Tissue Culture and Gene Transfer Approaches To Dry Bean Improvement

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The development of new variability for important agronomic traits that can be utilized by plant breeders is a primary goal of plant biotechnology. A key experimental procedure for these endeavors is a tissue culture regeneration system for the crop of interest.

With this sytem a piece of the plant is placed on a defined medium or a series of media and a new immature plant develops. This structure is nursed carefully and the plant eventually is transferred to a greenhouse where it is grown to maturity. The plant is termed a regenerated plant.

The procedure itself is known to induce or uncover variability (termed somaclonal variation) by some unknown mechanisms. For example, progeny of regenerated wheat plants exhibited variation from the parent for plant height, tiller number and heading date (Larkin et al. 1984).

A variation of this tissue culture approach is to add a selective agent in the tissue culture media. When potato was cultured in the presence of the herbicide MCPA, lines tolerant to the herbicide were identified (Wershun et al. 1987). Toxins developed by plant pathogenic organisms are another selective agent used in tissue culture media, and Gengenbach et al. (1977) developed corn lines resistant to the toxin produced by southern corn leaf blight. Thus tissue culture is potentially a valuable tool for generating and identifying novel germplasm.

Successful regeneration procedures have also been exploited for transferring genes of commercial importance into crop plants. This system involves the interaction of the plant part used in the tissue culture regeneration system and a biological agent to which a gene of commercial interest has been inserted. The biological agent transfers the gene to cells in the plant part from which the new plant will arise, and plants which contain the new gene are selected from those which do not. This procedure has allowed scientists to successfully develop plants resistant to viral attack (Powell et al., 1986), herbicide application (Shah et al., 1986) and insect predation (Vaeck et al., 1987). Thus, tissue culture has other important applications beside identifying somaclonal variation.

We have recently developed a tissue culture regeneration system for dry bean (McClean et al., 1989) and currently are assessing the degree of variation which it generates and testing its feasibility for gene transfer. This report aims to describe the regeneration system and the degree of variability detected to date. Also, we will briefly present preliminary results from our gene transfer experiments with this system.

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REGENERATION SYSTEM

The intial step in developing a tissue culture regeneration system is identifying a plant part which possesses the potential to develop new plants. Leaf pieces or hypocotyl sections (the stem region below the cotyledon of a seedling) have been quite responsive in many plant species but have not worked for dry bean tissue culture. A recent report indicated the cotyledonary node tissue of soybean was responsive in tissue culture (Wright et al., 1986). Since soybean had similar regeneration difficulties as dry bean, this tissue was tested on a series of media.

The cotyledonary node is the region about the cotyledons on the germinating dry bean seed where the cotyledons attach to the main stem. This node was trimmed and grown on several tissue culture media (McClean et al., 1989). A media which contained a basic mixture of sugar, inorganic salts and vitamins supplemented with the plant hormone benzyladenine induced new shoot development from the cotyledonary node. Benzyladenine belongs to a class of plant hormones called cytokinins which introduce shoot development. Several concentrations were compared and nodes grown in the presence of 5μM benzyladenine were the most responsive.

Two weeks after the nodes were placed on the medium, shoots were removed. The shoots do not contain roots and thus are placed in another medium to induce root development. These miniature plantlets were allowed to grow one month further in the culture room before transferring to the greenhouse. The plants obtained from these procedures express normal development and are sexually mature. Seed from these plants are then grown to determine if any cultured induced variation was generated.

Once shoots are removed from a node it can again be placed in culture for further shoot development. We have removed shoots every two weeks for three months at which time the node appears to lose the ability to initiate shoots. Since we remove two to three shoots each round, a single node can produce a large number of plants for analysis. This regeneration produce has been tested on the dry bean cultivars Othello, Nodak, Boz'n, Pindak, Midland, Olathe and Fleetwood. Only Boz'n performed poorly in culture.

SOMACLONAL VARIATION

Although the identification of genetic material which expresses improved agronomic traits broadens the genetic pool from which the plant breeder can choose, other tissue culture applications are best served when little or no variation is generated by the procedure. Once we had developed the regenerated procedure we examined the offspring of the regenerated plants to measure the extent of new variability we had generated. Two hundred fifty progeny lines from regenerated plants of Othello and Olathe, two pinto bean cultivars, were initially grown in a common blight nursery and in the Uniform Dry Bean Nursery during the summer of 1987. The goal of that experiment was to determine if resistance to either of these pathogens could be detected and if other morphological or agronomic changes had occurred among the material. Each line was investigated for symptoms of disease development but no variability could be detected. In fact, the only change detected among these 250 lines was an Olathe line which matured 10 days earlier in both nurseries.

Although the detection of traits of agronomic importance is one important goal of tissue culture, new material is occasionally produced which is of importance to basic genetic research. Within one line from a regenerated Olathe plant, five pink seeds were found. Olathe is a pinto type and thus some genetic change has occurred in this line. An extensive genetic system is responsible for the many dry bean seed coat color types and different interrelated factors interact to change the seed coat from one color to another. More specifically, two genetic changes are required to alter a pinto seed into a pink seed. This pink Olathe line thus may have arisen from two specific genetic changes in the known pathway, or alternatively by a genetic change which generated a new pink seed coat developmental pathway. We are currently performing the appropriate genetic crosses to investigate these hypotheses.

Another example of somoclonal variation was detected in the pinto cultivar Pindak, where among 22 progeny of plants regenerated from a single cotyledonary node, 11 were markedly shorter at maturity. Several of these short plants also displayed a smaller leaf size. In contrast, neither Olathe or Othello exhibited this reduced height characteristic. Although we have detected some differences in our regenerated material, we would have to conclude that our regeneration procedure generates minimal variation.

GENE TRANSFER

The second use for a regeneration system is the insertion of foreign genes into the DNA of a plant. This process is termed transformation. Currently, the most successful transformation procedure utilizes the naturally occurring plant pathogen *Agrobacterium tumefaciens*. This pathogen has the unique ability to transfer DNA into a plant where it will function as a normal part of the genetic material of the plant. Once this process was understood, Agrobacterium was engineered so that only useful genes were transferred. We thus sought to utilize our regeneration system and the Agrobacterium transfer system with the goal of eventually transferring beneficial genes into dry bean.

Our initial experiment was to determine which dry bean cultivars were susceptible to Agrobacterium infection. Three biotypes of *Agrobacterium tumefaciens* exist and all have been used for gene transfer experiments. We tested the infectivity of each biotype on 19 dry bean cultivars. All cultivars were susceptible to infection by each biotype in a seedling assay we have used in the laboratory. Since our regeneration system was quite productive with Othello, we concentrated on that cultivar in further transformation experiments.

Utilizing a regeneration system for transformation provides a screening step which distinguishes those newly emerging shoots which possess our transferred genes from those which do not. The essential feature of this step is a drug resistance marker which residues on the DNA which is transferred to the plant and allows the recipient plant to grow in the presence of the drug. Agrobacterium will transfer this DNA to the plant cell and allow the recipient plant to grow in the presence of the drug. After the bacteria and plant part are incubated together, any new shoot surviving in the presence of the drug may have received the DNA from the bacteria. Once this procedure is perfected for the crop species of interest, a useful gene (herbicide resistance, insect resistance, viral resistant) can be placed on the same piece of DNA as the drug marker and transfer of both the useful gene and drug marker occurs simultaneously. We currently have generated 100 plants which exhibit resistance to the drug marker and experiments are being performed to confirm the presence of the DNA in the plant.

Although transformation has the distinct advantage of adding genes of specific agronomic benefit into the plant, to date only a few genes are available. Currently research is being directed toward identifying new genes and locating the DNA which corresponds to them. This research will be central to biotechnology for years to come. At that point transformation technology will allow the biotechnologist to target crop improvement to specific traits.

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