RESEARCH REPORT SUBMITTED
TO
OREGON PROCESSED VEGETABLE COMMISSION
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AGRICULTURAL RESEARCH FOUNDATION
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TITLE: Genetic Transformation of Beans

PROJECT LEADERS: David Mok and Machteld Mok, Horticulture, OSU

PROJECT STATUS: Second of five years

PROJECT FUNDING FOR THIS PERIOD: $36,400

Funds were used to support a research technician, a student lab aid, to purchase chemicals and other disposable items.

OBJECTIVES:

1. To devise regeneration systems in beans adaptable to transformation using *Agrobacterium* infection.

2. To design and optimize conditions to deliver DNAs using particle bombardment.

PROGRESS:

Background:

Specific traits in plants can be modified by inserting either foreign genes or altered native genes (the process of delivering genes is called transformation). In plants, two general approaches are employed to obtain transformation although specific conditions of either approach vary greatly between species. The first approach utilizes the bacterium, *Agrobacterium*, which infects plants and inserts a piece of its own DNA (T-DNA) into the host chromosomes. If a foreign gene is spliced into the T-DNA, the foreign gene can be incorporated into the plant. This approach works well in conjunction with regeneration of plants from tissue culture (selection of transformed cells and plants derived from single cells to avoid chimeras). The second approach employs a "gene gun" which delivers DNA coated tungsten or gold particles directly to the growth points of the plant. Seeds are then obtained from treated plants to select for transformed progeny in subsequent generations. Methods to successfully transform beans (as well as other large-seeded legumes such as soybean) have yet to be devised.

An important consideration is which genes should be targeted for change. In recent years, many genes controlling a number of traits have been isolated from plants as well as other organisms. Regardless of the source of origin, these genes can be modified and transformed into plants. In beans, perhaps one of the most obvious ojective is high level of general resistance to

fungal and bacterial diseases. A number of genes encoding disease defense enzymes such as glucanase and chitinase have been isolated. In test plants such as tobacco and *Arabidopsis*, highly expressed genes of this type confer resistance. The utilization of such genes to increase disease resistance of beans will be the eventual goal of the project.

Progress:

In the past year, *Agrobacterium* infection and gene gun were used to deliver DNAs with marker gene (GUS gene) to devise the most efficient protocols for transformation. Successfully transformed cells developed a blue color when treated with appropriate reagents. Both immature embryos and seedlings germinated under sterile conditions were used. They were either infected with *Agrobacterium* after wounding, or subjected to particle (coated with DNA) bombardment directly. Plant materials were then grown on medium containing antibiotics to eliminate the bacteria, or on medium to allow for recovery if the gene gun is used. The tissues were assayed every week to assess the GUS gene expression (blue color development in transformed cells). Approximately 2% of the treated samples were GUS positive. In addition, a new method employing fine glass beads to generate minute wounds (to allow *Agrobacterium* penetration without severely damaging the tissue) was tested and found to increase the survivability of the target tissues. The next step will be to allow the treated material to grow and harvest seeds to determine if the transformed cells are included in meiosis (and gamete formation) to give transformed seeds. Although using the GUS gene is convenient in visually detecting transformation, the assay is destructive (cells are killed); therefore, additional selection markers, herbicide resistance genes will be used in addition to GUS. We are in the process of constructing DNA vectors containing both color and resistant genes for this purpose.

SIGNATURES:

Project Leaders:

Redacted for Privacy

David W. S. Mok Date
Professor

Redacted for Privacy

Machteld C. Mok Date
Professor

Redacted for Privacy

Charles Boyer, Head Date