7种RNA沉默抑制子对植物病毒载体表达系统表达水平的影响(点击查看pdf全文)

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Title: Effects of seven RNA silencing suppressors on heterologous expression of green fluorescence protein expression mediated by a plant virus-based system in Nicotiana benthamiana

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摘要: 目的探索不同植物病毒RNA沉默抑制子对植物病毒载体表达系统重组蛋白表达水平的作用，为合理高效地利用这一新型的外源基因表达平台奠定基础。方法构建了7种不同的RNA沉默抑制子瞬时表达载体，以农杆菌渗滤法，与马铃薯X病毒表达载体PVXdt-GFP共侵染寄主植物本明烟，通过对报告基因绿色荧光蛋白（GFP）的荧光观察，并以Western blotting、ELISA和RT-qPCR等测定GFP在烟草中的表达情况，分析不同RNA沉默抑制子对植物中外源基因表达水平的作用和特点。结果7种病毒RNA沉默抑制子对外源基因GFP在烟草中表达水平的作用效果和持续时间存在差异，其中，番茄丛矮病毒的P19蛋白的增效作用最好，作用时间也最长；非洲木薯花叶病毒的AC2蛋白和水稻黄斑驳病毒的P1蛋白无明显的增效作用。结论RNA沉默抑制子可以通过抑制病毒诱导的RNA沉默来提高外源基因在植物中的表达水平和表达持续时间，但是不同的植物病毒载体表达系统需要通过筛选获得其最佳的共表达沉默抑制子“伴侣”。

Abstract: ObjectiveTo test the effects of7virus-encoded RNA silencing suppressors (RSSs) for enhancement of a plant virus-based vector system-mediated heterologous expression of green fluorescence protein (GFP) inNicotiana benthamiana. MethodsSeven transient expression vectors for the7RSSs were constructed and co-inoculated on the leaves ofNicotiana benthamiana with PVXdt-GFP vector, a novelPotato virusX-based plant expression vector, through agroinfiltration. The protein and mRNA expression levels of the reporter gene GFP in the co-inoculatedNicotianaleaves were examined by Western blotting, ELISA and RT-qPCR to assess the effect of the RSSs for GFP expression enhancement.ResultsThe7RSSs differed in the degree and duration of enhancement of heterologous GFP expression, and

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the p19protein ofTomato bushy stunt virus (TBSV) induced the highest expression of GFP. African cassava mosaic virusAC2protein andRice yellow mettle virusP1 protein produced no obvious enhancement GFP expression.ConclusionTransient co-expression of RSSs suppresses host silencing response to allow high-level and long-term expression of heterologous genes in plant, but the optimal RSS has to be identified for each plant virus-based expression vector system.

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