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THE ROLE OF BIOTECHNOLOGY IN CROP IMPROVEMENT

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ABSTRACT

For thousands of years conventional breeding techniques have been used to improve crop plants. Emerging biotechnologies enable us to work at the whole plant as well as the organ, tissue, cell, protoplast, chromosome and gene levels in our efforts to modify plants. Biotechnology has loosely been defined to include a collection of techniques including gene mapping, recombinant DNA, Agrobacterium-mediated gene transfer of recombinant DNA, DNA and chromosome microinjection, microprojectile bombardment, protoplast fusion, selection for somaclonal variation, embryo rescue, anther culture and micropropagation. Plants with disease resistance, pest resistance, herbicide tolerance, drought tolerance and increased yield have been produced using one or a combination of these techniques. The application of several of these techniques to the improvement of peach will be discussed. Plant breeding in combination with biotechnology provide a bright future for the improvement of crop plants.

INTRODUCTION

This is the "Age of Biotechnology". To some, this phrase conjures up images of a Pandora's box, while to others, it conjures up images of "super plants" that will be a panacea for all our agricultural problems. These images are misleading and can lead to either overexpectations or fear of biotechnology. The objective of this paper is to present a more realistic picture of the types of research being conducted by presenting some of my own research.

Most of my research falls under the heading of "Plant tissue culture". Just as biotechnology is not one technology, plant tissue culture is composed of many technologies, i.e., embryo rescue, anther culture, protoplast fusion, selection for somaclonal variation, etc. These techniques enable us to conduct research at the whole plant, organ, tissue, cell, protoplast, chromosome or gene levels and can be used effectively for the improvement, propagation or preservation of plant species. What makes these techniques applicable to crop improvement is "totipotency" or the ability of plant cells, tissues and organs, under controlled conditions, to undergo morphogenesis. The nature of the problem to be solved and the amount of groundwork laid with respect to the technology will determine whether conventional approaches and/or biotechnological approaches should be utilized.

Micropropagation

A major obstacle to crop productivity is the occurrence of plant diseases. Over the years effective control measures have been developed for many pathogenic fungi and nematodes, but not for viruses which are present in virtually all food crop species and cause serious yield losses. Viral diseases in vegetatively propagated crops, i.e., fruit tree species, warrant special attention because of the rapidity with which these diseases spread by means of clonal propagation. Virus-indexed budwood of temperate fruit tree species is often in very short supply and the standard method of propagating these species, bud-grafting, is not an effective method for producing large numbers in a limited amount of time. Micropropagation is an extremely effective technique for propagating a wide range of plant species (Read and Hosier, 1986) and can also be used in conjunction with virus elimination procedures (Kantha, 1986).

A micropropagation system for peach has been developed (Hammerschlag, 1982; Hammerschlag et al., 1987) and numerous cultivars can now be propagated effectively in vitro. Field studies have demonstrated that tissue cultured, own-rooted trees produce a marketable fruit crop at least one year earlier than budded trees (Hammerschlag and Scorza, 1990). This research demonstrates the feasibility of using tissue culture techniques for propagation of peach trees and points out that crop productivity can be influenced by changes in methods of propagation.

Selection and Screening for Somaclonal Variants with Disease Resistance

The germplasm base is quite narrow for most commercial peach (Prunus persica (L.) Batsch) cultivars in the United States (Scorza et al., 1985) and reports indicate a scarcity of peach germplasm with resistance to bacterial leaf spot caused by Xanthomonas campestris pv. pruni (Werner et al., 1986) and bacterial canker caused by Pseudomonas syringae pv. syringae (Petersen, 1975). One approach for generating variation that has received a good deal of attention is to obtain somaclonal variants generated by the tissue culture cycle (Larkin and Scowcroft, 1981).

Selection at the cellular level for somaclonal variants that are insensitive to toxic metabolites produced by plant pathogens is an example of a tissue culture technique that has been used successfully to obtain disease resistant plants (Daub, 1986; Hammerschlag, 1984a). Cell cultures are exposed to the toxic metabolite produced by the plant pathogen and plants are regenerated from the cell(s) that survive the treatment. If the toxic metabolite is involved in disease development, then cells surviving the metabolite treatments

should produce plants that are resistant to the pathogen that produced the metabolite.

Before applying the above approach to peach cells, prerequisite studies were conducted to determine the feasibility of using this approach. Our studies demonstrated that 1) a toxic metabolite is produced by the pathogen that is involved in disease development and is active at the cellular level (Hammerschlag, 1984b); 2) peach plants can be regenerated from callus derived from immature embryos (Hammerschlag et al., 1985); and 3) peach plants can be propagated in vitro (Hammerschlag, 1982; Hammerschlag et al., 1987).

Recurrent selection studies were initiated utilizing embryo callus derived from the bacterial leaf spot-susceptible peach cultivar Sunhigh and a toxic filtrate (TF) of X. campestris pv. pruni (Hammerschlag, 1988). Two out of 400 calli survived treatments with progressively higher concentrations of TF, and two plants were regenerated from each of the two surviving calli. Each regenerant was micropropagated and tested for whole plant resistance to X. campestris pv. pruni. Results from bioassays on greenhouse-grown plants indicated that two out of the four selected clones were significantly more resistant than cultivar Sunhigh and one was significantly more resistant than moderately resistant cultivar Redhaven. These plants are currently being evaluated in the field for leaf spot resistance and ultimately, fruit production, fruit quality, and heritability of leaf spot resistance.

Screening unselected regenerants is another approach to obtaining disease resistance (Daub, 1986; Hammerschlag, 1984a). This approach can be used when either 1) a selective agent is not available, 2) a selective agent is not involved in disease development, 3) a selective agent does not act at the cellular level, or 4) organized tissue is required for the expression of resistance. This approach is much simpler to carry out than in vitro selection and may be chosen for this reason alone. One major disadvantage is that only limited numbers of regenerants can be screened at any one time. However, previous studies have indicated that large numbers of regenerants may not be needed in order to identify a desirable mutant (Evans et al., 1984; Irvine, 1984; Lorz and Scowcroft, 1983; Zong-Xiu et al., 1983).

Unselected peach regenerants were screened for resistance to X. campestris pv. pruni utilizing a modified detached-leaf bioassay (Hammerschlag, 1990; Randhawa and Civerolo, 1985). Of the 26 regenerants derived from two 'Sunhigh' embryos, nine were more resistant than 'Sunhigh'. Of the 19 regenerants derived from three 'Redhaven' embryos, one was more resistant than 'Redhaven'. Phenotypic stability of bacterial spot

resistance in regenerants was investigated to determine whether aging or propagation influences the disease resistance response (Hammerschlag, 1990). Our results indicate that spot resistance was retained in some regenerants over time and following propagation. These results together with the results from in vitro screening provide evidence that these approaches can provide much needed useful variation in peach.

Screening Regenerants for Nematode Resistance

Because organized tissue is required in order to detect the expression of nematode resistance, screening either whole plants or organ cultures has been used to obtain resistance to nematodes (Lauritus et al., 1982; Palys and Meredith, 1984). Recently, screening whole peach plants in vitro has been shown to be an attractive alternative for identifying plants with resistance to root-knot nematode (Meloidogyne incognita) because the procedure facilitates early detection of nematode resistance (Huettel and Hammerschlag, 1986). Studies are in progress to use this procedure to screen peach regenerants and own-rooted cultivars for root-knot resistance.

Agrobacterium-mediated Gene Transfer

Unravelling the nature of host-pathogen interaction in the crown gall disease syndrome (Chilton et al., 1977) has led to the use of the crown gall pathogen, Agrobacterium tumefaciens, as a vector for introducing genes into plant cells (Fraley et al., 1986). Transformation was once a fairly complicated procedure, involving regenerating calli from protoplasts transformed by co-cultivation with A. tumefaciens (DeBlock et al., 1984; Horsch et al., 1984). Recently, simpler methods have been devised utilizing organized tissues rather than protoplasts (Fillati et al., 1987; Horsch et al., 1985; Pua et al., 1987).

Peach has been shown to be a host of Agrobacterium (Kerr, 1969), which is an important prerequisite for Agrobacterium-mediated gene transfer. Transformation of peach cells derived from mature plants was recently described by Smigocki et al. (1989). This report demonstrated that peach cells from mature plants can be transformed. Previous studies on transformation of woody species have utilized explants from juvenile tissues (Parsons et al., 1986; Sederoff et al., 1986; Fillati et al., 1987; McGranahan et al., 1987). Juvenile tissues have been used because it is often impossible to regenerate plants from tissues derived from mature plants. Transformation of tissues from mature plants would facilitate rapid improvement of commercially important cultivars. The objective of the peach transformation study was to induce morphogenesis of peach cells from mature plants by transforming cells with the "shooty" mutant strain of A. tumefaciens. This strain contains a gene on the T-DNA region of the Ti plasmid that codes for

an enzyme responsible for cytokinin synthesis (Barry et al., 1984) and a mutation in the region coding for auxin synthesis (Schroeder et al., 1984). Infection with this strain leads to a shooty phenotype on tumors of tobacco (Ooms et al., 1981). By using this strain, we hoped to alter the endogenous cytokinin to auxin ratio to favor shoot regeneration (Skoog and Miller, 1957). Although in the above study, we were only able to obtain transformed cells, studies are in progress utilizing other strains of A. tumefaciens that may induce shoot organogenesis from cells derived from mature plants. More recently, we have obtained transgenic peach plants by co-cultivating immature embryos with the "shooty" mutant and then culturing the embryos on hormone-free medium (Smigocki and Hammerschlag, 1990). In future studies, we will concentrate on transferring genes for disease resistance. Genes of interest for peach improvement include the gene for the bacteriocidal peptide cecropin (Jaynes et al., 1987) and the gene for the coat protein of Prunus necrotic ringspot virus. The recovery of transgenic plants with tolerance to lepidopteran larvae (Fischhoff et al., 1987) or that are protected from virus infection (Cuozzo et al., 1988; Tumer et al., 1987) suggests that Agrobacterium-mediated gene transfer can be instrumental in obtaining resistance to pests and pathogens.

CONCLUSION

In conclusion, the above studies demonstrate how tissue culture and Agrobacterium-mediated gene transfer techniques can be used for peach improvement. These studies are representative of other studies that have been or are currently being conducted with other plant species. These approaches will not replace conventional techniques but rather serve as a useful adjunct to the conventional approaches.

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