Development of gene constructs for imparting grey mold tolerance

With the aim of testing the cumulative effects of multiple genes in imparting resistance to Botrytis, two polygene cassettes are being developed. In the first construct, three independent gene cassettes were cloned within a T-DNA in an iterative way. Two of the three target genes are involved in the signal pathway elicited by necrotrophic fungi and the other one is an antiapoptotic gene. All these genes are expressed under the inflorescence specific promoters from *Arabidopsis*. The three individual cassettes (AtACS4 –*BIK*1, AtACS5 – ERF1 and AtACS7- AtEBP1) were developed, confirmed and mobilized into *Agrobacterium tumefaciens* strain. The three gene cassettes were cloned within a single T-DNA region to develop the intended multigene cassette. In the process three double gene cassettes were also developed. Also, to ascertain that the isolated promoters (AtACS4, At ACS5 and AtACS7) drive elevated expression of the genes in the inflorescence parts, gene constructs were made with these three promoters driving the expression of gus gene. These gus based constructs could give the expression pattern as observed by GUS histochemical staining procedures.

In the second multigene construct, genes for three antifungal proteins were cloned to produce a self-disassembling poly-protein. Codon optimized endo-chitinase from *Trichoderma harzianum*, and two antifungal genes (Rsafp2 and AcAMP1) were synthesized including the transcription and translation enhancers. MARS duplicate repeats were also cloned into the binary cassette. The fusion poly-protein gene has been cloned downstream of double 35S promoter so that it expresses constitutively.