Progress Report #1 on SCRI-Funded Project on IYSV and Thrips Reactions and SNP Development in Onion
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This report documents our progress in 2008-2009 towards the identification of resistance or tolerance to *Iris yellow spot virus* (IYSV) and thrips, genetic and transmission studies on the virus, and identification and mapping of single nucleotide polymorphisms (SNPs) in onion. This research is part of a USDA-SCRI funded project. Support from your company was imperative for us to meet the required 100% non-federal match in order to receive this grant. To date:

- **Research by Dr. Chris Cramer (New Mexico State University):** Seeds of 78 plant introductions (PIs) of onion were provided by the USDA onion curator in Geneva, NY to the onion breeding program at NMSU in Las Cruces, NM (Table 1). Half of the seed from each accession was sent to Howard Schwartz at Colorado State University (CSU) while the other half remained at NMSU. Seeds were sown in flats on 18-19 Feb, 2009. Plants were later transplanted to the field on 30 Apr. These PIs, commercial cultivars, and NMSU breeding lines were evaluated for thrips pressure and IYSV symptoms using spreader rows and natural inoculum. IYSV pressure was high and many plants exhibited severe IYSV symptoms. Thrips counts, IYSV severity and incidence, agronomic traits, and bulb yield were measured for each entry. Individual plant selections were made for self-pollination and verification of phenotypes. As previously observed, vigorous plants generally performed better under IYSV pressure. Origins of promising accessions will be provided after re-testing in 2010.
- **Research by Dr. Howard Schwartz (Colorado State University):** Commercial cultivars and plant introductions of onion were grown Colorado for evaluation of thrips pressure and IYSV symptoms using natural inoculum. The Colorado site experienced unusually cool, moist conditions throughout much of the 2009 growing season, and IYSV was delayed until very late in the season resulting in only trace incidence. However a commercial cultivar trial in CO had excellent thrips pressure and 100% incidence of IYSV (Figure 1). There were differences among the cultivars and 6 entries were more vigorous than the other 28 entries in the replicated field trial. These advanced breeding lines and commercial cultivars, as well as the PIs, will be re-evaluated in 2010.


Figure 1. Stand decline due to IYSV in a trail of commercial cultivars in CO 2009.

- **Research by Dr. Hanu Pappu (Washington State University):** A set of indicator hosts were identified that respond by producing a specific host reaction following mechanical inoculation with IYSV (Bag and Pappu. 2009. Symptomatology of Iris yellow spot virus in selected indicator hosts. Online at Plant Health Progress doi:10.1094/PHP-2009-0824-01-BR). These hosts produced localized infection (i.e. the virus is able to infect/replicate and produce symptoms in the inoculated leaves, but fails to move to younger uninoculated leaves).

Using this set of IYSV-susceptible, differential indicator hosts, several field-collected IYSV isolates were screened to identify biologically distinct strains that differ in their disease severity. Preliminary data suggest that there are strains that differ in their ability to colonize and cause systemic infection in infected plants. Efforts to develop an efficient mechanical inoculation for IYSV on onion are ongoing. We have been able to reproduce IYSV symptoms in mechanically inoculated onion plants. Use of IYSV-infected *N. benthamiana* plants appears to be a better source of inoculum to establish IYSV in onion. Existing protocols provided relatively low rates of infection (10 to 20%). While the small and medium RNAs of IYSV have been characterized at the molecular level, the large (L) RNA has remained uncharacterized. We cloned and sequenced the complete ca. 9-kilobase L RNA that codes for the RNA-dependent RNA polymerase. (Bag et al., 200x. Archives of Virology, accepted for publication pending editorial revisions). Availability of the sequence information of all three RNAs of IYSV should facilitate design and use of individual RNA-specific primers to track the movement of the virus in onion after mechanical inoculation.

- **Research by Drs. Michael Havey (USDA-ARS and University of Wisconsin) and Foo Cheung (J. Craig Venter Institute):**
  - We grew and flowered plants from doubled haploid line 5225B (a red maintainer from long-day Spanish background) and OH-1 [an inbred maintainer population with high gynogenic haploid production described by Havey and Bohanec (2007) HortScience 42:1731-1732]. Roots, immature bulbs, leaves, and immature umbels were harvested and these tissues frozen in liquid N_2 (OH-1 did not flower after vernalization). Frozen tissues were shipped to the J. Craig Venter Institute (Rockville, MD). There, total RNA was isolated from each tissue and each line separately. The concentrations of the RNAs was determined spectrophotometrically and qualities assessed electrophoretically (Figure 2). Equal molar amounts of RNA from the sampled tissues were pooled for each line.

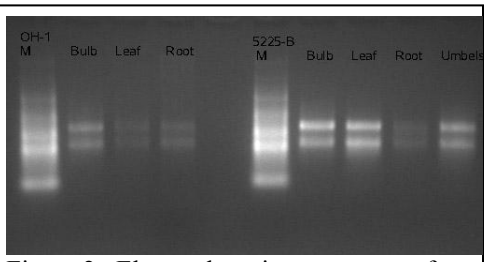

Figure 2. Electrophoretic assessment of RNA quality from onion tissues.

  - We contracted with Evrogen (Moscow, Russian Republic) for synthesis and normalization of cDNAs. Synthesis will use a modified poly-T primer with a *Gsu*I site that allows for removal of the poly-A tail, to avoid problems with pyrosequencing of long tracts of single nucleotides. We anticipate that the two cDNA libraries will be delivered to JCVI in early 2010.
  - We provided F_1 bulbs from OH-1 x 5225B to the laboratory of Dr. Borut Bohanec (University of Ljubljana, Slovenia), where they extracted over 400 gynogenic haploids. These haploids were grown in the greenhouse (Figure 3), bulbs produced, and will be asexually propagated off of the basal plate. Genetic mapping of SNPs will be efficient because there is no heterozygosity. If desired, DNAs from these haploids will be provided to companies supporting this research.
  - We anticipate that 454 sequencing of the cDNAs will occur at JCVI during 2010. We will initially sequence one-half plate of each cDNA library to determine the quality and total lengths of reads. At JCVI, sequences will be assembled, annotated, and putative SNPs identified in silico. Our plan is to initiate validation of SNPs in 2010. We are presently in contact with different companies (such as Illumina and K-Bioscience) regarding high throughput validation of the SNPs.


Figure 3. Gynogenic haploids from OH-1 x 5225B in the greenhouse.

  - The semi-glossy phenotype of onion is associated with reduced thrips build-up, ostensively due to reduced epicuticular waxes. We produced an F2 family segregating for the semi-glossy phenotype, using an inbred developed from the heirloom

cultivar 'Colorado #6'. Plants from this family will be evaluated in 2010 for the types and concentrations of epicuticular waxes. F3 families will be produced to evaluate under field conditions the build up of thrips on waxy versus semi-glossy foliages. We also grew 87 onion PIs, visually scored foliage types, and harvested glossy to semi-glossy types for genetic complementation studies.

- Outreach activities included a field day at CSU (Aug. 31 to Sep. 1), as well as presentations to be presented at a winter education meeting for the CO onion industry and Pacific NW Vegetable Growers meeting in the tri-cities WA in November. Presentations on IYSV and field evaluations will occur at the winter meeting of the National Onion Association in San Antonio, TX, in December 2009. An extension bulletin for IYSV on onion is under development at CSU.

Table 1. Onion accessions evaluated in CO and NM for reactions to *Iris yellow spot virus* and thrips.

G 32590	PI 248754	PI 293756
G 32787	PI 249899	PI 318886
PI 124525	PI 251325	PI 321385
PI 142790	PI 255557	PI 342943
PI 164361	PI 256048	PI 343049
PI 164807	PI 256049	PI 344392
PI 165498	PI 258956	PI 391509
PI 168962	PI 264320	PI 430371
PI 168966	PI 264321	PI 433330
PI 171475	PI 264631	PI 433332
PI 171477	PI 264648	PI 546096
PI 172701	PI 269306	PI 546100
PI 172702	PI 271039	PI 546140
PI 172703	PI 273211	PI 546115
PI 172704	PI 274780	PI 546162
PI 174018	PI 277349	PI 546174
PI 174024	PI 287540	PI 546188
PI 177242	PI 288073	PI 546192
PI 179164	PI 288270	PI 546201
PI 179627	PI 288272	PI 639911
PI 182138	PI 288902	PI 639912
PI 183660	PI 288903	PI 639913
PI 200874	PI 288908	PI 639914
PI 233186	PI 288909	PI 639915
PI 239633	PI 289689	PI 639916
PI 248753	PI 289690	

Publications

Refereed Journal Articles

Bag, S., and H.R. Pappu. 2009. Symptomatology of Iris yellow spot virus in selected indicator hosts. *Plant Health Progress.* doi:10.1094/PHP-2009-0824-01-BR.

Bag, S., K.L. Druffel and H.R. Pappu. 2009. Completion of the molecular characterization of the multipartite RNA genome of *Iris yellow spot virus* (IYSV, genus *Tospovirus*, family *Bunyaviridae*): Structure and genome organization of the large RNA of IYSV. *Archives of Virology* (accepted).

Abstracts and Conference Presentations

Bag, S., K.L. Druffel & H.R. Pappu. 2009. Molecular Characterization of the Large RNA of *Iris yellow spot virus*. The 1X International Symposium on Thysanoptera and Tospoviruses. Gold Coast, Queensland, Australia. 31 August – 4 September, 2009.

Bag, S. & H.R. Pappu. 2009. Symptomatology of *Iris yellow spot virus* in selected indicator hosts. The 1X International Symposium on Thysanoptera and Tospoviruses. Gold Coast, Queensland, Australia. 31 August – 4 September, 2009.

Pappu, H.R. 2009. IPM strategies for reducing the impact of *Iris yellow spot virus* epidemics in onion. The 1X International Symposium on Thysanoptera and Tospoviruses. Gold Coast, Queensland, Australia. 31 August – 4 September, 2009.

Pappu, H.R. 2009. Managing *Iris yellow spot virus* epidemics in onion: Progress and Challenges. Invited talk given in the weekly seminar series in the Department of Plant Pathology, UC-Davis, Davis, CA. September 28, 2009.

Bag, S., K.L. Druffel, and H.R. Pappu. 2008. Genome characterization and genetic diversity of *Iris yellow spot virus*. National *Allium* Research Conference, Savannah, GA. December 11-13, 2008.

Pappu, H.R. 2008. Progress toward managing *Iris yellow spot tospovirus* epidemics in onion. Invited talk at the 3rd International Conference of the Working Group on Vegetable and Legume Viruses. Ljubljana, Slovenia. August 20-23, 2008.