Gene Transfer and Interspecific Hybridisation: Two Approaches to Virus Resistance in Papaw (*Carica*)

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INTRODUCTION

Papaw or papaya (*Carica papaya* L.) is grown as a fruit crop in countries with tropical and subtropical climates. In 1988 the world production of papaw was 3.68 million tonnes. Papaya ringspot virus (PRSV) is the most serious disease of papaw (Purcifull, 1972) and poses the greatest single threat to papaw production in the world (Litz, 1985). It was first reported on the island of Oahu in Hawaii in 1945 (Lindner et al., 1945) and subsequently occurred in Africa, Bangladesh, Brazil, Colombia, Cuba, El Salvador, Sri Lanka, Venezuela, India, Thailand, Taiwan, and the Philippines. In the nineties it has been reported in Malaysia and South East Queensland.

PRSV is a member of the potyvirus family and infects plants in families Caricaceae, Chenopodiaceae, and Cucurbitaceae. Two closely related strains occur: P and W. Papaws are infected by strain P, which is aphid-borne and spreads rapidly in affected areas. The symptoms of PRSV-P in papaws are vein clearing and chlorotic spots followed by mottling and distortion of the leaves, and greasy ringspot patterns on the fruit and upper portion of the stem. Many trees die before fruit set and trees that do survive set few fruit. These fruit are of poor quality, have a low sugar content, are covered by greasy ring spots, and are unmarketable.

DEVELOPMENT OF PRSV-P TOLERANT VARIETIES

Since the pioneer work by Conover in the early seventies (Conover, 1976), there have been numerous attempts to produce tolerant lines by selection and crossing of various cultivars within the species *Carica papaya*. A number of lines that exhibit some tolerance have been produced including Cariflora (Florida), Know You, Red Luck, and Tainung (Taiwan). However, these lines have been ineffective and have not allowed re-establishment of commercial plantations in infected areas. When trialed in Australia, their performance against local strains of PRSV-P has been poor. Recently 'Sinta' has been produced in the Philippines and shows good tolerance to their PRSV-P strains (Mercado et al., 1995).

DEVELOPMENT OF PRSV-P RESISTANT VARIETIES

Currently two approaches are being used in Australia to produce papaw lines that are resistant to PRSV-P. Resistance to PRSV-P has been reported in the *Carica* species *cauliflora, pubescens, quercifolia*, and *stipulata* and an attempt to introgress genes from these wild species into papaw is being made using interspecific hybridisation. This work is a collaborative project between Queensland Department of Primary Industries and University of the Philippines at Los Banos and is being funded by the Australian Centre for International Agricultural Research. The second method involves gene transfer. Constructs of viral coat protein and viral replicase genes are being transferred to papaya via microprojectile bombardment.

This project is being undertaken by the Queensland Department of Primary Industries and the Queensland University of Technology and is being funded by the Queensland Papaw Industry and the Horticultural Research and Development Corporation.

COMPARISON OF TWO METHODS

The aim of this paper is not to present results but to compare the advantages and disadvantages of the two research methods. These are summarised in Table 1. Details of conventional breeding between papaw varieties are also presented, but since no resistance exists within *Carica papaya*, this technique is not discussed further.

Some wild relatives of papaya that are highly resistant, may even be immune to PRSV. After inoculation with high levels of PRSV followed by growth in the field with papaws exhibiting high levels of infection, no virus could be detected in either *C. cauliflora* or *C. quercifolia* by ELIZA testing (Persley, pers comm). Furthermore, this resistance has been stable for long periods in infected areas. Long-term field performance of coat-protein-mediated resistance is as yet untested. In some cases, other species transformed with coat protein constructs have shown reduced virus symptoms or tolerance rather than resistance. Single-gene disease resistance developed in plant breeding programs often breaks down in the field as virus strains mutate and this may occur with coat-mediated resistance. The useful life may be extended by the transfer of two genes for resistance (for example coat protein and replicase genes). To be successful resistance must be both stable and heritable.

When adding genes for PRSV resistance by either breeding or genetic engineering the genetic integrity of elite cultivars must be maintained. Interspecific hybridisation requires a long backcrossing programs (6 to 8 generations) to restore commercial cultivars. In theory, a major advantage of gene transfer is that one or two useful genes may be added to an elite genotype without altering the rest of the genome. However, in practice, transgenic plants are regenerated from cell cultures, which are prone to genetic variation resulting from somaclonal variation.

A laboratory phase is required for both methods. Embryo rescue and in vitro plantlet production are required following interspecific hybridisation as embryo abortion and death occur due to breakdown of the endosperm. Routine procedures have already been developed for embryo culture, embryogenesis, and plantlet production (Magdalita et al., 1996). Gene transfer requires the development of embryogenic cultures, high frequency gene transfer and stable expression, growth on medium containing kanamycin to select transformed cells, good regeneration of plants from these cells, and growth in vitro of a plantlet that can be acclimatised. These stages constitute lengthy laboratory phases and because this is a new field of research, unexpected problems and delays regularly occur. For example, transgenic papaw plants grow very poorly in culture and are extremely difficult to micropropagate.

Field evaluation of resultant plants is essential. Because interspecific hybrids are from wide crosses, incompatibility causes poor growth and development of plants and high levels of infertility. By comparison, in Hawaii transgenic papaw plants are growing normally in field plantings (Manshardt, pers. comm.). In Australia transgenic plants are growing poorly in culture and may lack vigour when grown in the field.

Transgenic plants pose environmental and legal problems and can be grown in the

Table 1. Advantages and disadvantages of conventional breeding, interspecific hybridisation and gene transfer as methods of developing PRSV-P resistance in papaw.

Conventional breeding within *Carica papaya*.	Interspecific hybridisation with *C. papaya*	Gene transfer of essential viral genes to *C.*papaya
No resistance only tolerance to PRSV	Species resistant over long period in field	Long-term field performance unknown
Requires long backcrossing program	Requires long backcrossing program	Potential to add 1 or 2 genes without altering remainder of genome
No laboratory phase, plants grow normally	Proven laboratory phase, no unexpected problems	Complicated laboratory phase with unexpected problems and delays
No growth problems	Hybrid breakdown, prone to growth problems in glasshouse and field	Reduced regeneration and poor growth after gene transfer and selection
Fertile hybrids	High levels of infertility	Infertility rare, somaclonal variants can occur
Field evaluation required - no complications	Field evaluation required - no complications	Field evaluation required - subject to legal and environmental restrictions
No patent limitations	No patent limitations	Patent obligations

glasshouse and field only after permission from (GMAC) Genetic Manipulation Advisory Committee. There may be patent obligations involving up front payments and/or royalties before these plants can be grown commercially. If PRSV resistance can be transferred from wild species there are no patent obligations or restrictions on field testing.

Both approaches should provide PRSV-P resistant papaya lines in the future although the first plants in the field are likely to result from the gene-transfer project.

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Getting the Most Out of Growing Media and Nutrition

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INTRODUCTION

Plants need carbon dioxide, water, mineral nutrients, oxygen in their root zone, and energy. In their natural state they acquire these for themselves. It ought not to be too difficult to help them along in a nursery, but somehow it often is. The typical Australian nursery is placed at various levels, somewhere between the maligned backyarder who recognises the simplicity of it all and the idealism of a Dutch grower nurturing a single variety with almost total environment control.

We can abridge the requirements of plant survival in nurseries into the following parts: the plant, the growing media, nutrition, the environment, pest, disease and weed control, and management.

THE PLANT

Universally, plants obey the same principle rules for survival but because they have to cope with different environments they modify their responses. In short, each plant is distinct and requires a different level of management. Most have wide tolerances of growing conditions. If this were not so then the nursery industry could