Artificial microRNA derived from the precursors of ananas comosus, arabidopsis thaliana, and oryza sativa effectively silences endogenous genes in md2 pineapple

ABSTRACT

Artificial microRNA (amiRNA) is considered the next generation of gene silencing vectors because it can be custom designed to silence any gene of interest in an organism. In the amiRNA mechanism, the precursor microRNA (pre-miRNA) plays an important role in transporting and ensuring that the amiRNA is processed through the endogenous miRNA biogenesis pathway, allowing for the amiRNA to be expressed and function as a gene silencing tool. However, the efficiency of expressing amiRNA between miRNA precursors and plant species varies, as there are no universal precursors that are suitable for use in all species. We therefore attempted to identify precursors that are compatible and efficient for use in the MD2 pineapple with the eventual purpose of studying gene function in the crop. The results showed that three endogenous precursors (aco-MIR156, aco- MIR399 and aco-MIR2673 of Ananas comosus) and two exogenous precursors (ath-MIR319 of Arabidopsis thaliana and osa-MIR528 of Oryza sativa) were compatible in the MD2 pineapple, all of which expressed amiRNA. However, the efficiency of these precursors differed, with the exogenous precursor ath-MIR319 found to be more efficient, that is, produced a higher level of amiRNA than the endogenous precursor itself. The endogenous precursors were then structurally modified to increase their efficiency. This resulted in precursor aco-MIR156 (with a modified stem structure) expressing the highest level of amiRNA. In conclusion, we identified suitable precursors for the amiRNA backbone in pineapple and showed that aco-MIR156 from pineapple was as equally efficient as established precursors, ath-MIR319 and osa-miR528 derived from Arabidopsis thaliana and Oryza sativa, respectively. This result opens the door to designing a set of precursors which are reusable as a backbone for amiRNA silencing in other non-model species. We recommend this newly developed pineapple amiRNA as a tool in gene silencing as it mimics the endogenous miRNA with high efficiency.