A Study of Molecular Interface of Grass-Herbivory Interaction in Grass

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A study of molecular interface of grass-herbivory interaction in grass

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Introduction

Grass-herbivore interaction is a complex process that involves wounding effects caused by herbivore feeding, defoliation effects due to leaf-surface loss during grazing, and the deposition of herbivore saliva onto the surface of plants (Chen *et al*., 2009). Wounding can stimulate plant growth but clearly differs from grazing (Mattiacci *et al*., 1995). Defoliation affects root development in grasses and alters the carbohydrate-metabolism pathway in rice. Saliva has been found to stimulate plant growth, enhance tiller and increase biomass. However, little is known about the molecular mechanisms of plant responses to grazing in molecular level. In our previous transcriptome studies, many genes relating with grazing were identified from sheepgrass (Li *et al*., 2013). In last IGC report, we proposed the concept of "molecular interface on grass-herbivore interaction" (Liu *et al*., 2013) to understand the interaction between plant and large herbivories on molecular level, which has significant importance on agriculture and grassland conservation. This paper will present some new results in the area.

Materials and Methods

Plant materials, growing conditions, and treatments: The sheepgrass (*Leymus chinensis*) variety Zhongke 3 was grown in a soil mix of peat moss and vermiculite (2:1, v/v) in a greenhouse at 23℃ with a photoperiod of 16 h light/8 h dark. All treatments were performed on eight-week-old sheepgrass seedlings. To induce defoliation, approximately two-thirds of the aboveground biomass was removed. And to induce wounding, tweezers were used to mechanically wound a site at the same position as the defoliation site. For the bovine serum albumin (BSA) deposition treatment, a BSA solution (1 mM) was daubed on the cut ends of the leaves. The remaining one-third of the aboveground biomass was collected 2, 6, and 24 h after defoliation, and the corresponding parts were collected for control seedlings and wounding at 2, 6, and 24 h. Each sample included 3 replicate pots and 21 seedlings. The samples were collected and immediately frozen in liquid nitrogen and stored at -80℃.

Illumina sequencing and differentially expressed genes (DEGs) analysis : Total RNA was extracted from individual control, wounded, defoliated and BSA deposition plants at 2, 6, and 24 h using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA fragmentation, cDNA synthesis, and ten cDNA libraries, which were isolated from mechanically wounded, defoliated and BSA deposition material at 2, 6, and 24 h and the control, were sequenced using the Illumina HiSeq 2000 System at the Chinese National Human Genome Center (Shanghai, China). The reads for each sample were counted and mapped back to previously generated sheepgrass reference genes using the software tool Bowtie.

Results and Discussion

Ren *et al*. (2000) put forward the theory of the interfaces within pratacultural system (grassland livestock production system), and identified three major interfaces, such as: plant-soil interface, grassland-animal interface, grassland and livestock-management interface. In order to complete the above pratacultural system theory and to deep the research in molecular mechanism of grassland- animal interface, the "concept of molecular interface on grass-herbivore interaction" was proposed (Liu *et* al., 2013). "Molecular interface" is a series of molecular process after the herbivore feeding plant, mainly including the molecular mechanisms of plant defense against wounding, the plant regrowth after defoliation, and the cell stimulation or death response to animal depositions (Fig 1). Those molecular processes contain signal transduction, gene expression, protein metabolism, hormone synthesis, sucrose transporters and cell physiology.

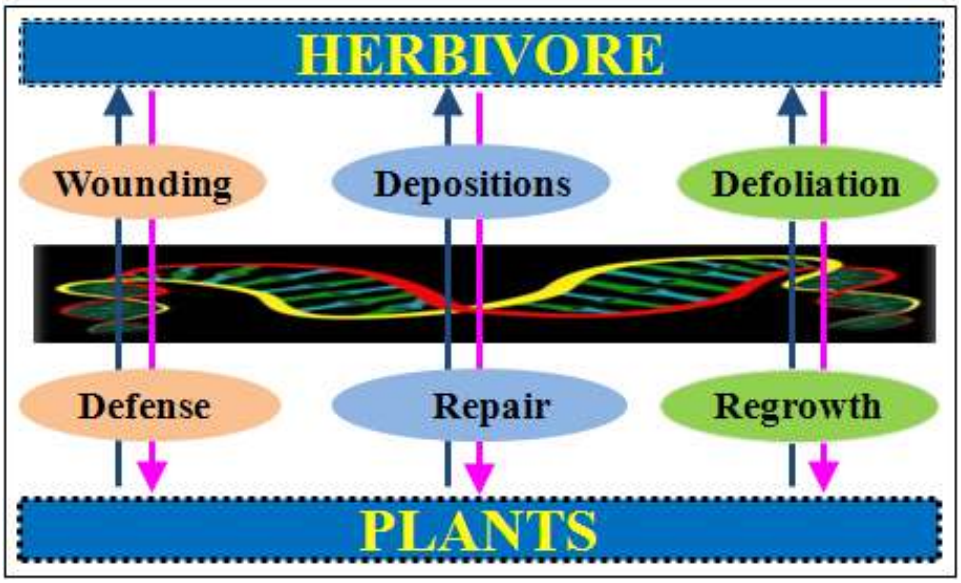

Fig 1. The model of molecular interface of the grass-herbivore interaction.

In our early study, we used rice as a model plant to discover the genes and pathways induced in response to defoliation (Chen *et al*., 2009). The results suggested genes encoding sucrose phosphate synthase and sugar transporter were up-regulated by defoliation due to amount of carbohydrate needed for re-growth. In sheepgrass study, β-amylase is activated by defoliation, and the sugar transporter *LcSUT1* gene was significantly up-regulated in leaf sheath after defoliation but not wound. We also cloned *FEH* gene from sheepgrass, and found it was induced by defoliation. In addition, our proteomics study indicated that the ovine saliva induced an early response in the rice seedling by stress-related pathways.

In the present study, we investigate the molecular interface between grass and large herbivores using Illumina GAIIx technology to sequence sheepgrass transcriptome after defoliation, mechanically wounded, and BSA (bovine serum albumin) deposition. 1,836, 3,238 and 2337 genes were differentially expressed (DEGs) under wounding, defoliation and BSA deposition treatments, respectively. Based on GO terms and KEGG pathway enrichment analysis of the DEGs, we observed that the DEGs involved in JA biosynthesis after wounding and defoliation were significantly enriched. The transcription levels of key genes in JA biosynthesis, including genes encoding lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), 12-oxophytodienoic acid reductase (OPR), acyl-CoA oxidase (ACOX1, ACOX3), acetyl-CoA acyltransferase 1 (ACAA1), and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (EHHADH), were significantly increased after wounding and defoliation. The results are consistent with the previous reports, demonstrating that wound-induced responses are triggered by the de novo synthesis of the plant hormone JA when plant tissues are injured by herbivores, pathogens, and mechanical stress (Koo *et al*., 2009). Based on functional analysis of the saliva-deposition DEGs, the cellular-antioxidant and apoptotic pathways apparently respond to grazing stress (Fig 2). This result is consistent with a proteomic analysis of rice after ovine BSA deposition.

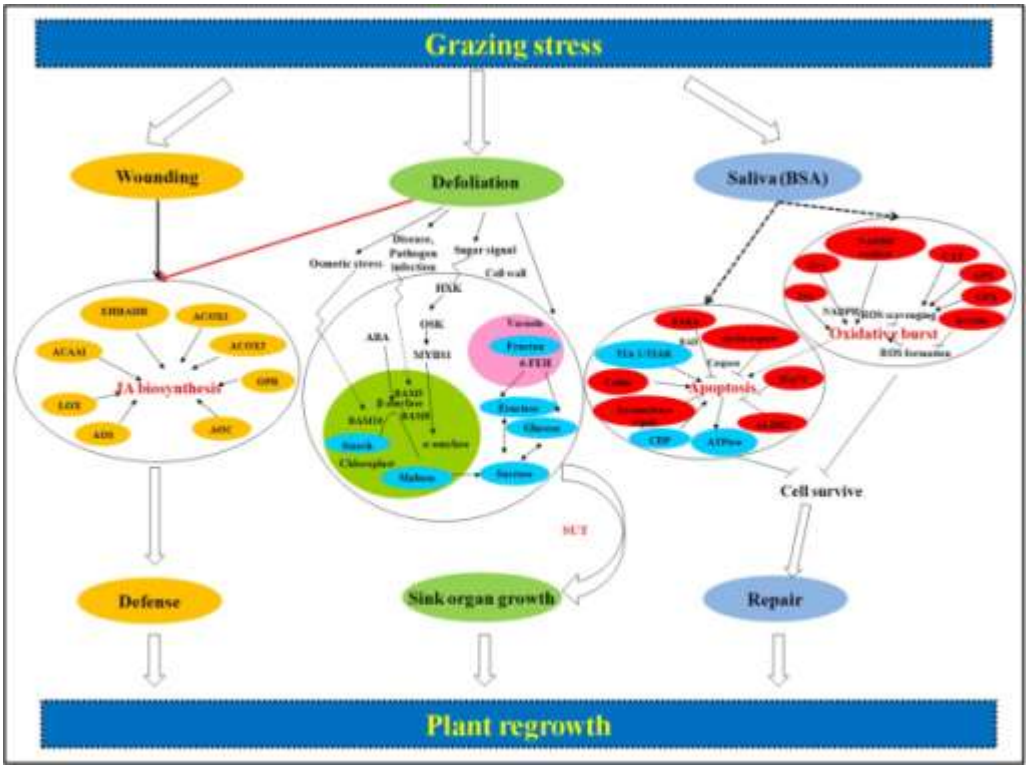

Fig 2. The differential molecular processes of plant response to wounding, defoliation, and BSA deposition. The full lines mean the interaction that has been demonstrated, and the dotted lines mean the deduced interaction or the interaction indirectly demonstrated.

Conclusion

To study the molecular interface of the grass-herbivore interaction, we performed GeneChips, proteomics, and transcriptome sequencing to identify DEGs response to wounding, defoliation and simulated BSA deposition. The results demonstrated that both wounding and defoliation activated the systemic synthesis of jasmonate (JA). The genes included in sucrose phosphate synthase and sugar transporter were up-regulated by defoliation due to amount of carbohydrate needed for re-growth. The effects of grazing and BSA deposition involved more apoptosis and cell oxidative changes compared to defoliation. Our research elucidates distinct molecular mechanisms of plant responses to defoliation, wounding and BSA deposition.

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