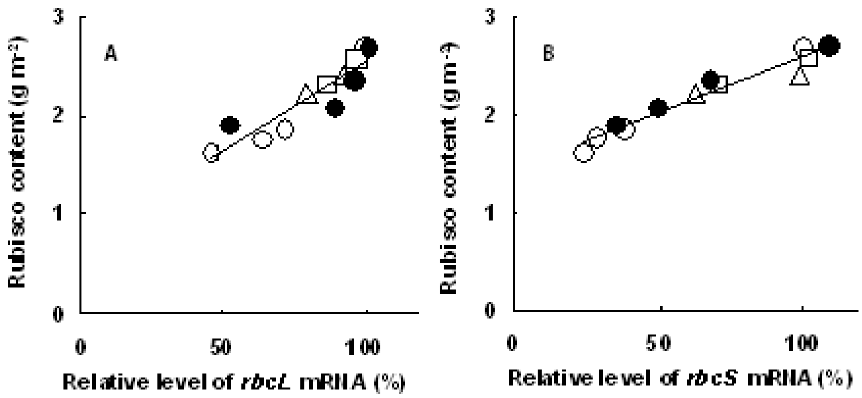

Fig.3. Relationship between the relative levels of *rbcL* mRNA (A) and *rbcS* mRNA (B) and the Rubisco content in flag leaves. ○, Control; □, BA; △, NF; ●, Akenohoshi.

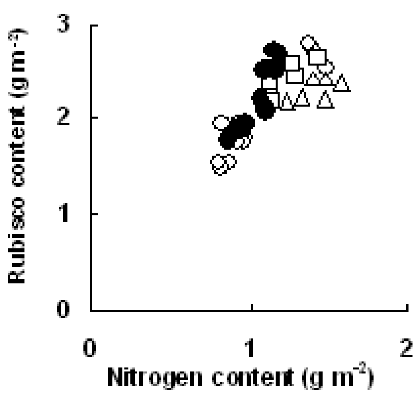

Fig. 4. Relationship between the nitrogen content and the Rubisco content in flag leaves. ○, Control; □, BA; △, NF; ●, Akenohoshi.

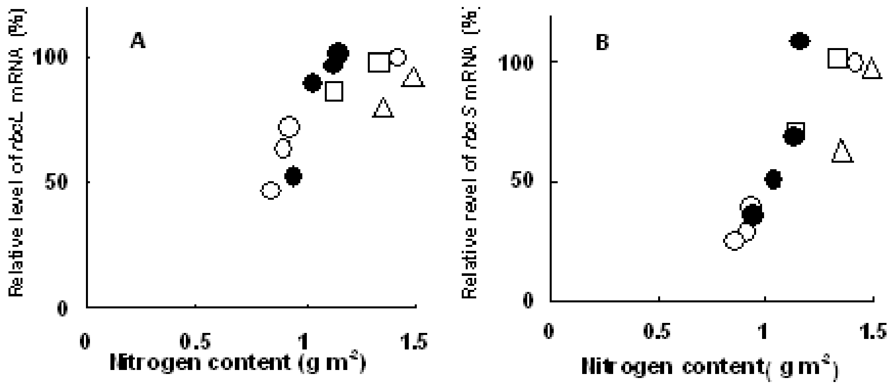

Fig. 5. Relationship between the nitrogen content and the relative levels of *rbcL* mRNA (A) and *rbcS* mRNA (B) in flag leaves. ○, Control; □, BA; △, NF; ●, Akenohoshi.

4. Effects of BA and NF on the accumulation and partitioning of nitrogen

The high leaf nitrogen content was maintained not only in NF-treated plants of Nipponbare but also in BA-treated plants of Nipponbare. The amount of total accumulated nitrogen at the late-ripening stage was much larger after NF treatment as compared with controls (Table 1), and the partitioning of nitrogen to leaves increased significantly as a result of NF treatment. Therefore, the maintenance of high leaf nitrogen content in NF-treated plants resulted not only from an increase in total nitrogen accumulation by the entire plant but also from an increase in nitrogen partitioning to leaves. There was no difference, in terms of the increase in the nitrogen content of entire plants, between BA-treated plants and controls from the heading stage to the late-ripening stage. The partitioning of nitrogen to leaves at the late-ripening stage was considerably higher in BA-treated plants than in controls, but less nitrogen was partitioned to panicles in the former than the latter (Table 1). These results showed that maintenance of the high leaf nitrogen content in BA-treated plants was caused by an increase in the nitrogen partitioning to leaves and a decrease in the partitioning to panicles, rather than an increase in accumulation of nitrogen by the entire plant.

Table 1 Comparisons of nitrogen content, changes in nitrogen content and nitrogen partitioning to various organs in entire plants.

	Nitrogen content (mg hill^{-1})		Changes in nitrogen content	Nitrogen partitioning (%)	
Organ	Heading (Aug. 31)	Late ripening (Sept. 25)	(mg hill^{-1})	Heading (Aug. 31)	Late ripening (Sept. 25)
			Whole plant		
Control	592.4?35.1	635.7?19.3 a	43.3	100	100
BA	—	653.9?15.8 a	61.5	—	100

NF	—	1024.6?6.2 b	432.2	—	100
Leaves					
Control	292.4?12.1	142.9?4.9 a	-149.5	49.4?1.0	22.6?0.3 a
BA	—	238.7?3.2 b	-53.7	—	36.5?1.3 b
NF	—	307.2?3.5 c	14.8	—	30.0?0.6 c
Culms + leaf sheaths					
Control	202.0?18.2	114.1?3.1 a	-87.9	34.0?1.0	17.9?0.5 a
BA	—	115.2?0.3 a	-86.8	—	17.6?0.4 a
NF	—	202.3?12.2 b	0.3	—	19.7?1.0 b
Panicles					
Control	58.2?1.6	328.7?8.9 a	270.5	9.8?0.4	51.5?0.7 a
BA	—	259.7?18.8 b	201.5	—	39.8?2.0 b
NF	—	459.4?3.1 c	401.2	—	44.8?0.5 c
Roots					
Control	39.8?4.2	50.1?7.4 a	10.3	6.8?0.4	8.0?1.0 a
BA	—	40.3?1.6 b	0.5	—	6.2?0.4 b
NF	—	55.7?0.6 a	15.9	—	5.5?0.0 b

Data represent means ? standard deviations of results from three replicates. Nitrogen partitioning is expressed as the nitrogen content of the indicated organs as a percentage of the total nitrogen content of whole plant. Means followed by different letters are significantly different at the 5% level of provability (LSD).

Conclusion

Cytokinin affects both the synthesis of Rubisco in leaves and the partitioning of nitrogen to leaves in rice plants during ripening. This suggests that cytokinin causes the differences in the decrease in Rubisco during senescence, resulting in differences in the reduction of photosynthetic rates between Akenohoshi and Nipponbare. Therefore, the capacity of roots for cytokinin synthesis is one of the important determinants of the ability of rice plants to maintain the rate of photosynthesis in leaves during ripening in the high-yield cultivars of rice.

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