High nitrogen conditions enhance the cooling damage to pollen in rice plants : proteome analysis of mature anthers

Takami Hayashi[1], Tomoya Yamaguchi[1], Katsuhiro Nakayama[1], Setsuko Komatsu[2] and Setsuo Koike[1]

[1] National Agronomy Research Center for Tohoku Region, Morioka 020-0198, Japan.
http://tohoku.naro.affrc.go.jp
Email takami@affrc.go.jp
[2] National Institute of Agrobiological Science, Tsukuba 305-8602 Japan. www.nias.affrc.go.jp

Abstract

We used proteome analysis to investigate the cooling damage on anthers of rice plants grown under high nitrogen conditions. Proteins were extracted from mature anther samples and separated by two-dimensional gel electrophoresis. The anther proteome maps of different treatments were compared and 20 protein spots, which were changed by the treatments, were found. These protein spots were identified based on the rice proteome database and/or digested with trypsin for peptide mass fingerprinting (PMF) analysis. Digested samples were analyzed by matrix-assisted laser desorption/ionization-time flight mass spectrometry (MALDI-TOF MS) to produce PMF data. Database searches using these PMF data revealed the identities of 16 proteins. These proteins included polypeptides involved in carbon metabolism, nitrogen metabolism and stress responses.

Media summary

High nitrogen conditions enhance cooling damage to pollen in rice. Proteome analysis was conducted to investigate proteins related to this phenomenon.

Key Words

Cool temperature sensitivity, pollen development, sterility, proteome analysis, rice

Introduction

In northern Japan, cool temperature in summer often causes a serious decrease in the yield of rice plants mainly because of unsuccessful fertilization due to damaged anthers. A sufficient nitrogen supply is necessary for optimal plant growth and yield in rice. In cool summers, however, a higher number of sterile spikelets are found in rice plants which are grown under high nitrogen conditions, resulting in a decrease in yield. This decrease in yield is a major problem in rice production, but the physiological mechanism of the increased sterility is still unknown.

The young microspore stage is most sensitive to cool temperatures during the reproductive period, (Hayase et al. 1969, Satake and Hayase 1970). Cool temperatures at this stage lead to decrease in the number of microspores and pollen grains (Satake 1991), and the number of pollen grains is highly correlated with fertility (Nishiyama, 1982). The decrease in the number of pollen grains in anthers caused by cooling treatments was enhanced under high nitrogen conditions (Hayashi et al. 2000). However, these anthers still contain over 100 pollen grains. Effects on the pollen germination ratio on the stigma suggested that high nitrogen supply enhanced the impact of cooling damage on pollen grains (Hayashi et al, 2000). In this study we investigated the combined effects of high nitrogen supply and cooling on protein patterns in mature anthers.

Methods

Plant materials

Hayayuki, a cold tolerant, early japonica rice variety from Hokkaido, was used. Twenty seeds per pot were sown in a circular pattern (Satake et al. 1969). Plants were grown under conditions of 14 hour day length and day/night temperature regime of 24/19 C until the young microspore stage. Plants were grown with a nutrient culture solution (Satake and Koike 1983) that included a standard level of nitrogen (10ppm). At the spikelet differentiation stage, half of the pots were transferred to high (80ppm) nitrogen conditions. To obtain uniform samples, the third to the fifth spikelets from the top on the first and the second primary branches of the main clums were examined.

At the young microspore stage, plants which were to undergo a cool temperature treatment were transferred to a cool chamber (12/12 C) for 5 days, then transferred back to the 24/19 C chamber. Nutrient solution supply was discontinued after the end of flowering.

2D-electrophoresis and protein identification

Mature anthers were collected and kept at -80 C. One hundred to 150 anthers were homogenized with lysis buffer and centrifuged twice at 13000g for 5 min. The supernatant was used for the two dimensional electrophoresis.

The proteins on preparative gels were stained with CBB solution. The stained gels were compared and protein spots changed by the treatments were investigated. Proteins were identified using the rice proteome database or were analysed by MALDI-TOF MS, then by PMF analysis.

Results

When plants were not cooled, 50 to 150 pollen grains were found on each stigma and fertility of those plants was over 95%, while in cooled plants there were 50 to 100 pollen grains and 50 to 80% fertility (Fig.1). In rice, over 50 pollen grains on the stigma is required for satisfactory fertilization. Cooled spikelets with this number of pollen grains on the stigma, however, showed lower fertility, especially under high nitrogen conditions. This suggests that pollen grains in cooled plants under high nitrogen conditions were damaged physiologically even if they appeared normal under microscopic observation.

Therefore, proteome analysis was conducted to investigate the physiological changes associated with high nitrogen treatments and cooling. The protein pattern of the 2D-electrophoresis is shown in figure 2. Twenty spots were changed by high nitrogen and cooling treatments and 16 protein spots were identified (Table 1). These proteins included polypeptides involved in carbon metabolism, nitrogen metabolism and stress responses.

(a) Carbon metabolism.

Glycelaldehyde 3-phosphate dehydrogenase(607) was upregulated by high nitrogen conditions and was downregulated by high nitrogen conditions combined with cooling treatments.
Fructokinase(722) and Starch synthase(921) were upregulated by high nitrogen conditions combined with cooling treatments. Phosphoenolpyruvate carboxylase(953) was downregulated by high nitrogen conditions combined with cooling treatments.

(b) Nitrogen metabolism

NADH dependent Glutamate Synthase(761) was upregulated under high nitrogen treatments.

(c) Stress responses

Calcium-dependent protein kinase(442) and heat shock protein 82(912) were upregulated by high nitrogen conditions and downregulated by high nitrogen conditions combined with cooling. Aldehyde dehydrogenase (839) was upregulated under high nitrogen conditions.

Several proteins related to stress responses were upregulated under high nitrogen conditions, suggesting that rice plants with high nitrogen supply were either responding in a manner indicative of stressful conditions or had higher rates of protein turnover. Plants over expressing calcium-dependent protein kinase have been shown to be tolerant to low temperature treatments (Saijo et al. 2000). In our study, this protein was downregulated by cooling, suggesting links to the cooling damage on pollen under high nitrogen conditions.

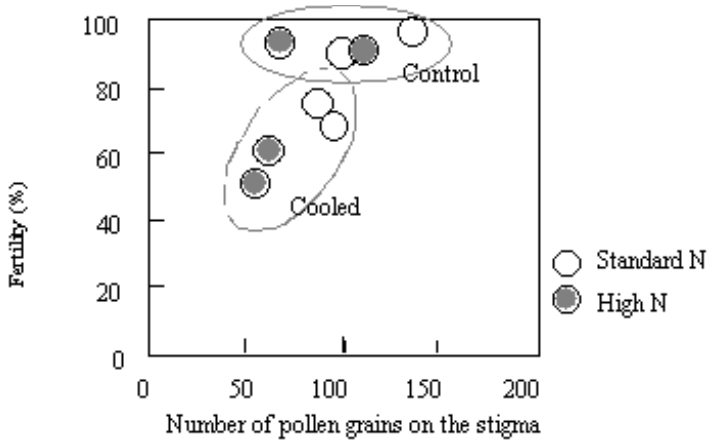

Figure 1. The relation between % fertility and number of pollen grains on the stigma for the experimental treatments (after Hayashi et al. 2000).

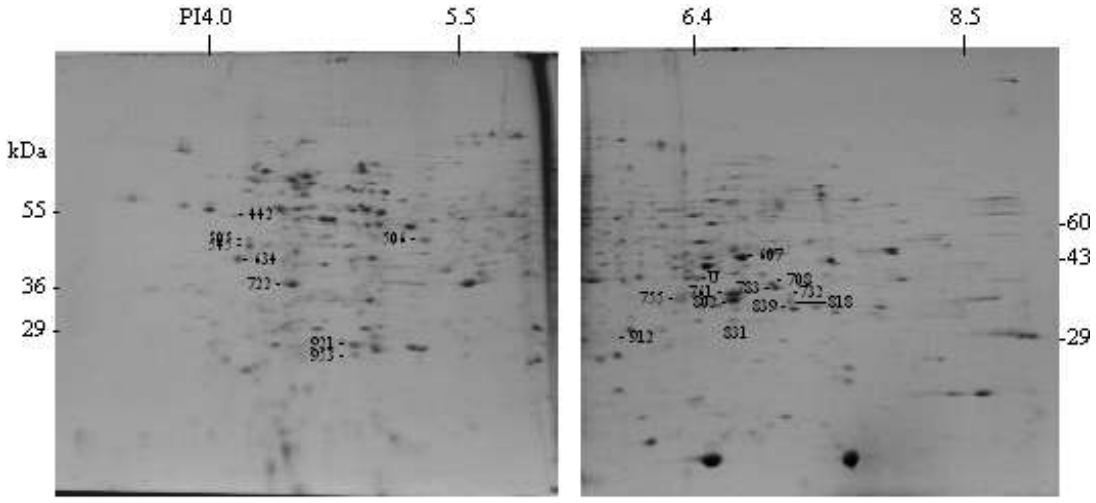

Figure 2. Protein patterns of mature rice anthers from the high N treatment. Additional spots are numbered based on the rice proteome database (http://gene64.dna.affrc.go.jp/RPD/)

Table 1. Results of protein identification

Spot No.		High N[1]	High N Cooled[2]	Accession	Homologous Protein
783	*	+	+	AF391106	Oryza sativa beta-expansin (EXPB13) gene

442		+	-	P53684	Calcium-dependent protein kinase, isoform 11 (EC 2.7.1.-)
505		+	-		not identified
607	*	+	-	Q09054	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic (EC 1.2.1.12)
708	*	+	-	Ab040052	mEF-G gene for mitocondrial elongation factor G, complete cds"
732	*	+	-	AF394453	Oryza sativa alpha-expansin
912		+	-	Z15018	O.sativa hsp82 gene for heat shok protein 82
634		-	+	AC1454	protein gp18 from Bacteriophage A118 homolog
802	*	-	-	P31417	Fatty acid binding protein
545		ns	+		not identified
722	*	ns	+	Q944F5	Putative fructokinase II.- Oryza sativa (Rice).
921	*	ns	+	AF395537	soluble starch synthase II-2 mRNA.(uncultured bacterium)
506		ns	-		not identified
953	*	ns	-	Q94QB2	Phosphoenolpyruvate carboxylase

761	*	+	ns	AB008845	mRNA for NADH dependent Glutamate Synthase
831	*	+	ns	AF323610	glucanase (GLU) mRNA
839	*	+	ns	AF148877	putative aldehyde dehydrogenase OS-ALDH
U1		+	ns		not identified
755	*	-	ns	U25664	histone H3 gene.
818	*	-	ns	X95271	matrix association region

[1] Results (enhanced (+), reduced (-), not different (ns)) based on comparison with control

[2] Results based on comparison with high nitrogen conditions.

* Indicates identifications based on the rice proteome database.

In the carbon metabolism category, fructokinase, which is upregulated several days before heading (Kerim et al. 2003), was upregulated in this study in the cooling treatments. Zujian et al. (2001) showed that number of pollen grains was decreased by gibberellin treatments and gibbellerin upregulated fruktokinase in the rice leaf sheath (Tanaka et al. 2004). This suggests a possible association of the cooling effect with hormonal triggers.

Conclusion

Proteome analysis on mature anthers of rice plants showed that proteins involved in carbon metabolism, nitrogen metabolism and stress responses were changed by high nitrogen and cooling treatments.

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