How do plants respond to grazing at a molecular level?

Gongshe Liu, Xin Huang, Shuangyan Chen

Key Laboratory of Plant Resources, Institute of Botany, the Chinese Academy of Sciences, Beijing, People's Republic of China

Contact email: liugs@ibcas.ac.cn

Abstract. Grazing is a multiple-component process that includes wounding, defoliation, and saliva depositing. The molecular mechanism for how plants respond to grazing in grassland is a new topic. To address this question, we performed gene expression activities within 2 to 24 hours of grazing and proteomics analysis of rice seedling, examining hundreds of genes and proteins. Some key genes in GeneChips analysis specifically researched were β-amylase, *LcSUT1*, *LcDREB3*, and FEH gene. BSA (bovine serum albumin), an important and abundant component in saliva was used to study the saliva-plant interaction in grazing. Combined with corresponding gene and grazing research by other laboratories, this will advance our knowledge of the molecular interface of the grass-herbivore interaction.

Keywords: Grazing, molecular level, proteomics analysis, gene expression activities, bovine serum albumin.

Introduction

Plant-herbivore interaction is an old but interesting field in biology. Plant growth stimulation from herbivory has been found for 50 years. Generations of scientists have devoted to unfold the mechanism's using ecology and statistics methods, but conflicting results were found. For large animal feeding, many studies demonstrate plant use saliva to stimulate the growth and initiate compensation (Paige and Whitham 1987, McNaughton 1979, Poveda *et al*. 2010), but there are some research demonstrating that saliva from herbivores had no or even negative impacts on plants (Johnston and Bailey 1972, Reardon *et al*. 1974, Capinera and Roltsch 1980, Detling *et al*. 1981, Fan *et al*. 2011). Till now, there has been limited knowledge on the molecular mechanism of how plants respond to large herbivory feeding. Understanding the molecular interface of plant and large herbivore interaction has significant importance for agriculture and grassland conservation.

This paper summarises a number of studies conducted to investigate the molecular interface between grass and large herbivores to understand grazing, which is a complex stress process that includes wounding, saliva depositing, and defoliation.

Gene expression in response to grazing

In the first study (Chen *et al*. 2009) rice was used as a model plant as the genome sequence is available. When seedlings were 18 cm in height, the top 12 cm was removed by simulated grazing. The gene expression activities within 2 to 24 hours in the remaining aboveground tissues were profiled using the Affymetrix Rice GeneChips and confirmed by RT-qPCR. In total, the expression activity of 466 genes, involved in signal transduction, miRNA regulation, cell wall modification, metabolism, hormone synthesis, and molecule transporters, had been significantly changed at least two fold. The results suggest that 1) remaining aboveground tissues used anaerobic respiration as an emergency measure for energy/substrates supply; 2) Sink tissues reduced its demand after 2 hours; and 3) Sucrose synthesis enhancement that occurs around the 24th hour is likely driven by shoot re-growth.

Several receptor-like kinase genes in the signal transduction pathways were activated, including AUX/IAA family proteins, and gibberellin-20 oxidase gene. The gibberellin-20 oxidase gene up-regulated by grazing in rice seedling encodes a gibberellin synthesis-required key enzyme that catalyzes the conversion of GA53 to GA20 via a three-step oxidation at C-20 of the GA skeleton. The upregulation of this gene suggests that grazing may increase gibberellin biosynthesis within two hours after grazing.

A cytochrome P450 74A1 gene, a lipoxygenase 2.1 gene, and three other genes were up-regulated by grazing. Each of these genes is involved in jasmonic acid (JA) synthesis. Among the genes induced by grazing, there are also those involved in cell wall construction, modification, cell wall expansion, maintenance of cell wall architecture, and cellulose degradation, such as alpha-expansin, endoglucanases, class III peroxidases, and glucan endo-1,3-beta-glucosidase.

Genes encoding several enzymes involved in the flavonoid biosynthetic pathways were significantly induced after grazing, including 19 functional genes. Several defense related enzymes, including regulatory protein NPR1, pathogenesis related protein 1, and nematode resistance protein were also induced by grazing. We also found carbohydrate metabolism related genes induced by grazing. It is notable that the expression of alpha-amylase, which is involved in starch degradation, was strongly activated, and we studied its family gene-β-amylase in another experiment.

For sucrose synthesis and transportation, genes encoding sucrose phosphate synthase and sugar transporter were up-regulated. Among the activated genes, there were also pyruvate anaerobic metabolism pathway genes,

including those encoding enzymes of pyruvate dehydrogenase, aldehyde dehydrogenase, and L-lactate dehydrogenase. These genes and their biochemical pathways identified provide insights into how plants respond to grazing at the molecular physiology level.

Seedlings response to ovine saliva

We researched rice seedlings response to grazing and ovine saliva at the protein level (Fan *et al*. 2011). A total of 54 protein spots were responsive to ovine saliva treatment and 37 of them were identified. The identified proteins were involved in biostress defense, photosynthesis, energy, metabolism, signal transduction, transcription, and other biological functions that were either related to the resistance to pathogen attack or seedling growth including catalase, peroxiredoxin, ATP-synthase, glyceraldehyde-3-phosphate dehydrogenase, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Moreover, RT-PCR data showed that most of the genes were also regulated at the transcriptional level. Our results indicate that the ovine saliva induces an early response in the rice seedling by stress-related pathways.

To distinguish the effects of defoliation and wound on carbohydrate metabolism in grazing process, another experiment was carried out. In this study, we showed that the expression of β-amylase gene was induced by defoliation but not wound on rice leaf sheath. β-amylase is activated by defoliation due to the amount of carbohydrate needed for re-growth. Starch and soluble carbohydrates stored in sheaths and roots were mobilized at the early stage after defoliation but not after wounding. It is in agreement with some results of previous studies in *Lolium perenne* (Donaghy and Fulkerson, 1997, Morvan-Bertrand *et al*. 2001, Rasmussen *et al*. 2009). The mobilization lasts until the plant has adequate leaf area. The sugar supply study provided clues on transcription activation of three kinds of β-amylase subfamily genes after defoliation (unpublished data).

Recently, we performed RNA-Seq in *Leymus chinensis* to explore the effects of BSA (bovine serum albumin), which is an abundant component in bovine saliva. Using BSA avoids the instability of naturally acquired saliva and the disturbance of bacteria in saliva which may conceal the actual function of saliva. This study may help to understand the effect of animal saliva component on the plant recovery and provide insights in the co-evolution between plants and herbivores in grazing systems.

Besides these expression profiles, some grazing responsive genes were cloned, such as FEH gene, *LcSUT1*, and *LcDREB3*. Tamura *et al*. (2011) reported that 6-FEH gene plays a role in the degradation of fructose and the mobilization of carbon sources for re-growth after defoliation in timothy. We also cloned FEH gene from *L. chinensis*, and found it was induced by defoliation. Berthier *et al.* (2009) found defoliation in *Lolium perenne* led to the significant increased expression of *LpSUT1* in leaf sheath. Our laboratory cloned a functional sucrose transporter *LcSUT1* from *L. chinensis*. The *LcSUT1* was significantly up-regulated in leaf sheath after defoliation, but not induced by wound signal. The expression level of *LcSUT1* was also increased on N6 medium without sucrose that suggested the increased expression of *LcSUT1* in leaf sheaths after defoliation may be the results of sucrose starvation. Meanwhile, an *LcDREB3a* transcription factor was isolated from *L. chinensis*. The gene function analysis indicate that LcDREB3a involved in the ABA (abscisic acid) depended on signal transduction in the stress responsive process, and this stress responsive process is thought to be related to mowing.

Conclusion

Studies on plant responses to grazing in the molecular interface of grass-herbivore interaction have been advanced to a great extent. Two important conclusions are drawn: 1) Plants respond to grazing by signal transduction, miRNA regulation, cell wall modification, metabolism, hormone synthesis, and molecule transporters. 2) Plants may have different response mechanisms to three components of grazing, including wounding, saliva depositing, and defoliation. Despite these studies, there is still much knowledge to be gained on the molecular interface of grass-herbivore interaction. Therefore, further studies are warranted to further explore this interesting field.

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