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New antimicrobial gene promoters from chickweed (*Stellaria media*) for biotechnology of cultivated plants

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Earlier we with colleagues found that hevein-like peptides genes from chickweed (*Stellaria media*) are the source of strong constitutive promoters for biotechnology of cultivated plants. E. g., in transient expression in *Nicotiana benthamiana* pro-SmAMP1 and pro-SmAMP2 promoters were 2–4 times more effective than the CaMV35S promoter and in rape (*Brassica napus*) and sugar beet (*Beta vulgaris*) plants were comparable to it. The functionality of the pro-SmAMP2 promoter was shown in the calluses of flax (*Linum usitatissimum*). In the homozygous lines of transgenic tobacco (*Nicotiana tabacum*), the pro-SmAMP1 and pro-SmAMP2 promoters are twice as strong as the CaMV35S promoter. The both promoters are at least as effective as the duplicated CaMV35S promoter for neomycin phosphotransferase II gene control in the selection of transgenic tobacco and *Arabidopsis* plants on media with kanamycin antibiotic at recommended concentrations. In present research we focused on the fact that pro-SmAMP1 and pro-SmAMP2 promoters are identical by 94 % differing by point mutations outside canonical cis-elements. Additional deletion analysis showed that in transient expression the minimal variant of pro-SmAMP1 (–119 b. p.) is twice stronger than minimal variant of pro-SmAMP2 (–121 b. p.). Along with this, pro-SmAMP2 is significantly more efficient than pro-SmAMP1 in control of selective gene *nptII* being comparable with duplicated 2xCaMV35S promoter. We employed the 9-nucleotide point polymorphism between sequences of two minimal promoters from chickweed for creation of new effective promoters featuring simultaneously high active and constitutive.

Significant similarity of nucleotide sequences in promoters of hevein-like genes precludes from using both of them within the same genetic construct so that recombination between the repeats be excluded. To create novel regulatory elements, we cloned the promoters of α-harpinine gene (*pro-SmAMP-X*) and defensine gene (*pro-SmAMP-D1*) from the chickweed using genome walking method. These novel promoters do not have a homology with any other known promoters being comparable with promoter CaMV35S in efficiency of transient expression.

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