Plant Breeding Experiences with the BeanCAP Program

University of Nebraska Lincoln

Scout Wilson

About Myself

- 20 years old
- Sophomore at Western Nebraska Community College
- Major: Biology
- Future Interest: Plant Breeding
- Live in Scottsbluff, NE

BeanCAP Program

- Began as a high school student in summer of 2010
- Worked from planting till harvest
- Came back as college student for summer of 2011
- Worked planting and harvest, continued through winter in greenhouse
- Back again in fall 2012


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Dry beans take area student on educational journeys

By SANDRA HANSEN
Ag Editor

When Scout Wilson decided to study plant science in college, he really didn't expect to learn so much about one plant, nor did he anticipate the many adventures he would have because of the common dry edible bean.

Wilson, a 19-year-old sophomore at Western Nebraska Community College, enrolled in the BeanCAP program three years ago to learn more about plant science, his chosen field for college study. While still a student at Scottsbluff High School, Wilson took advantage of the educational opportunity being offered by Dr. Carlos Urrea, plant breeder at the UNL Panhandle Research and Extension Center in Scottsbluff. Urrea had originated the Bean Coordinated Agricultural Project (BeanCAP) program idea as a way to get more young people interested in plant breeding and beans in general.

Wilson signed up in part because he was interested in what makes plants do their thing. Why are some disease resistant while others are not? Why are some varieties more drought tolerant, while others are not. Although he didn't expect to apply what he learned while working with Urrea to his own home garden and greenhouse, he did believe that he would gain some very important knowledge that would be useful in his future.

Well, the future is here, and Wilson said he is amazed at all of the things he has learned, the knowledgeable people he has met and had an opportunity to work with, [illegible]

Scout Wilson, a sophomore at Western Nebraska Community College, enjoys a few minutes in the sunshine during Thanksgiving break from school. Wilson spent most of the summer at Delaware State University as a result of his [illegible]

North Platte River irrigators hope for wet winter

By SANDRA HANSEN
Ag Editor

MILLS, Wyo. — Of course the wettest months are still ahead, and water users along the North Platte River are hoping for some real old-fashioned snow fall and rain storms in the northern Colorado and south central Wyoming Rocky Mountains, as well as along the river as it flows from Colorado through Nebraska.

The seven reservoirs along the river are at 45 percent of the total average capacity of 2,815,800 acre-feet. Guernsey Reservoir stands at 5 percent capacity, with 2,300 a-f. Glendo is at 31 percent, or 152,900 a-f. Seminoe Reservoir is at 53 percent of capacity, with 543,900 a-f, and Pathfinder, with 410,050 a-f, is at 38 percent of capacity. Seminoe and Pathfinder hold just over 1 million a-f each.

October is the first month of the annual Water Year, so actual numbers are expected to be lower than later in the year. However, with the end of an extremely hot and dry summer, drawdown was more than projected, and precipitation amounts have failed to improve conditions.

flow from upstream.

Seminoe and Pathfinder, the two largest [illegible] on the system, have gained less than 50 percent of the average inflow. Seminoe has picked up 14,300 a-f, while Pathfinder has accumulated 1,800 a-f.

Outflows have also been below average. Pathfinder released 8,000 a-f last month, or 27 percent of average, while Seminoe sent 32,100 downstream, or 76 percent of average.

As of the beginning of November, Seminoe held 89 percent of the average storage, or 543,900 a-f. Pathfinder contained 410,000 a-f or 38 percent of its storage capacity.

Total storage as of Oct. 31, was 1,271,400 a-f, 86 percent of the 30-year average of 1,485,200 a-f. Total storage in the system is 2,815,800 a-f.

The big question for irrigators in the North Platte River Valley is the amount of ownership they can draw [illegible] spring. As of Oct. 1, North Platte Project irrigators had 169,[illegible] their disposal, or 61 percent of the 30-year average.

The Inland Lakes and the new Water

bean fields first lab studies fo

By SANDRA HANSEN
Ag Editor

Three years of coordinated, hands-on studies have landed a local youth in a paying summer internship, and provided quality assistance for a scientist at the University of Nebraska Panhandle Research and Extension Center. Scout Wilson, 19, will study this summer at Delaware State University through a National Science Foundation program, and his local mentor, Dr. Carlos Urrea, who initiated the idea, hopes that Wilson will continue working with him at the end of the paid internship.

The opportunity is a result of the BeanCAP (Coordinated Agricultural Project) program, which Urrea initiated three years ago as a means of training future bean breeders. "There is a lack of plant breeders," Urrea explained during an on-site interview Wednesday. "There are openings in private industry as well as in university research, and with the increasing demand for food, there will be a greater need for these people in the future.

"I'm pleased that Scout is moving ahead with this program," Urrea said of Wilson's summer learning opportunity that will focus on the lab side of the plant research he has experienced so far.

Wilson entered the BeanCAP program three years ago, the summer after his junior year at Goring High School. He has spent two summers and one full year in BeanCAP, and will return later this summer to continue his studies under Urrea.

While in Delaware, he will be able to apply what he has learned at Scottsbluff in a full-scale lab experience. The work will focus on genotyping.

See FIELDS, page 3

Student Sco[illegible] check a pac[illegible] did last yea[illegible] the next fi[illegible] opportunitie[illegible] him to Dela[illegible]

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Sequence Identification of Mock Inoculated and Inoculated Common Bean *(Phaseolus vulgaris)* Chromatin Immunoprecipitated (ChIP) DNA

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Research Experience for Undergraduates (REU) Program, Delaware State University 2012

ABSTRACT

Phaseolus vulgaris, also known as common bean, is an important economic crop of the world that is affected by the fungal pathogen *Uromyces appendiculatus* that causes bean rust. The Plant Molecular Genetics and Genomics (PMGG) laboratory at DSU is interested in understanding epigenetic mechanisms in disease resistance and susceptibility to bean rust race 53, a common race of bean rust seen in US common bean production. By studying epigenetic factors which determine the regulation of bean rust resistant genes such as *Ur-3* and *Crg*, we can learn more about how the plants react to this fungal pathogen. The ChIP assay is used to study DNAs bound to a specific location on histones, while ChIP-sequencing uses high throughput methods to isolate and sequence these DNAs. Much of the epigenetic modification happens on histone tails and since these modifications influence the regulation of genes associated with the specific locations on the histone tails, this is an important area of study in epigenetics. Samples are obtained from inoculated and mock inoculated plants at different time periods: zero, 12, and 84 hours after inoculation. These samples are used to obtain ChIP DNA using two different antibodies: H4K12ac and H3K9me2. H4K12ac is an antibody which is specific to histone regions of active genes, while H3K9me2 is an antibody specific to histone regions of suppressed genes. The resulting ChIP DNA will be sequenced and analyzed to reveal any genes present in these regions and their role in the bean rust resistance response.

INTRODUCTION

The Plant Molecular Genetics and Genomics Lab's research focuses on common bean (*Phaseolus vulgaris*) and the epigenetic factors that play a role in disease resistance and susceptibility to bean rust (*Uromyces appendiculatus*, race 53). One area of epigenetics that is of interest to the lab is histone modifications which play a role in the plants response to a pathogen by activating the expression of disease resistant genes or by suppressing these genes. Previous research suggests epigenetic mechanisms are responsible for the formation of heritable epigenetic gene variants (epialleles) and can contribute to stress adaptation in plants[1]. Histone modifications have been of considerable interest as they have been shown to occur in a successive order indicating cross-regulation[2]. Additionally previous research on common bean has yielded valuable information on the gene regulation upon infection of bean rust[3]. Two histone sites have received considerable interest and are important sites to be studied in common bean response to bean rust, these being H4K12ac and H3K9me2[4, 5]. These sites will the focus of this study to provide information on the genes present at these sites and the role they play in plant response to the fungal pathogen.

OBJECTIVES

- Sequence ChIP DNA from mock inoculated and inoculated Sierra.
- Reveal any of the eight stress genes in the ChIP DNA.
- Real-time PCR from Sierra inoculated and mock inoculated leaves will be used for future research.

MATERIALS AND METHODS

- Sierra- rust resistant common bean cultivar, hypersensitive resistance (HR) to *Uromyces appendiculatus* (rust race 53).
- Leaf samples were flash frozen with liquid nitrogen at 0hr, 12hr, and 84hr, and stored in -80 Celsius.
- ChIP DNA extracted using ChIP assay protocol (modified protocol from our lab).
- Eight stress genes tested in ChIP DNA using PCR.
- Seedlings, roots, leaves, flowers, and pods collected for testing spatial expression using PCR.
- Inoculated and mock-inoculated Sierra leaves were collected at 0, 12, 84hrs for Real-time PCR.

Sierra: Inoculated & Mock-Inoculated

Stress/ Defense Genes Upregulated in *Phaseolus vulgaris* during *Uromyces appendiculatus* infection

EST's annotation	EST's potential function	Forward primer sequence	Reverse primer sequence
6-24 HPI			
CL1004Contig1	Wound-induced protein WIN2 precursor	TCATATCAGAGTGCAGAGTTCCA	CAGTGCCCGCAATATTATGAC
Pv011A_M1SF_B01	Phenylalanine Ammonia lyase 1	GACACACAAGTTGAAGCACCA	TGCAGCTTCTTAGCATCCTTC
CL113Contig1	Hypersensitive-induced response protein	ATTGCATGGTTCATAGCCAGT	CCTCCACACAAGTATCAAAGGA
CL907Contig1	Xyloglucan-specific fungal endoglucanase inhibitor protein	CTGCTTATGTGCCCCTCTATG	CCAAAATTGCAACAATGGAG
72-96 HPI			
CL1350Contig1	Pathogenesis-related protein PR1	GTTGTGAGCGTTGAAGGAAGTC	CGATCCTTTTAGCCACATCAA
Pv022C_M1SF_B09	Glutamine synthetase PR-1	TGCCAAGTATTCAGCAATCC	CAACAGGCCAGTTCACTTCTT
CL1032Contig1	Nodule-enhanced sucrose synthase	AGCATGTGACCAACCTTGAAC	GGGAACAGACTCAGCCAATTT
CL2176Contig1	Salicylic acid-binding protein 2	TCTTGTTTGGTGTGTGTTTCG	TTGCCCAGTTATTGGTTTGTC

Table 1: Names of eight stress genes and primers used.

Chromatin immunoprecipitation (ChIP) Protocol

Leaf tissues: Sierra (inoculated & mock-inoculated)
Spatial Expression Tissues: Seedlings, roots, leaves, flowers, pods

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Chromatin extraction and isolation

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Sonication- chromatin broken up into smaller fragments

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Immuno-Precipitation(IP)- histone specific antibodies bind to their site on the chromatin (H4K12ac and H3K9me2)

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Proteinase K dissolution of histones

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Extraction of ChIP DNA by Chl/IAA method

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Precipitation of DNA by ethanol

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ChIP DNA stored at -80C for future use

RESULTS

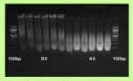

Figure 1: Sonication verification. Before sonication (BS) and after sonication (AS).

Figure 2: Actin PCR to verify ChIP DNA amplification and spatial expression.

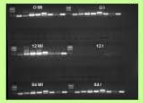

Figure 3: Eight stress genes PCR. Inoculated (I) and Mock Inoculated (MI) Sierra

DISCUSSION

- ChIP DNA from H4K12ac and H3K9me2 immuno-precipitated chromatin were amplified using Actin PCR for sequencing.
- ChIP DNA from samples ran through PCR using primers from the eight stress genes revealed the presence of all genes.
- Spatial tissues (seedlings, roots, leaves, flowers, pods) were run through Actin PCR and successfully amplified.
- Sierra plants were inoculated and mock inoculated for collection at 0hrs, 12hrs, and 84hrs to be used in real-time PCR.

CONCLUSIONS AND FUTURE DIRECTIONS

- Good ChIP DNA was extracted and isolated from all samples which will be sent for sequencing.
- The presence of some of the eight stress genes in the ChIP DNA may indicate a role in plant response to the fungal pathogen.
- To measure the expression of the stress genes Real-time PCR will be used. ChIP DNA will be extracted and isolated from inoculated and mock inoculated Sierra leaves.
- Mutants (crg, Δ 2, Δ 3) were observed to be healthy compared to Sierra which was wilted when transferred from greenhouse to growth chamber. Sierra may have hypersensitivity to heat while the mutants do not. Could be an area of interest for furture research?

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Interest in Plant Breeding

- BeanCAP program activities have influenced by interest towards plant breeding
- Making crosses in the greenhouse
- Getting hands on experience in a working environment throughout the year has exposed me to all activities involved at different times of the year.
- Hands-on training has helped critical thinking during activities which is more inviting to on the spot questions.

The Future

- Plan to transfer to 4 year university for B.S. in Plant Science
- Graduate school with focus on plant breeding.

Thank You!

- Any questions?