New Discoveries in Resistances to Columbia Root-knot Nematode and Corky Ringspot Disease.

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Introduction:

The Columbia root-knot nematode (*Meloidogyne chitwoodi*) is a serious pest of potato in the Pacific Northwest. In the warmer zones, with longer growing seasons, this nematode builds up to high populations and damages the potato tubers by invading and causing discoloration and galling. It is presently controlled by fumigation. About 20 million dollars is spent each year to prevent 40 million dollars of loss. Corky ringspot disease is caused by the transmission of tobacco rattle virus into potato tubers by the stubby root nematode (*Paratrichodorus allius*). Damage appears most often as dark brown arcs or blotches in the flesh (Brown and Mojtahedi 2005). *P. allius* is controlled by fumigation by a second product. Together the two fumigations may cost as much as 350 dollars per acre, an expense that may jeopardize the profitability of the crop.

Columbia Root knot Nematode Resistance:

We started looking for resistance to Columbia root-knot twenty years ago. Resistance was found in Mexican wild species. A breeding program ensued and has resulted in long russet processing types that are in the Tri-State Trial System starting in 2006 (Brown et al. 2006).

Originally, we thought of the resistance as pertaining only to the root system. Upon hatching, the juveniles would enter the roots and be stymied by a resistance reaction that did not permit further development. The limitations of this resistance lay in the diversity of the nematode. Resistance to race 2 (the less common race) was not incorporated and resistance-breaking races have appeared in two instances. We also found that the resistance in some lines, although functional in the root system, did not extend to the tubers. If a supply of hatching juveniles was present from a weed host (*Solanum sarrachoides* or hairy nightshade) tuber damage would occur. Interestingly, this discouraging result lead to the most important and promising observation of all. Some of the breeding lines apparently had a second genetic factor, extracted from the wild species donor, that controlled a strong and broad resistance to root-knot nematodes in the tuber. In Figure 1 are displayed tubers of Russet Burbank and two breeding lines that were grown in the presence and absence of *Solanum sarrachoides* (*SS*). Russet Burbank, since it has neither root or tuber resistance, is damaged regardless of the presence or absence of *SS*. This can be observed in the galling which is expressed as a bumpy surface on an unpeeled tuber and brown spots apparent on the surface of a peeled tuber. Breeding line PA95B4-67 resists nematode damage when *SS* is absent, and suffers tuber damage when *SS* is present. This is despite the fact that the nematode itself is unable to reproduce on the root system of PA95B4-67. In stark contrast, the tubers are undamaged in the case of PA99N82-4, as both root and tuber resistances are independently expressed.

Figure 1. Skin intact and peeled Russet Burbank, PA95B4-67, and PA99N82-4, from field trials in *M. chitwoodi* (race 1) infested soil. A and C result from plots where *Solanum sarrachoides* (*SS*, hairy nightshade) is present while in B and D, *SS* is absent. The tubers of PA95B4-67 are penetrated and damaged despite the root resistance, in the presence of *SS*, because it lacks tuber resistance. In contrast, PA9N82-4 possesses both root and tuber resistance and is undamaged in the presence or absence of *SS*. Russet Burbank suffers damage under both treatments.

Examination of the breeding behavior of the co-segregation of root and tuber resistance indicated that each is controlled by a separate gene, but that those two genes are closely linked (Figure 2). Consequently, the root and tuber resistance will coincide most of the time if a doubly resistant parent is used in the crossing. This also means that the molecular markers already developed for root resistance alone will serve adequately to select both traits.

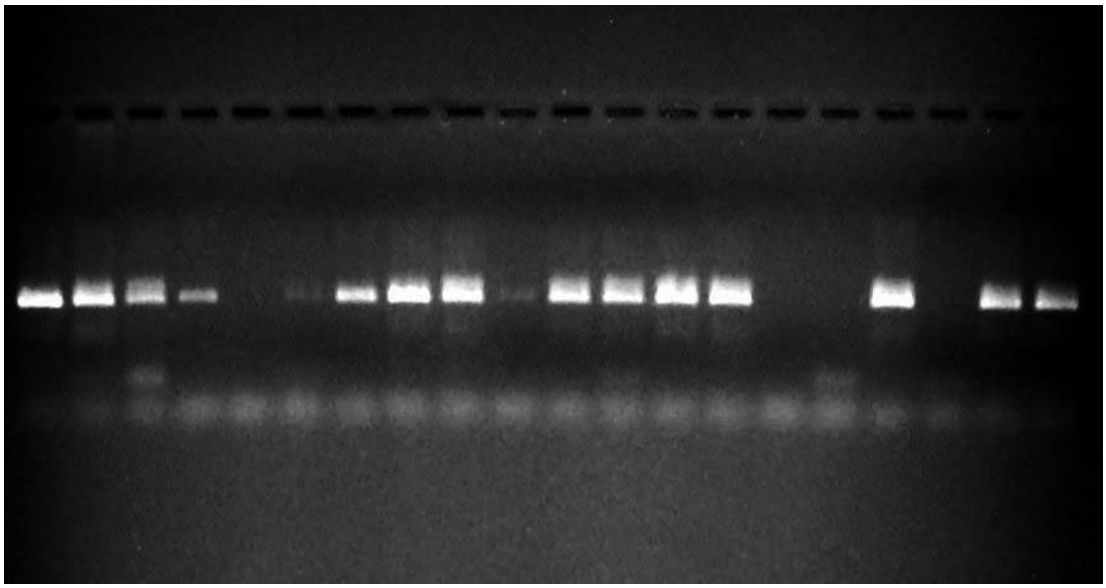

Figure 2. The DNA fragments resulting from the amplification from Sequence Tagged Site (STS) marker 406L19, co-segregate 98 % of time with the combined root and tuber resistance in progenies in a cross with PA99N82-4, a combined tuber and root resistant parent.

Corky Ringspot Disease Resistance

Recognition of Corky Ringspot Disease as a serious problem in the Columbia Basin first occurred when aldicarb became unavailable. It had apparently been controlling the disease sufficiently in problem fields for years. Although aldicarb became available again for at-planting use only, this limited application regime is not sufficiently effective to control the stubby root nematode that vectors the virus which incites the Corky Ringspot Disease.

Resistance to corky ringspot was found in advanced breeding clones in the Aberdeen breeding program. Aberdeen and Prosser locations have worked together to introduce resistance into new varieties (Brown et al., 2000). It is well established that corky ringspot is caused by infection by tobacco rattle virus. The nature of resistance is less well understood. Is the lack of symptoms due to presence of virus in latent status, or due to failure of the virus to infect? Alternatively, is the appearance of symptoms due to a strong hypersensitive resistance?

To examine this question we used the polymerase chain reaction to detect virus in symptomatic and asymptomatic tubers in both resistant and susceptible clones grown at two locations. The PCR uses particular stretches of viral RNA converted to DNA as master templates for amplification of DNA (Figure 3). It is extremely sensitive, needing only a small amount of virus to start the reaction.

After harvest, tubers of test clones were sliced longitudinally into quarters and scored for symptoms. If symptomatic, a 0.5 cm dia. cylinder of tissue was excised from the lesion, and, if asymptomatic, a cylinder of tissue was excised from basal and apical regions, and stored at -20° C. The test was run according to the procedure of (Crosslin et al., 1999).

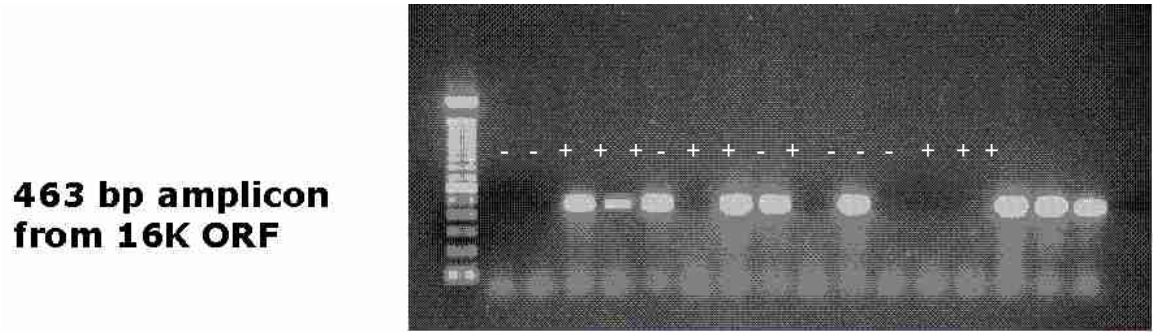

Figure 3. The bands indicate the presence of tobacco rattle virus in potato tubers. The amplicon is a 463 bp band from the 16 kD ORF of RNA 1.

Most of the resistant clones were confirmed as asymptomatic after field testing, while the PCR tests also failed to detect virus (Table 1). In contrast the varying percentages of tubers of susceptible varieties showed symptoms which were matched in a majority of cases by positive PCR results. Two clones that had shown resistance, in earlier testing, converted to susceptibles in the 2004 and 2005 tests. This unexpected and inexplicable outcome was confirmed by visual scoring and PCR testing. The only way to be more secure in a resistance evaluation is through more testing. However, the rest of the resistant clones have shown stability through at least four years of testing. Further, we can regard potato clones resistant to TRV as resistant to infection. The risk that they will be latent carriers of virus, contaminating the field, and providing inoculum to susceptible varieties during subsequent crops, appears to be zero.

Conclusions:

The combination of root and tuber resistance to Columbia root-knot nematode promises to address the problem caused by this nematode. This resistance united with resistance to Corky Ringspot Disease in new varieties opens the door for growers to consider both forms of fumigation as optional. This gives them greater opportunity to be in control of the profitability, and to be more competitive globally.

Breeding lines or Varieties	Prosser. WA		Klamath Falls, OR	
	2004		2005	
	No. PCR postive/ No. asympto-matic	No. PCR positive/No. symptomatic	No. PCR positive/No. asympto-matic	No. PCR positive/No. symptomatic
PA00N6-1	0/40	0/0	0/29	0/1
PA00N2-3	0/40	0/0	0/40	0/0
PA00N1-9	0/40	0/0	2/40	0/0
PA99N36-1	0/40	0/0		
PA99N46-1	0/40	0/0	0/40	0/0
PA99N82-4	0/40	0/0	0/40	0/0
PA00N1-1	13/36	3/4	3/23	14/17
PA99N2-1	1/40	0/0	0/37	0/3
PA99N13-1	1/40	0/0	0/40	0/0
PA98NM22-2	1/40	0/0	040	0/0
PA98NM21-14	20/35	5/5	7/33	3/7
PA00N14-2	0/40	0/0		
PA98NM2-3	0/40	0/0	0/40	0/0
PA00N15-2	0/40	0/0	0/39	0/1
PA99N31-1	0/40	0/0	0/32	1/8
PA00N10-5	0/40	0/0	0/30	0/0
Russet Burbank	3/4	36/36	4/24	13/15
Dark Red Norland	2/2	34/38		
Shepody	4/9	29/31		
Yukon Gold			1/2	35/38

Table 1. Number of tubers PCR positive from symptomatic and asymptomatic tubers over the number tested (i.e. Number of tubers tested RT-PCR positive / Total number tested). The plants were grown in two different fields with viruliferous stubby root nematodes (*Paratrichodorus allius*).

References:

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