Molecular breeding of white clover for transgenic resistance to *Alfalfa mosaic virus* and natural resistance to *Clover yellow vein virus*

P. Chu[1], G. Zhao[2,3] and G.C. Spangenberg[2]
[1]*CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia;* [2]*Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia;* [3]*Department of Grassland Science, Gansu Agricultural University, Lanzhou 730070, China. Email: paul.chu@csiro.au*

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Introduction *Trifolium repens* L. (white clover) is one of the most important pastoral plants in temperate Australia. Its productivity and persistence is being reduced significantly by *Alfalfa mosaic virus* (AMV), *Clover yellow vein virus* (ClYVV) and *White clover mosaic virus* (WClMV). These viruses are also widespread in other legumes and are inflicting large economic losses to farmers throughout the world (Campbell, 1984). To reduce the economic impact of these viruses, white clover plants resistant to both ClYVV and AMV are being developed for future commercial release. Since introducing viral transgenes from two or more viruses into a transgenic plant has the potential threat of viral recombination, we have decided to develop white clover with transgenic resistance to AMV and natural resistance to ClYVV.

Materials and methods Transgenic white clover plants expressing the AMV coat protein (CP) gene and showing immunity to that virus have been produced. White clover plants with natural resistance to ClYVV were identified by screening various cultivars of the white clover against different isolates of the virus. To combine the transgenic AMV immunity and natural ClYVV resistance into a single transgenic white clover cultivar, crosses between singly AMV immune and ClYVV resistant white clover plants were performed. The offspring plants were subjected to molecular analysis and infectivity screens to identify plants carrying AMV CP and showing immunity to both viruses. The best of three AMV + ClYVV resistant genotypes was selected for crossing with 12 Sustain-type elite plants in order to produce a new white clover cultivar with double virus resistance.

Results Crosses between singly AMV immune and ClYVV resistant white clover plants produced offspring plants that were resistant to both viruses. After crossing with the Sustain-type parents, almost 100% of the offspring plants carrying the AMV CP showed immunity to that virus, irrespective of the Sustain-type parent used. In contrast, there were significant variations in the percentage of ClYVV resistant progenies produced by the different Sustain-type parents (Figure1). Diallele crossing between these first generation double virus resistant progenies (T1 plants) increased the proportion of ClYVV resistant plants among the T2 population from 34% to 49% (Figure 2). Furthermore, T2 population showed a higher proportion of plants with milder symptoms than plants in the T1 population.

Figure 1

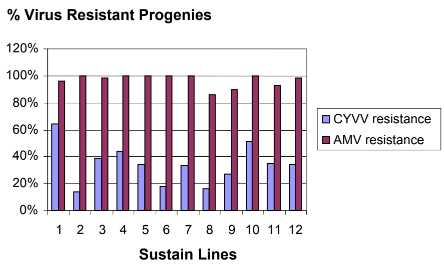
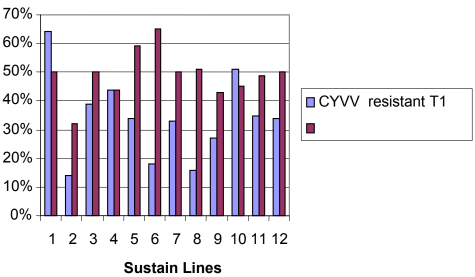


Figure 2



Conclusions The results showed that it is possible to produce white clover plants with transgenic resistance to AMV and natural resistance to ClYVV. The differential inheritance of ClYVV resistance from different Sustain genotypes suggests that multiple resistance genes may be involved in the natural ClYVV resistance.

References

Campbell C.L. and J.W. Moyer (1984). Yield responses of 6 white clover clones to virus infection under field condition. *Plant Disease*, 68, 1033-1035.