HYPOXIC RESPONSE IN THE GRAIN STORAGE INSECT PEST CALLOSOBRUCHUS MACULATUS

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The cowpea bruchid, *Callosobruchus maculatus*, is an important storage pest of grain legumes. Infestation of cowpea by bruchids starts in the field and proliferates in storage. The females lay their eggs on the surface of legume seeds, and larvae feed and develop inside the seeds. Adults start to lay eggs soon after they emerge, initiating yet another round of infestation. Due to its high fecundity and short life cycle, even a few months of storage can result in 100% grain loss in unprotected cowpea seeds.

Use of modified atmospheres with depleted O_2 (hypoxia) is an environmentally friendly alternative to currently used chemical fumigation for control of storage insect pests. Many insects, however, can adapt to O_2 deprivation and recover from hours to days of hypoxia. This poses a difficulty in using the modified atmosphere technique. We studied the effects of low O_2 on cowpea bruchid development, profiled transcriptomic responses to hypoxia, and investigated HIF1-regulated hypoxia-induced genes.

To determine the impact of hypoxia and hypercapnia (high CO_2) on cowpea bruchids, two $O_2/CO_2/N_2$ combinations were used; (a) 10% O_2 + 10% CO_2 + 80% N_2, (b) 2% O_2 + 18% CO_2 + 80% N_2 (80% N_2 is equivalent to normal atmospheric concentration). When exposed to 10% O_2 + 10% CO_2 + 80% N_2, eggs, larvae and pupae were able to complete their growth and successfully enter the next developmental stage, although developmental time and mortality varied at different stages. However, under more severe hypoxic/hypercapnic conditions, i.e. 2% O_2 + 18% CO_2 + 80% N_2, development of all stages ceased. Effects on eggs and adults were most dramatic. The older larvae (3rd and 4th instars) were least sensitive and could survive up to 20 days treatment, although in ambient atmosphere, the rate of O_2 consumption and CO_2 output are greatest in the 3rd and 4th instar larvae compared to other stages. Results suggest that cowpea bruchids are able to tolerate a certain level of O_2 deprivation.

It is likely that the bruchids encounter hypoxic conditions because they live in confined grains in storage. Possibly they have evolved adaptive strategies to cope with such environmental stresses and survive suboptimal levels of O_2. To gain some molecular insight into the hypoxic/hypercarpnic response, we performed transcriptomic analysis and used qPCR reactions on selected metabolic genes involved in the TCA cycle and in protein digestion, as well as genes encoding stress-responsive heat shock proteins. Results suggest that cowpea bruchids suppress their metabolic activity and increase stress tolerance when challenged by O_2 deprivation.

We then investigated the role of HIF1 in regulating transcript expression of representative heat shock proteins (HSPs) by promoter analysis. *HSP*s were induced by hypoxia treatment. Promoter analysis of representative *HSP* genes suggested that HIF1 positively regulates these genes. Its transcription activation function has been confirmed by transient co-transfection into S2 cells of constructs of HIF1 subunits and the *HSP* promoter-driven reporter.

Knowledge obtained from cowpea bruchids can be extended to other economically important insect pests. Understanding the effects of alternative pest management measures on target insects, as well as molecular mechanisms of the remarkable plasticity of the insects will ultimately help us design more effective strategies for pest management.