Cysteine proteases activity and gene expression studies in soybean nodules during development and drought stress

By

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ABSTRACT

Activity and transcription profiles of two classes of cysteine proteases, papain- and legumain-like cysteine proteases, as well as their potential inhibitors, cysteine protease inhibitors (cystatins), were investigated in soybean nodules during nodule development and after drought inducing premature senescence. During nodule development total protease activity increased with major activity bands detected protease zymography in older nodules. Expressed cysteine proteases during nodule development were detected by tagging proteases with the cysteine protease inhibitor DCG-04 with major DCG-04 tagged bands found in both young and old nodules. Increase in protease activity was associated with a significant decrease in nitrogenase activity of nodules measured as acetylene reduction. Semi-quantitative RT-PCR for cysteine protease and cystatin transcription profiling showed a decrease in transcription during development and also after drought treatment of several papain-like cysteine proteases (Glyma04g04400, Glyma17g05670, Glyma10g35100, and Glyma04g03090). In contrast, transcription of three legumain-like cysteine proteases (Glyma17g14680, Glyma05g04230 and Glyma14g10620) increased during nodule development and also after drought treatment. Transcription of two cystatins (Glyma13g27980 and Glyma05g28250) increased during nodule development with Glyma13g27980 strongly up-regulated after drought treatment and Glyma05g28250 constitutively strongly expressed in both well-watered and drought treated nodules. Overall, the study has contributed in establishing an expression profile of cysteine proteases and cystatins in soybean nodules. This knowledge provides a basis which can be used to determine the importance of the individual components of the cysteine protease – cystatin system, during soybean nodule development and during stress-induced premature nodule senescence.

COMPOSITION OF DISSERTATION

Chapter 1 of this dissertation provides an overview about legume nodule biology and the current knowledge of the protease-protease inhibitor system in soybean and its possible biological function in plants. Also outlined are the mechanisms of how plant senescence and stress affects this system. The objectives and aim of the study are provided at the end of this chapter. **Chapter 2** outlines the materials and methods used in this study. This includes growth and drought treatment of soybean plants and the different molecular and biochemical techniques applied to characterize plant tissues and nodules. **Chapter 3** outlines the results obtained from characterizing nodule material of different age and after drought treatment in particular the various expression studies carried out for cysteine proteases and cystatins. **Chapter 4** focuses on the outcomes of the study and new results obtained are discussed in greater detail. The chapter also outlines possible future actions to further advance the function of the cysteine protease-cystatin system in nodule development and under stress. In **Chapter 5** the appendix provides information about all DNA sequences used during this study. Finally, literature cited in this study is listed in **Chapter 6.**

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