Functional study of cocoa cystatins (*Theobroma cacao*) by expression in tobacco plants (*Nicotiana tabacum*)

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In the databases of libraries of interaction cocoa - *Moniliophthora perniciosa* have been identified four cystatins (*TcCYS1, TcCYS2, TcCYS3 and TcCYS4*) biochemically characterized exhibiting inhibitory activity against *M. perniciosa*. The cystatins are cysteine protease inhibitors of papain-like proteases and are related to different mechanisms of growth and plant development, biotic and abiotic stress and inhibition of PCD (Programed Cell Death). The use of cystatin as a biotechnological tool via transgenic plant has been the focus of several studies. In the process infection a fungus protein, NEP (Necrosis Ethylene-inducing Proteins), induces PCD by activating cysteine proteases. In the present work two *T. cacao* cystatins were over expressed in *Nicotiana tabacum*. For so, *TcCYS3 and TcCYS4* cDNAs were amplified by PCR using specific primers. The DNA fragments cloned at sense orientation to the modified pCambia 1390 vector, under the control of CAMV 35S promoter. Transformation process was performed by *Agrobacterium tumefaciens* strain EHA105. The relative expression of TcCYS3 and TcCYS4 were evaluated by qRT-PCR using the ∆∆Ct method showed an expression up 3.2 and 8.9 times than the caliper respectively. The protein analysis by western blot showed that plants transformed with TcCYS4 were up to 1.5 higher than in non-transformed plants. Leaves were infiltrated with NEP and the results revealed that plants over expression TcCYS4 in qRT-PCR were capable of inhibiting the activity of NEP. This work will give further support to witches’ broom disease program once cystatin level can be related to plant improved tolerance to several pathogen interactions.

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