Evaluating for Susceptibility to Stem Rust in Irradiated *Hordeum vulgare*

Abby Olson, Dr. Brad Mogen - UW-River Falls
Brian Steffenson – University of Minnesota 4/26/05

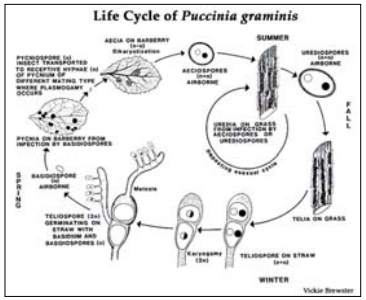

Figure 1: Life cycle of the pathogen responsible for stem rust.


Figure 2: Field layout including rows (test and spreader), ranges, and trays

Abstract

Several pounds of barley seed normally resistant to the disease stem rust were irradiated with fast neutrons. Second generation seed following irradiation was planted in Rosemont, Minnesota and inoculated with stem rust in hopes of finding a naturally resistant plant with signs of susceptibility to stem rust.

Mutations in a number of plants in the field prove the irradiation was a success but a limited number of plants became infected with stem rust. Those plants infected with stem rust were collected and will be analyzed further in hopes of locating one, or possibly more, genes coding for resistance in Barley.

Introduction

A mutant is an individual possessing a new heritable characteristic as a result of an accidental change in a gene or chromosome (1). Morex is a common barley species that is coded genetically to resist stem rust. The Morex seed used in this experiment was intentionally irradiated with fast neutrons in hopes of creating deletions in the gene that codes for resistance. Mutations in the field plants signal that the irradiation had an effect on the plants. It isn't until after the introduction of stem rust that it would be known if the specific gene for rust resistance was affected.

Stem Rust was among the most devastating diseases of barley in the northern Great Plains of the U.S. and Canada before the deployment of the stem rust resistance gene Rpg1 in 1942 (2). After harvesting, Morex plants that have been infected with stem rust can be mapped and it will be better understood whether the Rpg1 gene is the only gene needed to cause the resistance in Morex, or if it is simply a step in a long cascade resulting in resistance.

Materials and Methods

The M2 irradiated generation was planted April 26th and 27th of 2004 using a 4-row Hege planter. The total plot size was 55,000 sq. ft and consisted of 27 rows. Each row has two ranges. Within each range there are four rows, two continuous spreader rows (70% 80-tt-30/steptoe and 30% Washington State certifies Steptoe) on the outside, and two inner test rows that were divided into 40 3.5ft trays of 1-3 grams of seed. In every range, tray number 19 was the non-irradiated form of Morex and tray 20 was the Steptoe mix for use to train the eye to both ends of the spectrum, resistant and susceptible respectively, and to better score for mutants.

Three weeks after planting, scoring for mutants began. For seedlings, scoring consisted of walking through the rows and looking for any abnormalities. The most common mutants found were the albinos, chlorinas, and yellow dwarf.

On the 8th of June, .75 ml of the rust inoculum (0.5g of urediniospores, 300ml of distilled water, and 4 drops of the surfactant tween 20) was injected into the stems of 2 plants in the spreader rows directly to the left or right of every test tray. Beginning about 10 days after the inoculation the infection was observed on the spreader rows. 2-3 weeks after the inoculation rust pustules were present on the stems of the spreader row but no evidence of the rust was found in the test rows.

The field was scored at least once a week for mutations and rust infection. Scoring for mature plants consisted of first training the eye with the Morex and Steptoe trays and then basing judgment of abnormalities on any variation from that. I had to walk through the trays bending the plants back in order to look at the stems, leaves, and heads, all the time looking out for rust pustules, discoloration, necrosis, etc.

When the plants had reached maturity in early August, every tray with a recognized mutant was harvested separately. Every tray without mutants was harvested anonymously. The bags were brought back to the lab and dried for three days in large drying ovens at 950C. They were then passed through a thresher and stored at low temperatures over the winter until they can be planted again next spring.

Results

Some of the most interesting mutations found were the white striped leaf, tubed leaves, necrotics and tillering mutants. The leaves of the white stripe mutant were a darker green with varying thicknesses of white stripes running parallel down the leaves. The ratio of striped versus normal for the tray was 1:4. The tubed leaf mutant had several leaves with large, black necrotic spots and the edges curled completely under. In that tray, for every six normal plants there was one plant with tubed leaves (1:6). The bleach spot necrotic spots displayed large bleached spots that occurred in three of the 15 plants in the tray, with a ratio of 1:4. Lastly, the tillering mutant involves plants with minimal internodal space.

When I left in mid-August, there were five mutant plants of interest and two rust infected Morex plants. Just under half the field had yet to be harvested, but most of the mutants and rusted plants had already been documented and retrieved. There was no loss due to any other pathogen and the field was, despite the substantial yellow dwarf infection, quite healthy.


Figure 3: Inoculating plants with rust Inoculum.


Figure 4: Reduced ligule on mutant plant.


Figure 5: Yellow dwarf plant.


Figure 6: Rust infected Morex plant due to deletion in genome.


Figure 7: Tissue necrosis on leaves. One of the many signs of mutation.

Discussion

Overall the five mutants were important finds. They allowed us to gauge the effect of the irradiation and one plant, the tubed leaf mutants was of considerable interest to another researcher in the plant pathology department because of it lessened ligule. The ligule is found at the node of a plant where the leaf disconnects, the researcher is studying the lack of ligules and other protective structures in plants.

The weather was not adding to the ideal conditions necessary to spread the fungus. Whether or not the lab will get any information out of the plants I collected that did have rust on them is yet to be seen. This winter, though, the lab will grow the seeds of the plants labeled as mutants in hopes to see the same mutation in the next generation. The seeds of the plants with Rust infection will be planted and inoculated again in the greenhouse to recheck their susceptibility.

Next spring all the seeds will be planted again in Rosemount with the hopes that the weather will allow the rust to infect the test rows. There is no way to tell what the outcome of the project will be, but there is no doubt that it will take many seasons before any assumptions can be made and published.

References:

1. Agrios, George N. Plant Pathology 4th ed. Academic Press, 1997.
2. Brueggeman, R. et. all. "The Barley stem rust-resistance gene Rpg1 is a novel disease- resistance gene with homology to receptor kinases." PNAS 99 (2002): 9328-9333.

Acknowledgements:

Dr. Brad Mogen, Biology Department, University of Wisconsin – River Falls
Ronald E. McNair Post-Baccalaureate Achievement Program
Brian Steffenson, Senior Researcher, University of Minnesota – Twin Cities