Dissecting Interaction Partners of the *Nicotiana tabacum* Stigma Cell-Cycle Inhibitor Protein, SCI1, by Two-Hybrid Assays

Strini, E.J.[1,2], De-Paoli, H.C.[1,2], Cossalter, V.[1], Quiapim, A.C.[1], Avanci, N.C.[1], Goldman, G.H.[3], Goldman, M.H.S.[2]
[1]Departamento de Biologia, FFCLRP/USP BRAZIL; [2]Departamento de Genética, FMRP/USP BRAZIL; [3]Departamento de Ciências Farmacêuticas, FCFRP/USP BRAZIL.

Plant reproduction success is dependent on proper pistil development, which is coordinated by the proliferation and differentiation machinery. We have recently reported a *stigma cell-cycle inhibitor* gene, *SCI1*, that controls cell division and differentiation on tobacco stigma/style. This gene encodes a small lysine-rich protein with two putative cyclin interaction domains and 15 putative phosphorylation sites. Experiments with SCI1-GFP fusion demonstrated its nuclear localization. To examine whether SCI1 interacts with tobacco cell-cycle regulators and signaling molecules, we performed two-hybrid assays between this protein and the cyclin D3;2, CDKA and MAPK proteins. The growth of the yeast Mav203 strain, expressing the bait BD-SCI1 and the prey AD-MAPK proteins, when compared to the different interaction controls suggests that SCI1 has a weak interaction with MAPK. The results showed that SCI1 does not interact with CDKA, while the experiments with cyclin were not conclusive, due to self-activation of BD-cyclin. To survey additional SCI1-interacting proteins, we have constructed a tobacco stigma/style cDNA expression library in the prey vector, which will be screened in the near future. A recombinant SCI1 HIS-tagged protein (rSCI1) was expressed in *Escherichia coli* BL21(DE3) Rosetta strain at 37ºC/20ºC. The rSCI1 was present in the soluble cellular fraction during the initial hours of induction and gradually accumulated in the insoluble fraction. The rSCI1 band was excised from polyacrilamide gel and was used to immunize BALB/c mice. The polyclonal antibody obtained successfully recognized the rSCI1 protein at 1:750 dilution and opens new perspectives for the validation of the SCI1 two-hybrid assays by co-immunoprecipitation.

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