RESEARCH REPORT SUBMITTED
TO
OREGON PROCESSED VEGETABLE COMMISSION
VIA
AGRICULTURAL RESEARCH FOUNDATION
December 1995

TITLE: Genetic Transformation of Beans

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

PROJECT STATUS: Third 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 which involves selection of transformed cells and subsequently deriving plants 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 many other large-seeded legumes) 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 transferred into

plants.

In beans, perhaps one of the most obvious ojectives is resistance to viral, bacterial and fungal diseases. For viral resistance, overexpressing either coat protein genes of the virus in plants, or generating a number of untranslatable viral RNAs in transgenic plants confer immunity to viral infection. For bacterial diseases such as *Pseudomonas* infection, a gene-for-gene relationship governs the resistance via hypersensitivity reaction (infected cells die thereby limiting the spread of the bacteria). Resistance genes isolated from one host plants can be transferred to others and confer resistance. For fungal diseases such as *Botrytis*, genes encoding proteins inhibiting growth of fungus have also been isolated recently and presumably can be utilized to confer resistance. It is not known at present if such proteins have an effect on inhibiting the growth of white mold (*Sclerotinia*), a serious problem in Oregon. In addition, a number of genes encoding general disease defense enzymes such as glucanase and chitinase can also be overexpressed to elevate the degree of general resistance to pathogens. In test plants such as tobacco and *Arabidopsis*, these approaches have been tested and found to be successful. Therefore, the utilization of such genes to increase disease resistance of beans will be the eventual goal of the project. (An oral presentation of the background and progress will be made at the January 16 Vegetable Growers meeting in Salem.)

Progress:

In the past years, we have utilized both *Agrobacterium* infection and gene gun to successfully deliver DNAs with a reporter gene (GUS gene) to a variety of bean tissues including hypocotyls, callus and meristem of germinating seeds. Transformed cells are evidenced by the formation of a blue color when treated with appropriate reagents, the result of the expression of the foreign reporter gene, GUS. Immature embryos and seedlings germinated under sterile conditions were used. Approximately 2% of the cells in treated samples were GUS positive. Although using the GUS gene is convenient in visually detecting transformation, the assay is destructive (cells are killed). Therefore, in the past year, additional selection markers, herbicide resistance genes were incorporated into the DNA in addition to the GUS gene. These genes confer resistance to kanamycin or the Bialaphos and treated tissues were grown on medium containing these selective agents to allow only transformed cells to grow. Samples of surviving cells were then used for the GUS assay to reconfirm the presence of foreign DNA. This modified procedure resulted in recovering transformed cells in all tissues treated. However, shoots derived from treated meristems did not give rise to transformed seeds. The exact cause of transformed cells not included in meiotic cells (derived from layers 2 and 3 cells of the meristem which give rise to pollen and eggs) is not clear. One of the most likely explanation is the escape of larger number of non-transformed cells from herbicide selection which out competed the low number of transformed cells. Future efforts will be directed at increasing the frequency of transformed cells by using much younger tissues (such as immature embryos under 1 cm in length) and stronger selection pressure (higher concentration of herbicides) to eliminate non-transformants.

SIGNATURES:

Project Leaders:

Redacted for Privacy

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