

Harnessing RNA silencing to protect peanuts from stripe disease

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Abstract

Genetically modified peanut plants that carry copies of the viral coat protein gene exhibited high levels of resistance to peanut stripe virus (PStV). Fertile plants of the Indonesian cultivar Gajah were regenerated following microprojectile bombardment of embryogenic callus with an untranslatable version of the PStV coat protein gene and 3' untranslated region. The hygromycin gene was co-bombarded on a separate plasmid for initial selection of transformed embryos. Segregation of the two genes allowed identification of marker-gene free, virus resistant progeny. The resistance mechanism is based on RNA silencing, an anti-viral defense mechanism intrinsic to all plants. Fifth generation progeny contain resistant lineages and the inheritance of the resistance trait appears to be linked to particular transgene copies.

Media summary

Improved peanut lines with strong resistance to peanut stripe disease will reduce a major constraint to peanut production in Indonesia and protect the Australian industry.

Key Words

Genetically modified crops, post-transcriptional gene silencing, virus resistance, groundnut

Introduction

Peanut or groundnut is an important oilseed crop and food legume in many sub-tropical regions worldwide. PStV belongs to the potyvirus family. It causes significant economic losses in southeast Asia and China due to poor peanut seed quality and yield. PStV strains have a coat protein sequence variability of below 10% and can be defined according to geographic origin and symptom type (Higgins et al. 1999). PStV is difficult to control because it can be transmitted in seed and through aphids, and there is no natural resistance in genetically compatible germplasm. Pathogen-mediated resistance has been deployed successfully to provide resistance to potyvirus infection in several crops. High level resistance or immunity can be induced in plants by triggering RNA silencing, an intrinsic defense mechanism against viruses (Waterhouse et al. 2001). An international collaborative research program funded by the Australian Centre for International Agricultural Research has now applied this technology to peanuts to control stripe disease in commercial peanut cultivars (Higgins et al. 2004).

Methods

A full-length untranslatable modification of the coat protein gene and 3' untranslated region (UTR) of an Indonesian blotch strain of PStV was cloned into the plant expression vector pRTL2 (Restrepo et al. 1990). Peanut somatic embryos were transformed and regenerated as described by Livingstone and Birch (1999) and Higgins et al. (2000). Transformed plants were analyzed by Southern blot hybridization to determine gene copy numbers and small RNAs were extracted and detected by RNA blot. Coat protein accumulation was measured by enzyme-linked immunosorbent assay (ELISA). Resistance was tested by mechanical challenge inoculation with PStV under glasshouse conditions

Results

Selected transgenic peanut plants carrying an untranslatable PStV coat protein gene and 3' UTR sequence were highly resistant to PStV infection. These lines showed no symptoms of stripe disease and virus was not detected in inoculated as well as systemic leaves when assayed by ELISA. Susceptible lines showed clear systemic blotch symptoms and PStV was detected throughout the plants. There was no correlation between transgene copy number and phenotype. All regenerated plants analyzed with resistant and susceptible phenotypes carried three or more transgene copies. None of the lines expressed detectable amounts of PStV coat protein when grown in tissue culture or in soil in a glasshouse. Transgene-specific

small RNAs, which are considered hallmarks of RNA silencing, were detected in R0 and progeny R2 plants of highly resistant lines, but not in a susceptible transformed line.

Most plants of 5th generation progeny derived from a multi-copy transgenic peanut parent propagated in Indonesia carried single copies of the resistance gene in specific locations of the genome. This allowed us to establish links between the presence of particular transgene copies and the stable resistance phenotype. Resistant lines lacking the independently segregating hygromycin gene were also identified. Such lines are expected to yield 100% resistant progeny in the 6th generation.

Conclusion

RNA silencing was successfully induced in transgenic peanut plants to specifically eliminate PSTV RNA. These plants were highly resistant to PSTV infection and the resistance was stably inherited over at least 5 generations. These plants will be useful for Indonesian growers to combat a major constraint in production and may provide a source of resistance in peanut breeding programs. The genetic improvement of the major Indonesian cv. Gajah for PSTV resistance is of particular significance, since this cultivar is also resistant to bacterial wilt, another economically important disease in southeast Asia (Mehan et al. 1994).

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