Detection of *Xanthomonas translucens* on barley seed

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Abstract

Bacterial blight of barley, caused by the bacterium Xanthomonas translucens, occurs sporadically in Arizona in sprinkler-irrigated barley. The pathogen is seed borne, and there are no resistant varieties of barley. Bacterial blight has been severe when contaminated seed is planted and favorable weather conditions, including spring rains and late frosts, occur in March and April. Methods for detection of the bacteria on seed have been established at The University of Arizona using both standard pathogenicity trials on barley seedlings and immunoassay techniques.

Introduction

Bacterial blight of barley, caused by *Xanthomonas translucens*, can be a serious problem in barley grown with overhead irrigation. Infections begin as water soaked lesions on leaves and/or chaff and can progress to necrotic streaks in leaves, seed decay and seed blanking. The pathogen is seed borne or carried over from one crop to another in crop residue. *X. translucens* occurs on wheat, barley and other grasses. Although different pathovars have been described to distinguish strains that are identical except in their host preference, distinctions are not clear. For example, strains isolated from barley may infect wheat and those from wheat infect barley (Forster, 1997).

Disease progression requires high moisture. Rain and sprinkler irrigation water spread the bacteria from infection sites. Since the bacteria are seed borne, wounding is not required for initial infections, but field observations in Arizona indicate that wounding of foliage from cold damage may play a major role in disease progression. There is little resistance to Bacterial blight in barley, and varieties commonly grown in Arizona are susceptible. Control is dependent on clean seed and rotations. Bacterial blight usually occurs only sporadically in furrow-irrigated barley in Arizona, but has been common in areas where barley is grown under center pivot. In spring 2001, outbreaks in southeastern Arizona were severe and probably caused reduced yields in several fields. Since rotations from barley are practiced routinely in these fields, the source of inoculum that initiated these outbreaks was assumed to be seed borne.

Barley seed planted in Arizona normally is not tested for presence of *Xanthomonas translucens*, but some growers would like to be able to have their seed tested after harvest and/or before planting. Protocols have been established for seed assays (Schaad, et al., 2001), but they are laborious and slow. However, immunoassay (ELISA) and DNA-based testing are available. The efficacy of these ELISA and DNA-based test kits are being tested for identification of *X. translucens* isolates and for use in seed assays. Our objectives were to establish procedures for conventional dilution plate tests and to initiate trials with ELISA and DNA-based technology for barley seed assays that, once proven to be reliable, would be available to Arizona growers.

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Materials and Methods

Seed samples used in these studies were collected from eight different sources in Cochise County. Samples were from fields known or suspected of having disease during 2001. Seed grown in furrow irrigation in southwestern Arizona and known to be non-infected (Barkley Seed, "Barcot") was used as a standard. For dilution-plating assays, seeds from each sample were taken from bags by reaching to the bottom of the bag and pulling a subsample up through the bag. This was repeated several times until 500 g seed had been collected. The 500 g sample was divided into 5 sets of 100 g in 250 ml Erlenmyer flasks and 120 ml cold sterile buffered saline solution added. Buffered saline was prepared from 0.1 M phosphate buffer pH 7.0 in 0.85% saline and 3 drops Tween 20 per liter. Flasks were put on a rotary shaker at 200 rpm for 5-10 minutes.

Solutions from each flask were serially diluted from 10^0 to 10^{-3} on XTS medium (Schaad et al., 2001, p.184) with 3 replications of each dilution. Cultures were incubated for 3 to 5 days at 28 C. All colonies typical of the *Xanthomonas* genus, 1-2 mm in diameter, clear, yellow, convex and smooth, were transferred to YDC medium (Schaad et al., 2001, p.179) and incubated for 4 days at 30 C. Yellow mucoid colonies suspected of being *X. translucens* were then subcultured to new YDC plates. Pure cultures on YDC plates were used for pathogenicity tests on barley plants according to established procedures (personal communication from Dr. Alan R. Poplawsky, Dept of Plant Pathology, University of Idaho, Moscow, Idaho) and for indirect ELISA (Agdia, http://www.agdia.com/) and DNA based screening (D^2 Technologies, http://www.d2biotech.com/) according to manufacturers recommendations. Isolates used as controls were from known *X. translucens* cultures obtained from the University of Idaho (Dr. Alan R. Poplawsky, Dept of Plant Pathology, University of Idaho, Moscow, Idaho). Dilution plating of all eight seed samples, as well as pathogenicity, indirect ELISA and DNA based tests, were repeated once.

Pathogenicity was tested on Barcot barley seedlings in the greenhouse. Pots containing five seedlings at the 3-leaf stage were inoculated using a hypodermic syringe with 26-gauge needle. The needle was inserted into the base of each plant and a bacterial suspension forced into the lower stem until the suspension began to ooze from leaf whorls. Bacterial suspensions were prepared from overnight cultures in NBY broth diluted 1:100 with sterile buffered saline. Inoculated plants were sealed in plastic bags to create a saturated atmosphere moist chamber and left in indirect light for 4 days. Plants were then taken out of the bags, put on a greenhouse bench and observed after 4-5 days for initial symptoms, water soaked areas in the leaf tissue, and after 7-10 days, for development of reddish brown necrotic lesions.

Tests also were done with both the ELISA and DNA based tests using seed directly. Seed from the two samples known to be infested and the non-infested control seed were crushed in sterile buffered saline with mortar and pestle and the extract tested according to testing procedures.

Results and Discussion

In dilution-plating assays, two samples out of eight were positive for *X. translucens*. The two seed sources that tested positive were sampled from stored seed that originated from fields in which Bacterial blight had been identified in May 1991. These isolates induced symptoms in barley seedlings and were positive using an ELISA test for the genus *Xanthomonas*. The other six samples were negative in both pathogenicity and ELISA tests. Results were identical in repeated trials. Since occurrence of disease had not been ascertained in the fields or origin of these samples previously, it is not known if the six negative samples were from clean fields or if the pathogen was not detectable in these assays.

The seedling assay for pathogenicity was time consuming, but it was not difficult to induce disease symptoms routinely once inoculation protocols were established. The ELISA test was fast and reliable. Although it identified bacteria in the genus *Xanthomonas* and not *X. translucens* specifically, any *Xanthomonas* species isolated from barley seed were presumed to be *X. translucens* since all the members of the genus *Xanthomonas* are considered

plant pathogens. Therefore, we found the ELISA to be specific enough in this case for seed testing using isolates from the dilution plate assays.

The DNA-based technique was problematic. We were never able to get expected results with known cultures or of isolates from Arizona barley previously shown to be pathogenic using the seedling inoculation test. The variability within *X. translucens* may cause difficulties since the commercially available DNA-based test from D^2 Technologies may not be sensitive to all strains of X. translucens. The routine use of nucleic acid-based diagnostic kits will not be used until further tests demonstrate they are accurate and reproducible.

Trials using seed directly for assays were encouraging but not conclusive. In one trial, only one of the two known infested seed samples was positive in the ELISA test. However, this trial should be repeated to determine reliability of the technique since it is an easy and fast way to test seed suspected of being infected. In the DNA-based study, the results were similar. We had hoped the DNA-based tests would be sensitive and reliable enough to use to test seed directly, but that has not been the case to date. As in the pure culture assays, the ELISA tests were the most dependable, but more testing and new assay kits that become available in the future may be more reliable and easy to use.

Literature Cited

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