Viroids in Grapevines: Transmission via Seeds and Persistence in Meristem-regenerated Vines

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SUMMARY

This general aim of the work described in this thesis was to study viroids in grapevines, especially their vertical transmission via seeds, during meristem culture and micropropagation, and to produce viroid-free vines by shoot apical meristem culture (SAMC). Because of the widespread occurrence of viroids in grapevines, major emphasis has generally been placed on the production of viroid-free vines to understand the true biological significance of viroids in grapevines.

The five viroids in grapevines are the grapevine yellow speckle viroids 1 and 2 (GYSV1 and GYSV2), which are highly specific to grapevines, the hop stunt viroid (HSV), the citrus exocortis viroid (CEV) and the Australian grapevine viroid (AGV), all of which are present in vines with no apparent deleterious effects, except for GYSV1 and GYSV2 which cause the grapevine yellow speckle disease in hot weather.

A prerequisite for this study was a sensitive means of viroid detection. In the first part of this research, an efficient RNA extraction protocol suitable for RT-PCR analysis for all five viroids was developed. The high quality of the RNA extract enabled the development of a highly sensitive RT-PCR assay for the diagnosis of grapevine viroids, significantly more sensitive than other viroid detection techniques currently in use. Careful selection of primers and optimization of the conditions for amplification by fast cycle times was essential for the detection of all five viroids. The amplification of viroids was found to be highly sensitive to the position of PCR primers in relation to viroid structure and the PCR product length. Amplification by the optimized RT-PCR protocol developed here was found to be 25,000-fold more sensitive than dot-blot hybridization analysis using ^{32}P-labelled riboprobes, for a high GYSV1 titre Sultana H5 vine. However, sequence variation was observed to influence the sensitivity of detection of GYSV1 by RT-PCR.

Applications of this RT-PCR-based viroid detection technique included assays for the five viroids in 10 grapevine varieties and for GYSV1 and HSV in SAMC-derived vines and in seedlings. Dot-blot hybridization analysis using ^{32}P-labelled riboprobes

was carried out for GYSV1, HSV, AGV and CEV to confirm the results for viroids present in higher titre. GYSV2 was not assayed separately because it had been reported to cross-hybridise with GYSV1.

Dot blot hybridization assays detected GYSV1, HSV and AGV in the 10 grapevine varieties tested. CEV was not detected. In contrast, the RT-PCR assay detected GYSV1, GYSV2, HSV, AGV and CEV in the 10 grapevine varieties including CEV which previously could not be detected directly by others in vine tissue and required passage through alternative hosts to build up the CEV titre.

Sixty four vines regenerated by SAMC, derived from 14 wine grape varieties and nine rootstocks, were obtained from two separate sources in Australia and assessed for their viroid content. Dot blot hybridization assay showed the persistence at low level of GYSV1 and AGV in almost all the vines tested. HSV was reduced to a level detectable, in most cases, only by RT-PCR. The results obtained from the vines from both sources provided strong evidence that viroids were not eliminated in grapevines by SAMC as reported by other workers, but rather, viroids were differentially reduced. A similar effect was observed in 19 vines regenerated by fragmented shoot apex culture (FSAC).

The indexing of viroids in 11 seedlings demonstrated a viroid content for seedlings, with only GYSV1 and AGV detected in most cases by dot blot hybridization assay, and indicated a differential transmission of viroids via seeds. The detection of viroids in seedlings by RT-PCR was achieved only with the use of an amplification protocol in which the sensitivity was further increased by "Hot Start" PCR. The RT-PCR results confirmed the transmission of GYSV1 in all 11 seedlings and revealed the presence of HSV in 10 seedlings.

A study of the effect of micropropagation on viroid content in grapevine samples generally showed an increase in viroid titre in micropropagated vines compared with their respective stock vines.

The newly-developed RT-PCR assay for viroids was furthermore used in conjunction with SAMC in an attempt to produce viroid-free vines. Stock vines, specially selected for their low viroid titre, were treated in low light and low temperature

conditions for up to 7.5 months prior to meristem culture to favour viroid elimination. However, GYSV1 and HSV were detected by RT-PCR in most of the subsequently regenerated vines.

The detection of viroids in grapevines using two different assay techniques highlighted the critical influence of the sensitivity of diagnostic methods. The data obtained so far provided ample evidence that viroids were not easily eliminated from grapevines. The high sensitivity RT-PCR assay was extended to the detection of grapevine leafroll-associated virus-3 (GLRaV-3) and the putative GLRaV-1 in infected grapevines.