Potato Progress

Research & Extension for the Potato Industry of Idaho, Oregon, & Washington

Andrew Jensen, Editor. ajensen@potatoes.com; 509-760-4859
www.nwpotatoresearch.com

Volume XVII, Number 15 | *19 September 2017*

Genetics and Genomics of Host Resistance to Columbia Root-knot Nematode in Potato

Sapinder Bali[1], Ryan C. Graebner[1], Chuck Brown[2], Kelly Vining[3] and Vidyasagar Sathuvalli

[1] Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR 97838
[2] Irrigated Agricultural Research and Extension Center, USDA-ARS, Prosser, WA 99350
[3] Department of Horticulture, Oregon State University, Corvallis, OR 97331

Introduction to CRKN

The Columbia root-knot nematode (CRKN; *Meloidogyne chitwoodi*) is an important pest of potatoes growing in the Columbia Basin of Oregon and Washington. It is a soil-borne microscopic pest that infests both potato roots and tubers (Fig. 1 & Fig 2.)

Figure 1: CRKN infested potato root showing male and female (Acid Fuchsin stained under 10X) (Pictures: Sapinder Bali, HAREC, OSU)

In potato roots and tubers, each nematode establishes a feeding site, where it modifies potato host cells to send a constant supply of resources to the nematode's vicinity (Fig 3). This causes pimple-like bumps to form at infection sites in the infected tubers, which dramatically reduces their fresh-market appeal, as well as increased sugar concentrations in the tissue surrounding each infection site, which browns when fried, making these tubers generally unsuitable for the French fry and potato chip industries.

Figure 2: Potato tubers showing (a) external and (b) internal defects due to CRKN invasion (Pictures: (a) www.inspection.gc.ca (b) and (c) CRKN Trials, HAREC, OSU)

Current control measures and breeding for resistance to CRKN

Currently, the predominant method to control CRKN is the use of fumigants and nematicides. Soil fumigation is done by applying chemicals into the soil where it forms gases that kill the nematodes living in the soil spaces. Fumigation does not completely eradicate the nematodes but helps to control the population levels.

An alternative to the direct application of chemical control agents is the use of biofumigant cover crops, most commonly a mustard, which decompose when tilled into the soil to release chemicals that act as soil fumigants.

Crop rotations have had little success in controlling CRKN, due mainly to the nematode's wide host range; the CRKN can reproduce on corn, wheat, alfalfa, and many other crops commonly grown in rotation with potatoes. However, control of CRKN may be possible through well-designed crop rotations, where the resistant crop is continuously changed, and care is taken to grow specific varieties of the rotation crops that have been shown to hold resistance to the CRKN.

At this point, no released potato cultivars have been shown to be resistant to the CRKN. However, the $R_{Mc1(blb)}$ and $R_{Mctuber(blb)}$ genes, which offer root and tuber resistance, respectively, are actively being used in the Northwest Potato Variety Development Program (*a.k.a* Tri-State Program) efforts.

New Sources of Resistance

Work conducted by our program has recently identified new sources of resistance to CRKN in seventeen clones from three wild potato species: *Solanum bulbocastanum*, *S. hougasii*, and *S. stenophyllidium*. However, much is unknown about these new sources of resistance, including how many unique resistance genes are present in each of these 17 clones, the strength of each resistance gene present, and the breadth of CRKN pathotypes that each resistance gene confers resistance to. Future research regarding these new sources of resistance will focus on better understanding the genes

conferring resistance for each clone, and introgression of these resistance genes into elite potato germplasm to develop new resistant varieties.

Development of genomic tools to facilitate the CRKN breeding program

Breeding programs are key to development of improved varieties containing the resistance trait. The genetics of the resistance trait provides valuable information, which can be utilized to facilitate breeding programs. We have a special interest in breeding for host resistance to CRKN from *S. bulbocastanum* clone SB22, a diploid wild relative of potato from Mexico. This resistance has already been successfully introgressed to tetraploid breeding clone PA99N82-4.

DNA-based marker technology offers promise for facilitating and speeding up the conventional breeding process, which is relatively slow. This technology utilizes the DNA sequences that are closely linked to the resistance "R" gene(s), and those markers, once known, can be utilized to keep track of the R gene(s). These markers will help to make phenotypic screenings simpler, thus saving time and labor. It will be possible to make selections at the seedling stage with higher accuracy. The markers can confirm beyond doubt which of the individuals in the segregating population contain the R-gene(s), making it easy for the researchers to identify the resistant and susceptible clones.

We are working on developing molecular markers linked to CRKN resistance and identifying genes that regulate the resistance mechanism.

We are employing high-throughput Illumina DNA sequencing technology and bulked segregant analysis (Zou et al. 2016) to identify single nucleotide polymorphism (SNP) markers linked to CRKN resistance. We have used ten CRKN resistant and ten CRKN susceptible individuals, which were developed from a cross between CRKN resistant and susceptible parents. Resistant and susceptible clones' sequences were pooled together to form an R pool and an S pool, respectively. Upon analysis of sequences of resistance and susceptible pools, we have shortlisted 84 longer length DNA sequences (contigs) containing 232 single-nucleotide polymorphisms (SNPs) that can differentiate resistant and susceptible pools (Fig 3). We are validating these SNPs for their potential use in the molecular breeding program.

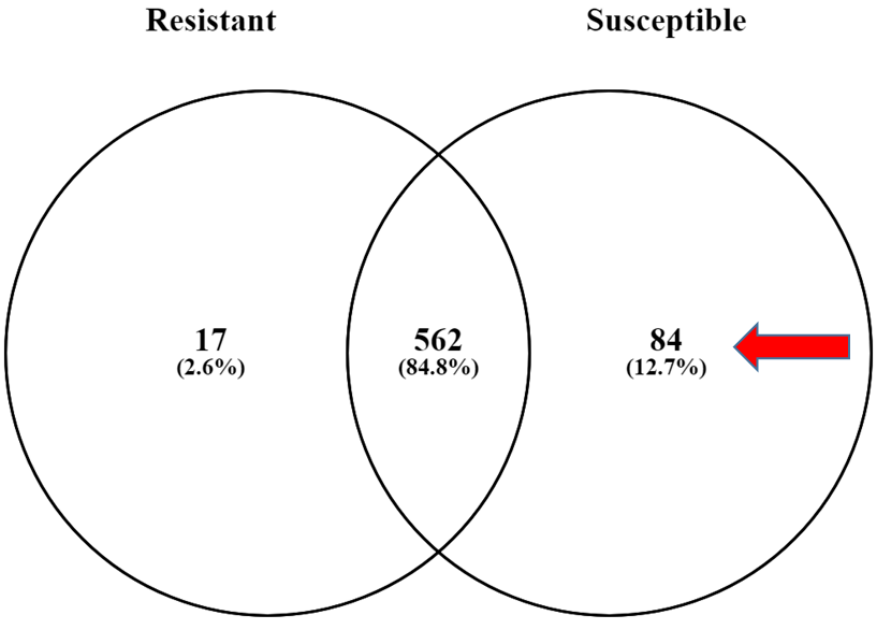

Figure 3: Summary of unique contigs in susceptible progenies that harbor the SNPs of interest.

In order to identify genes regulating the CRKN resistance mechanism, we are looking at differential gene expression between the CRKN resistant and susceptible clones during the progression of nematode infection. Gene expression data (transcriptome) from RNA extracted at different time points of nematode infestation (Fig 4) was generated by Illumina high throughput sequencing technology.

The RNAseq resulted in 30-35 million sequencing reads per time point with lengths of 151bp. Bioinformatic analysis identified many differentially expressed genes with disease resistance properties (Figure 5). Currently, we are in the process of validating the set of candidate genes that might be contributing to the CRKN resistance mechanism.

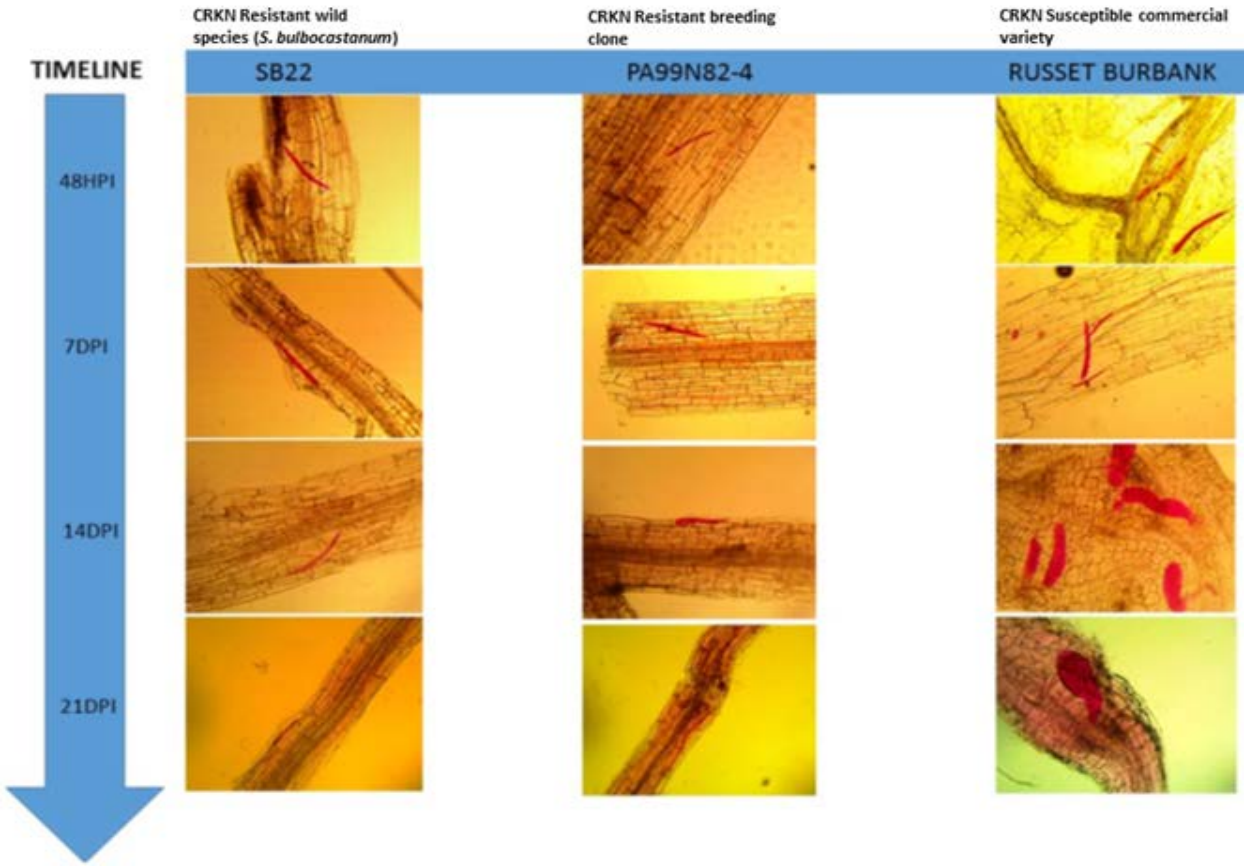

Figure 4: Microscopic examination of the progression of CRKN infection- Resistant versus Susceptible potato clones (Picture: Sapinder Bali, HAREC, OSU)

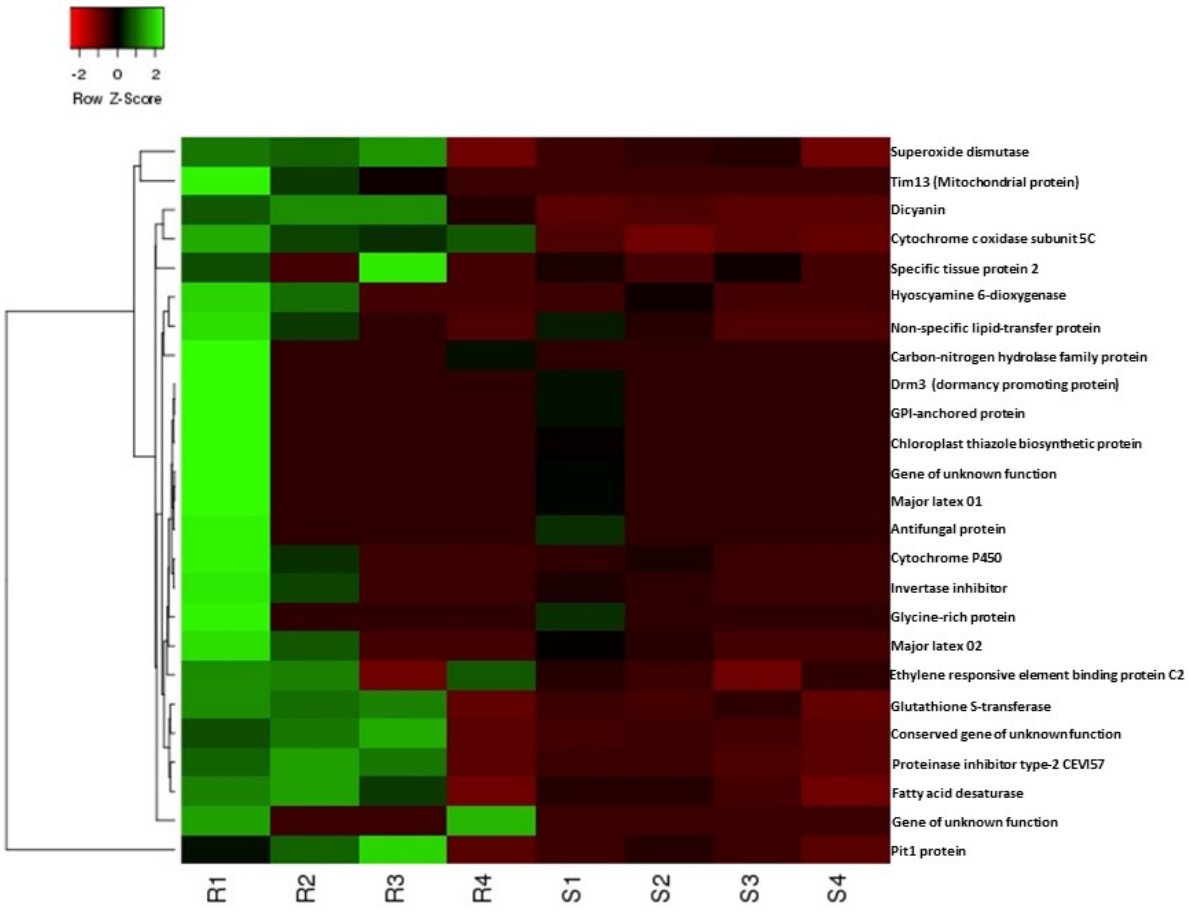

Figure 5: Heatmap of top 25 differentially expressed genes in Resistant (R) and Susceptible (S) clones. R represents PA99N82-4 and S represents R. Burbank clone.
1: 48 hpi; 2: 7dpi; 3: 14dpi and 4: 21dpi
Hpi and dpi represent hours past inoculation and days past inoculation respectively.

Literature sited

Zou, C., Wang, P. and Xu, Y. (2016), Bulked sample analysis in genetics, genomics and crop improvement. Plant Biotechnol J, 14: 1941–1955. doi:10.1111/pbi.12559