RESEARCH REPORT SUBMITTED
TO
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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: Fifth year

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. For bean production in the Willamette Valley, perhaps one of the most obvious ojectives is resistance to white mold (*Sclerotinia*) since this pathogen is difficult to control using chemicals and no native resistance has been found in common bean (*Phaseolus vulgaris*). It has been shown that the production of oxalic acid by the fugus *Sclerotinia* is the primary cause of pathogenicity. If the oxalic acid can be degraded rapidly by the plant, the symptom of infection can be inhibited. This approach has been tested by inserting and over-expressing a gene encoding oxalate oxidase (which converts oxalic acid to carbon dioxide and peroxide) into rape seeds and the test plant *Arabidopsis*. In both cases, resistance to mold was achieved. Similar solution should be applicable to beans if transformation techniques can be devised. Our research is centered on establishing such techniques.

Progress:

In the past years, we have utilized both *Agrobacterium* infection and the 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. The next step is to select meristems (growth points) containing the transformed cells and allow them to grow into whole plants and collect seeds from those plants, with the objective of recovering seeds that are transformed. This is necessary to avoid chimera especially in sexually propagated crops. The major challenge that remains is to enable transformed cells to go through sexual cycle and produce seeds.

The process consists of two steps. First, getting transformed cells into meiosis (cell division to form pollens and eggs). Second, allowing the transformed gametes (pollen and eggs) to be fertilized to form transformed sees. In the past year, we were successful in getting transformed somatic cells into meiosis and gametes with the reporter gene inserted into its DNA were formed (evidenced by the blue pollen grains). The result is of critical importance since it demonstrates that transformed vegetative tissues can lead to gametes containing the foreign gene. The next step we will pursue is to enhance the probability of the transformed pollen to achieve fertilization to produce seeds. Selective fertilization in beans may require some manipulation as well since the number of seeds per flower is very limited (in contrast with corn or other crops with high number of seeds per flower).

SIGNATURES:

Project Leaders:

Redacted for Privacy Redacted for Privacy

David W. S. Mok Date — Machteld C. Mok Date
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Charles Boyer, Head — Date