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Abstract Antisense (AS) and hairpin (HP) RNA interference (RNAi) targeted gene suppression technologies have been used to modify seed oil composition. Larger numbers of AS transgenics have to be screened to achieve a targeted level of suppression compared to RNAi. We hypothesized combining AS with RNAi might result in enhanced gene suppression compared to either method individually. AS and HP-RNAi were combined as hairpin

antisense

(HPAS) constructs containing ~125 bp sense and

antisense

portions of an untranslated region of the target gene separated by an intron containing an antisense

copy of a portion of the target coding region. The ?12-desaturase FAD2, the ?3-desaturase FAD3 and ?-ketoacyl-ACP synthase (KAS) II were targeted in *Arabidopsis* 

to evaluate changes in oil composition with AS, HP and HPAS constructs driven by the phaseolin promoter. Modest but statistically significant enhancements in oilseed phenotypes were observed with HPAS relative to AS and HP-RNAi. Phenotypes for HPAS suppression of FAD2 and FAD3 were indistinguishable from their strongest mutant alleles. Our data suggest that HPAS may be useful for: (1) achieving levels of suppression comparable to those of gene knockouts in a tissue specific manner. (2) Maximizing suppression of suboptimal RNAi constructs and (3) minimizing the screening of transgenics to achieve desired oilseed composition.

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