Engineering of Yellow Mosaic Virus Resistance YMVR in Blackgram

The project has aimed to generate blackgram (Vigna mungo) plants resistant to Mungbean yellow mosaic virus-Vigna (MYMV). We have designed two anti-viral strategies: (1) RNA interference (RNAi) targeting both DNA and RNA forms of the virus by stable plant transformation with RNAi transgenes and transient plant immunisation with dsRNA cognate to the virus; (2) “virus-induced cell death” using a split toxin gene inducible by the virus. These strategies have been tested in model transgenic systems using “easy-to-transform” tobacco and cassava plants and in transient systems based on tobacco and blackgram seedlings and plant protoplasts. Stable transformation of blackgram has been attempted using Agrobacterium-mediated transformation of embryonic explants and a direct gene transfer by biolistic delivery of anti-viral DNA constructs to the explants using gene gun.

The RNAi constructs targeting the viral promoter, the AC1 and AC2 genes were constructed for MYMV and for African cassava mosaic virus (ACMV) infecting cassava. The MYMV constructs have been tested transiently by biolistic delivery or by agroinoculation to MYMV-inoculated blackgram seedlings and shown to induce recovery from MYMV infection to varying degrees depending on the construct design: intron-containing constructs had better anti-viral effect. While stable transformation of blackgram with the MYMV constructs proved to be difficult, multiple transgenic cassava lines carrying the ACMV constructs were generated in collaboration with Prof. Gruissem group, ETH, Zürich, and some of them were shown to be resistant to ACMV.

The split barnase construct was designed, from which the toxic barnase gene can be reconstituted and expressed only upon MYMV infection. Transgenic tobacco plants carrying the split barnase construct were obtained and tested for MYMV resistance using leaf disc assay: compared to control plants MYMV replication was reduced about 3-fold in transgenics.

We have been applying both the Agrobacterium-mediated transformation method and a direct gene transfer method by biolistic delivery. So far, the transformation of blackgram leaf segments was successful, yielding kanamycin (Km)-resistant calli that express GUS and MYMV dsRNA fragments. These calli could be used for virus resistance assays and serve as controls for transformed plants, but could not be used to regenerate plants.

Large-scale experiments on blackgram transformation with anti-viral constructs using several thousands cotyledonary node explants and Km-selection originally led to Km-resistant plants that, however, eventually lost their markers upon further growth, probably because of their chimeric nature. Experiments were continued with a more suitable selection marker PPT (phosphinothricin resistance) gene, using variations of transformation and regeneration protocols and different blackgram cultivars. So far all attempts to produce stable transgenic plants were not successful.

The transient application of dsRNA-expressing plasmid DNA to cure blackgram plants has been shown by us in...
the first phase of ISCB. Although successful, this method required bombardment of plant material using the gene gun. Direct application of dsRNA would require large amounts of dsRNA, which can be produced only with high costs using the standard technologies. We therefore initiated collaboration with Prof. Bamford (Helsinki) to produce dsRNA using a Pseudomonas syringae bacteriophage Φ6-based system. Both components of MYMV genome were cloned under the control of replication signals directing the production of dsRNA in presence of the bacteriophage polymerase complex and are presently being tested. Given current difficulties of stable transformation of blackgram plants, the transient immunization approach holds promise for future control of yellow mosaic and other viral diseases.