GENE TRANSFER FROM WILD TO CULTIVATED POTATO: RESISTANCE TO COLUMBIA ROOT-KNOT NEMATODE AND POTATO LEAFROLL VIRUS

by
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Introduction:

The wild species of potato provide a reservoir of traits that can be incorporated into future varieties. The first step in gaining access to this valuable gene pool is to screen species for resistance. The methods that follow depend on the closeness of relationship between the wild species and cultivated potato. Certain species, like Solanum chacoense, when crossed with cultivated potato, produce F_1 hybrids that are comparable to selections from crosses between cultivars. Hence it is likely that extraction of traits from this wild species would be a rapid process using traditional breeding methods. Other species, like S. etuberosum, do not bear tubers. Initial steps of hybridization must be done with other wild species. Progress will be slow as repeated crossing and selection gradually produce types that resemble potato as we know it. The purpose of this paper is to present progress in identifying and transferring resistance to Columbia root-knot nematode, and potato leafroll virus from wild species sources to cultivated potato.

Columbia Root-knot Nematode Resistance:

Different accessions of wild potato were screened for resistance to Meloidogyne chitwoodi, or Columbia root-knot nematode. Screening was performed by transplanting rooted cuttings into pots while simultaneously inoculating the soil around the roots with 5000 nematode eggs. Both races of the nematode were used. Resistance was found in the Mexican species, S. bulbocastanum. This is a diploid species (2n=24) which is not directly crossable with diploid or tetraploid cultivated potato. Initially six seedlots of S. bulbocastanum, representing different sites of collection in its area of origin in Mexico, were screened. Twenty-two clones were further screened in replicated experiments. Nineteen were highly resistant to race 1. Out of these, four were also highly resistant to race 2. Table 1 shows the egg production of the nematode on Russet Burbank compared to two clones of S. bulbocastanum that have high resistance to both races. The importance of these findings is that we now have observed a biological mechanism for resisting this nematode in a relative of cultivated potato, and the possibility of introducing this into future varieties seems very good.

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Nematode Resistance: Selection In Vitro:

In the past, one of the obstacles has been the great length of time required to get reliable results by screening for nematode resistance in the field. To ameliorate this we have been exploring selection in in vitro culture. The objectives are: 1) to speed up progress by observing directly the response of the potato genotype to the nematode, 2) to control in a repeatable fashion the environment which affects the infestation process, and 3) to optimize the condition and quantity of eggs or larvae that attack the root system of the plant.

Roots of each genotype have been cultured and inoculated with either sterile recently hatched larvae. The larvae were obtained from ordinary greenhouse pot multiplication and sterilized by washing in dilute bleach and short-term exposure to streptomycin in agar cultures. The viability of sterilized larvae was monitored to be sure that inoculum suspensions would be effective.

Forty-five days after inoculation the eggs were extracted from the root mass in the same manner as with pots, i.e., washing, shaking, and passage through sieves.

Preliminary data are available on the relationship between resistance/susceptibility in pot versus in vitro culture (see Table 2). The results from the two environments on exised roots of the highly susceptible Russet Burbank are compared to three highly resistant genotypes. Pepper is a host differential since M. chitwoodi does not parasitize it. The level of resistance of the two accessions of S. bulbocastanum is equal to that of pepper and is expressed in pots as well as in in vitro culture.

For the purpose of large-scale screening, large quantities of inoculum will be needed, but virtually nothing is known of the logistics of doing this. To obtain more data about the production of eggs by nematodes feeding on excised roots in sterile culture, experiments were conducted. It was assumed that the roots would exhaust nutrients after an undetermined period of growth without transfer and that this would in turn limit the nematodes. Since the extensive root proliferation prevented serial transfers to fresh medium, treatments were set up which involved adding supplemental minerals, carbon-source, and vitamins at various time intervals. The results in Table 3 show that although some supplementation of the cultures is better than none, the degree of supplementation did not make a significant difference. These results were for 55 days of incubation. In a continuation of this experiment where cultures were allowed to incubate for 85 days, no significant increase in population size of eggs or hatched juveniles was found. This indicates that limiting factors which were not counteracted by adding nutrients.

Gene Transfer - Crossing:

The next challenge, having found a wild species source of nematode resistance, is to determine the inheritance of resistance and begin the process of transferring the gene(s) into the cultivated gene pool.

To study the inheritance of resistance, hybrids between S. bulbocastanum and another wild species that is susceptible to the nematode, S. cardiophyllum, have been produced. Screening of the hybrids and progenies from backcrosses to the S. cardiophyllum should elucidate the number of genes involved. At the same time, several alternative routes for introgression into cultivated potato are being explored (Figure 1 Scheme A). The 24-chromosome form of S. bulbocastanum does not cross with 24-chromosome cultivated potato, however, an artificially produced 48-chromosome derivative is expected to be crossable. By incorporating a meiotic mutant, donated from the cultivated diploid which produces triplandroids (functional triploid pollen), nematode resistance will be transferred into tetraploid cultivars (Brown, 1988). Three "doubled" clones (2n = 48 chromosomes) have been produced and are being crossed to the appropriate diploids.

Gene Transfer - Protoplast Fusion:

A second hybridization methodology is to use protoplast fusion between cultivars of current interest and nematode resistant S. bulbocastanum (see Figure 1 Scheme B). Leaves of both species are treated so that they disperse into single cells in sterile culture. In this form they are very much like bacterial cultures. Protoplasts of nematode resistance S. bulbocastanum make contact with and fuse with protoplasts of cultivated potato. Metribuzin, the active ingredient in the herbicide Sencor, acts as a selection factor which aids in the selection of protoplasts that are fusion hybrids. The protoplasts of S. bulbocastanum die unless they fuse with cultivated potato protoplasts. Attempts will be made to fuse nematode resistant S. bulbocastanum protoplasts with those of Russet Burbank, Norgold Russet, Shepody, and HiLite Russet. This work will be done in collaboration with J. P. Helgeson, USDA-ARS plant pathologist, who developed this fusion technique.

Resistance to potato leafroll virus from wild species:

Resistance to potato leafroll virus (PLRV) could have a large impact on the cost of growing potatoes in the Northwest. Some degree of relative resistance has been identified in cultivated potato, but much stronger resistance is known in several wild species. Resistance from S. etuberosum, a non-tuber-bearing native of Chile, is being introduced with aid of tissue culture into the cultivated gene pool. Likewise, resistance identified in S. acaule and S. chacoense, natives of Bolivia and Argentina, respectively, is being introduced through a process of repeated backcrossing to cultivated potato. The level of resistance to PLRV in various materials is shown in Table 4. Results are from replicated experiments in which viruliferous aphids were manually transferred to plants and allowed to feed for 7 days before being killed with insecticide. The level of resistance of the wild species derivatives is compared to that of Russet Burbank and an indicator host, Physalis floridana. The number of plants infected, out of eight inoculated, and the mean absorbance in the Enzyme Linked Immunosorbent Assay (ELISA) are indicated. All of the wildspecies accessions tested were clearly more resistant than Russet Burbank.

In addition, the virus titer level as indicated by the absorbance in ELISA tests was much lower in the wild species than in Russet Burbank. The S. acaule and S. etuberosum clones are in second and first backcross generations, respectively, indicating that progress is being made in transferring this resistance to the cultivated gene pool.

In summary, wild potato sources of resistance to Columbia root-knot nematode and potato leafroll virus have been found. Work is in progress to transfer the genes that control these resistances to future cultivars. Incorporation of these resistances would facilitate major savings to growers in chemical costs. Attempts to better the quality of water and the general environment also would benefit from crop plant genotypes requiring less chemical protection.

Citation:

Brown, C. R. 1988. Characteristics of 2n pollen producing triploid hybrids between Solanum stoloniferum and cultivated diploid potatoes. Am. Potato J. 65: 75-84.

Table 1. Egg production of Columbia root-knot nematode (Meloidogyne chitwoodi) on cultivated potato (Russet Burbank) and two clones of S. bulbocastanum.

	Race 1	Race 2
Russet Burbank	140,083 a	52,051 a
S. bulbocastanum (1.7)	11 b	85 b
S. bulbocastanum (6.1)	0 b	21 b

All values are geometric means. Means not sharing a letter are significantly different at 5% level.

Table 2. Egg production of Columbia root-knot nematode (Meloidogyne chitwoodi) on tomato, pepper, Russet Burbank, and two clones of S. bulbocastanum.

	No. eggs in pots	No. eggs in culture
Russet Burbank	36,314 a	447 a
Tomato	140,083 a	1,224 a
Pepper	11 b	0 b
243505.6 (blb)	0 b	0 b
243518.9 (blb)	11 b	0 b

All values are geometric means. Means not sharing a letter are significantly different at the 5% level.

Table 3. Effect of nutrient supplementation on production of nematode eggs on excised tomato roots in sterile culture.

Supplementation at:	No. eggs/plate	No. eggs/mass
2 and 4 weeks	1863 a	337 a
2 weeks only	1339 ab	344 a
2, 4 and 6 weeks	1287 ab	198 bc
4 weeks only	1224 ab	276 ab
4 and 6 weeks	1200 ab	299 ab
None (control)	750 b	145 c

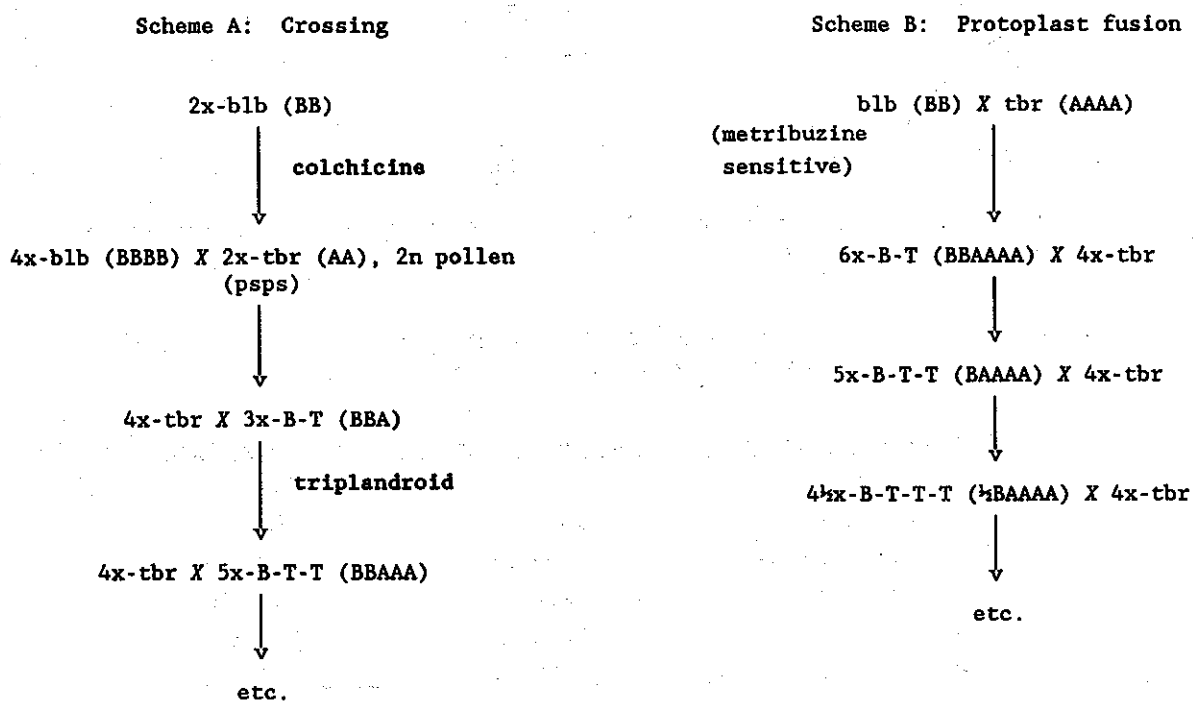
Means not sharing a letter are different at 5% level. Duncan's Multiple Range Test.

Table 4. Resistance to PLRV derived from S. chacoense, S. acaule, S. etuberosum compared to Russet Burbank Physalis floridana (host indicator).

	No. infect /No. inoc.	ELISA Mean absorbance
S. chacoense #1	0/8	.018 a
S. chacoense #2	0/8	.022 a
S. chacoense #3	0/8	.013 a
S. acaule #8	0/8	.009 a
S. acaule #12	1/8	.049 a
S. etuberosum #1	1/8	.034 a
Russet Burbank	5/8	.710 b
Physalis floridana	8/8	1.780 b

Means not sharing a letter are significantly different at 5% level.

Figure 1. Alternative schemes for introducing Columbia root-knot nematode resistance from S. bulbocastanum to cultivated potato gene pool. A represents x=12 chromosomes from cultivated species. B represents x=12 chromosomes from S. bulbocastanum.



blb = *S. bulbocastanum*
2x-tbr = cultivated diploid
4x-tbr = cultivated tetraploid