IMPROVEMENT OF POTATO DISEASE AND PEST RESISTANCE

by
C. R. Brown and C. P. Yang
Research Geneticist and Biological Technician
USDA, ARS, Prosser, Washington

Introduction:

The modern arena in potato breeding is likely to include a mix of traditional crossing and genetic engineering techniques. The purpose of this paper is to describe genetic engineering experiments in progress at the USDA/ARS Potato Genetics Laboratory at the Prosser Research Station. The aim of this work is to provide "new" genetic options for the solution of economic problems facing the potato industry through variety development.

Most of the disease and pest problems in potato production are currently addressed with chemical products. Some of these are likely to lose regulatory clearances in the future. Obviously a highly desirable course of action would be to switch to varieties that are genetically resistant to the problem in question. The traditional approach has been to survey available breeding parents, identify resistance, and try to incorporate this into new breeding material by making progenies of crosses between the resistant parents and clones that are successful varieties or are advanced clones with many desirable traits. Combining all the characters necessary for commercial success is a difficult process. Most of the potential varieties with good processing characters have relatively little resistance to most prominent pests and pathogens. Nonetheless, traditional breeding has much to offer in the coming decades and definitely deserves sustained funding.

One of the new routes for introduction of genetic variation involves the insertion of cloned genes by means of Agrobacterium-mediated transformation. Agrobacterium naturally has the ability to place a portion of its genetic code into plants. Transfer of bacterial genes into the plant genetic code was evolved by the bacteria to take over the metabolism of plant cells at the infection site and provide the bacterium a good home in the form of a tumor or gall (hence the name "crown gall bacterium") and an abundant food source in the form of unique amino acids that only the bacterium has the enzymes necessary to catabolize them. The secrets of the Agrobacterium gene transfer became known to science around 1980 just as molecular geneticists were perfecting their ability to isolate genes and deploy them in a precise fashion. This led to the placement of genes of interest in Agrobacterium and the further shuttling of these genes into plants. Fortunately the potato is a willing recipient of genetic material from Agrobacterium and is therefore the logical subject of a large amount of effort in this regard.

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The problem that we are addressing in Prosser is the creation of Colorado potato beetle resistant germplasm by this method. To introduce this topic it is necessary to describe another bacterium, Bacillus thuringiensis (Bt). Bt is a soil bacterium that produces crystalline proteins when it sporulates. These proteins exhibit highly specific insecticidal properties. Different strains of the bacterium produce distinct classes of proteins which in turn have diverse insect toxicity spectra. We are interested in two types of toxins produced by two different bacterial genes. The cryIA(a) gene produces a protein that kills some Lepidopetera (moths and butterflies) and the cryIIIA gene kills some Coleoptera (beetles) and, in particular, Colorado potato beetle (CPB). Although the gene products are called toxins they are not harmful in anyway to humans. They are considered benign and natural, and are officially acceptable to certified organic growers as insecticides. The objective is to get potato plants to make these proteins and thus enable them to control insect pests.

Procedures:

The engineering of this feat involes several steps. First the Bt genes have to be isolated from the bacterial host. Next the genes must be placed in the Agrobacterium in the section of DNA that is transferred to the plant called transfer-DNA. In addition, the bacterial gene must be placed with regulatory sequences that tell it to be expressed in a plant cell. The gene must be next to a selection gene that provides the plant cell with the ability to survive and grow in the presence of an antibiotic called kanamycin. The last step is to move the Bt genes into the plant cell using the marvelous mechanism that nature already provided in the Agrobacterium. We are fortunate to have a close collaboration with Dr's. Helen Whiteley and Doug Bradley of the Department of Microbiology at the University of Washington, who isolated the Bt genes and constructed the Agrobacterium vectors.

To obtain whole plants it is necessary to expose plant cells to Agrobacterium infection, select out the cells that have received the gene, and induce these cells to regenerate into whole new "transgenic" plants. This is done in tissue culture. Little plants are grown in glass tubes on special media in the absence of bacterial and fungal contaminants. Pieces of stems and leaves are dipped into a suspension of Agrobacterium cells that are ready to donate their transfer-DNA to plant cells exposed at the cut surface. Then the pieces are cultured on medium that contains kanamycin. Only the few plant cells that have received the transfer-DNA with the resistance gene can grow in the presence of kanamycin.

Results:

A binary Agrobacterium vector containing the cryIA(a) gene was inoculated onto in vitro internodes and leaf-pieces. Numbers of pieces and dates of inoculation are indicated in Table 1. Cultivars "Russet Burbank" and "Lemhi Russet" were tetraploid (2n=48) potatoes important to the processing industry of Washington. Clones J and DM56-4 were experimental diploid (2n=24) clones from the cultivated gene pool.

Various media were tested. After inoculation internodes were placed on callus induction medium (CIM) to build-up callus prior to placing calli on shoot regeneration media. Accordingly, developed calli were placed on various shoot induction media (SRM's and SA4, a summary of ingredients of CIM and other media that will be mentioned is supplied in Table 2). In addition to the ingredients listed, all media contained 50 mg-L^{-1} kanamycin sulfate and 200 mg-L^{-1} cefatoxime (bacteriostat for controlling Agrobacterium).

When shoots appeared on calli they were excised, taking care not to include undifferentiated callus. Shoots were kept only if they initiated roots when excised and transferred to shoot propogation medium containing kanamycin. A summary of the yield of shoots from shoot regeneration media, that also initiated roots on kanamycin-containing shoot medium (a sign that they were truly transformed) is given in Table 3. Media SRM2 and SRM3 were the only effective media for "Russet Burbank" while SRM1, SRM2, and SRM3 were all effective with DM56-4. Media SRM2 and SRM3 differed from SRM1 in having higher levels of zeatin. In addition, an auxin/cytokinin ratio experiment was carried out with three potato genotypes. Indole acetic acid (IAA) was the auxin and zeatin the cytokinin. This had an additional objective of determining the necessity of initiating tissue culture with callus induction medium. The results are presented in Table 4. The predominant factor again seemed to be concentration of zeatin. Maximum shoot formation was found at 10 micromolar regardless of the concentration of IAA. Also, this experiment showed that it was clearly not necessary to begin the tissue culture process on callus induction medium.

Results with binary vectors pWTC304 and pWTC350, which contain the Bt gene whose product controls Colorado potato beetle, indicated that putatively transformed shoots began appearing six weeks after inoculation. This has been achieved with "Russet Burbank" and "Lemhi Russet" by omitting callus induction step and culturing inoculated internodes directly on the shoot regeneration media SRM2, SRM3, and 3C5ZR, a medium described by Sheerman and Bevan (1988). It appears that 3C5ZR is better than SRM2 and SRM3.

Summary:

Methods for handling large numbers of genotypes needed to be developed in order to incorporate foreign genes into potato for breeding purposes. The inoculation with Agrobacterium of in vitro internodes of various potato gentotypes, and culture on various high zeatin shoot regeneration media provided a convenient method. The apparently low escape rate, as evidenced by resistance of most excised shoots to kanamycin in shoot propogation medium ensures that the production of hundreds of transgenic plants is feasible. A reasonable evaluation of transgenic potato includes testing of the transgenic clones under field conditions. Plans for 1990 include the field-testing of transgenic "Russet Burbank," "Lemhi Russet," and DM56-4 in Washington state.

References

Sheerman S., and Bevan M. W. (1988) A rapid transformation method for Solanum tuberosum using binary Agrobacterium tumefaciens vectors. Plant Cell Reports 7:13-16.

Table 1. Number of in vitro internodes or leaf-pieces inoculated with binary vector with PWTC101 (Cry1A(a)) insert on various dates.

Date of inoculation	Plant tissue	RB	Lemhi	DM56-4	J
7-3-89	Internodes	28	23	19	22
	Leaf pieces	51	51	36	34
7-10-89	Internodes	47	41	44	55
	Leaf pieces	24	--	--	--
7-25-89	Internodes	65	39	30	48
	Leaf pieces	--	--	--	--
8-9-89	Internodes	53	44	16	--
	Leaf pieces	--	--	--	--
8-16-89	Internodes	68	86	40	44
	Leaf pieces	--	--	--	--
8-23-89	Internodes	68	47	32	36
	Leaf pieces	--	--	22	17

Table 2. Ingredients in different media in weight or volume per liter.

	Shoot medium**	CIM	SRM1	SRM2*	SRM3*	SRM4	SA4	3C5ZR
MS salts	1 pk*	1 pk	1 pk	1 pk	1 pk	1 pk	*	1 pk
Vitamins	JHMS 1 ml	JHMS 1 ml	JHMS 1 ml	JHMS 1 ml	JHMS 1 ml	JHMS 1 ml	N&N 1 ml	3R 1 ml
Myo-inositol	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Kinetin	0.04 mg	--	--	--	--	--	0.5 mg	--
IAA	0.10 mg	--	--	--	--	--	0.1 mg	3 1M
NAA	--	2.0 mg	0.02 mg	0.02 mg	0.02 mg	0.02 mg	--	--
BAP	--	--	--	--	--	2.25 mg	--	--
GA_3	0.20 mg	--	0.10 mg	0.10 mg	0.10 mg	10 mg	0.5 mg	--
Casein	--	--	1000 mg	1000 mg	1000 mg	--	100 mg	--
Sucrose	30 g	30 g	30 g	30 g	30 g	30 g	2.5 g	30 g
D-Mannitol	--	--	--	--	--	--	35 g	--
$zeatin^R$	--	1.0 mg	2.5 mg	5.0 mg	7.5 mg		2.0 mg	(1.76 mg) 5 1m
pH	5.6	5.6	5.7	5.7	5.7		5.8	5.9
Agar	6 g	6 g	8 g	8 g	8 g	8 g	10 g	8 g

Vitamin Stock Solutions (L^{-1})

Vitamins (100 ml)	JHMS	N&N	3 R
Glycine	200 mg	200 mg	
Nicotinic acid	50 mg	500 mg	50 mg
Pyridoxin.HCl	50 mg	50 mg	50 mg
Thiamine.HCl	40 mg	50 mg	100 mg
Folic acid	20 mg	50 mg	
d-Biotin	5 mg	5 mg	

* pk = 1 package of Gibco Murashige-Skoog salts
Shoot medium used for propagation
CIM = Callus induction medium
**SRM = Shoot regeneration medium

Table 3. Number of shoots excised from PWTC101 inoculated internodes on various media from various potato genotypes.

	No. of shoots excised/no. calli			
Medium	RB	Lemhi	DM56-4	J
SRM1	3/30	0/37	74/6	0
SRM2	100/30	0/37	44/6	0
SRM3	69/30	0/37	116/6	0
SRM4	2/30	0/37	0/6	0
SA4	*32/24			0
SA4/SRM2	**11/6	9/37	1/6	-
Total no. shoots	217	9	235	

* 32 RB shoots were excised from calli which were in SA4 medium before changing to SRM2.

**11 RB shoots were excised from SRM2 plates after changing from SA4.

Table 4. Regeneration of shoots from PWTC101 inoculated internodes in three potato genotypes over different concentration ratios of auxin (IAA): cytokinin (zeatin riboside).

Medium				Initial	No. shoots/No. calli		
Code	MS salts	IAA/1M	Z^R/1M	medium	RB	Lemhi	DM56-4
ck	1 pk	0	0	SRM	0/6	0/5	0/3
				CIM	0/3	0/5	0/5
1	1 pk	0.3	1	SRM	0/6	0/5	0/3
				CIM	0/3	0/5	0/5
2	1 pk	0.3	5	SRM	4/6	2/5	0/3
				CIM	0/3	0/5	0/5
3	1 pk	0.3	10	SRM	14/6	0/5	0/3
				CIM	0/3	0/5	0/5
4	1 pk	3	1	SRM	0/6	0/5	0/3
				CIM	0/3	0/5	0/5
5	1 pk	3	5	SRM	7/6	0/5	0/3
				CIM	0/3	4/5	0/5
6	1 pk	3	10	SRM	11/6	0/5	0/3
				CIM	4/3	0/5	0/5
7	1 pk	10	1	SRM	1/6	0/5	0/3
				CIM	3	1/5	0/5
8	1 pk	10	5	SRM	3/6	0/5	0/3
				CIM	0/3	0/5	0/5
9	1 pk	10	10	SRM	9/6	3/5	0/3
				CIM	0/3	1/5	3/5

Z^R Zeatin riboside

IAA Indole acetic acid