The peptide linker between variable domains of heavy (V_H) and light (V_L) chains is one of important factors that influence the characteristics of scFv, including binding activity and specificity against target antigen. The scFvs against daidzin (DZ-scFvs) with different linker lengths were constructed in the format of V_H-$(GGGGS)_n$-V_L (n = 1, 3, 5, and 7). They were expressed in the hemolymph of silkworm larvae using the *Bombyx mori* nucleopolyhedrovirus (BmNPV) bacmid DNA system, and their reactivity against daidzin and related compounds were evaluated using an indirect competitive enzyme-linked immunosorbent assay (icELISA), which is applicable for quantitative analysis of daidzin. The results showed that the reactivity of scFvs against daidzin was increased, whereas specificity slightly decreased when their peptide linker was lengthened. These results suggested that the linker length of DZ-scFvs contributes to its reactivity. In addition, the results emphasize that the linker length could control the reactivity of DZ-scFvs.

Construction, expression, and characterization of multiform scFvs against daidzin (DZ-scFvs) with different linker lengths.