The Search for Novel Resistance Alleles:

Screening Teosinte-Maize Introgression Lines for

Resistance to Northern Leaf Blight

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by
Oliver O. Ott

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Commonly Used Acronyms

AUDPC: Area Under the Disease Progress Curve
DLA: Diseased Leaf Area
IP: Incubation Period
NAM: Nested Association Mapping Population
NIL: Near Isogenic Line
NLB: Northern Leaf Blight
QTL: Quantitative Trait Loci
SNP: Single Nucleotide Polymorphism

Introduction

Wild teosintes (*Zea* spp.) are the ancestors of domesticated maize (*Zea mays* ssp. *mays*) and are native to Mexico, Guatemala and Nicaragua. As early as the nineteenth century, botanists realized that there was a relationship between the teosintes and maize. It is now known that the subspecies *Z. mays* ssp. *parviglumis* is the direct ancestor of modern cultivated maize (Fukunaga, 2005). Currently four species and three subspecies of *Z. mays* are collectively called teosinte: *Z. diploperennis*, *Z. nicaraguensis*, *Z. luxurians* and, *Z. perennis* and the subspecies, *Z. mays* spp. *huehuetenangensis*, *Z. mays* ssp. *mexicana* and, *Z. mays* ssp. *parviglumis* (Funkunaga, 2005).

Cytologically, all of the teosintes have chromosome lengths and centromere positions similar to those of modern maize, which facilitates cross-species study as hybrids can be easily made. The subspecies *Z. mays* spp. *huehuetenangensis*, *Z. mays* ssp. *parviglumis*, and *Z. mays* ssp. *mexicana* are all diploid with 10 chromosomes, which distinguish them from the other species which have 20 chromosomes. Teosinte morphology is also very similar to that of maize, but teosinte produces tillers more profusely than maize, and the tillers are often as tall as the main stalk. Teositne also has a

tendency to produce branches at the upper nodes of the plant. The largest differences between teosinte and maize occur in the female inflorescence. In maize, the female flowers are born singly and are solid, thus forming the ear. In teosinte, the female flower develops as a spike, is very brittle, and shatters at maturity for seed dispersal. The teosinte seeds are enclosed in hard triangular fruit cases, which are indigestible. The maize ear bears naked seeds, having lost this hard fruit case. Maize has also lost the trait of seed shattering, so kernels remain attached to the ear at maturity (Manglesdorf, 1974).

Despite the differences in ear and seed morphology between teosinte and maize, all species of teosinte can form hybrids with maize under natural conditions. Crosses of maize with *Z. mays* ssp. *mexicana* and *parviglumis* are the most common and fertile. The progeny of these hybrids show a range of morphological traits intermediate between the parents. Since the 1920s, maize breeders have collected teosinte throughout Mexico and Central America. From these collections, introgression lines were developed to further understand the domestication process of maize. George Beadle observed that about one in 500 F_2 progeny from a maize – teosinte cross were either completely maize-like or teosinte-like. These ratios correspond to the expectation of four or five major genes controlling the modern maize growth habit (Doebley, 2004). Most the work done with teosinte has focused on understanding domestication; there has been little investigation of the use of teosinte as a source of alleles for improved traits in maize breeding.

As teosinte crosses readily with maize to produce fertile hybrids, introgression lines have been used for years to study the inheritance of differences between species (Desjardins, 2000). Many of the domestication characteristics of maize are recessive. The

recessive alleles are fixed in maize and the dominant alleles are found only in teosinte. It is estimated that during domestication maize lost about 25% of the genetic diversity that is found in teosinte (Doebley, 2004). The teosintes represent an important potential resource for maize breeding that has not yet been widely used to improve many traits of interest in elite maize. This makes exotic teosinte germplasm a potential source of new and valuable alleles that are capable of improving agronomic qualities in maize, such as disease resistance, yield, and increasing nutrient levels. Today, with the use of genetic linkage maps, it is possible to identify, map, and study the effect of individual loci that control quantitatively inherited traits or quantitative trait loci (QTL), such as quantitative disease resistance. Due to hidden genetic variation, phenotypically inferior plants may contain favorable alleles that can be utilized for breeding and crop improvement. The use of markers has the potential to affect the way that wild and primitive germplasm can be utilized (Tanksley, 1997). It has been found that the advanced backcross QTL method can be applied to identify and manipulate useful QTLs in elite maize. The gains that are made genetically by using the backcross approach are more useful when combining them with more traditional maize improvement methods like mass selection for favorable gene complexes by (Ho, 2002).

Rationale

The use of genetic resistance is the most important strategy for reducing yield-limiting diseases in maize production. Fungal pathogens are especially harmful in Africa where there is little distribution of resistant seed stocks, the environment is highly conducive for disease progression, and preventive cultural methods are not practiced

(Tripp, 2006). Northern leaf blight (NLB) caused by *Exserohilum turcicum*, has been one of the most consistent economically damaging foliar disease of field corn in the U.S. Corn Belt and in developing countries. The disease is typified by cigar-shaped lesions that are gray to tan in color, which reduce photosynthesizing leaf area, resulting in yield loss (Corn Compendium; White, 1999).

Until now there has been little work done evaluating resistance to fungal pathogens in wild teosinte populations Teosintes are reported to be susceptible to some fungi that are pathogenic to maize, but the potential of teosinte species as sources of genes for improving resistance to northern leaf blight is unknown (White, 1999). As teosinte grows in a tropical environment with no winter freeze to keep pathogen populations under control and as it is a perennial, teosinte must tolerate significant disease and insect exposure because its long life span renders it more likely to come into contact with pests. Thus we hypothesize that teosinte may be a resource that harbors a large range of defense related alleles different than those found in modern, annual temperate maize.

For this study, a set of near isogenic lines (NILs) derived from five teosinte accessions were used to study genetic disease resistance for NLB resistance from teosinte. NILs are genetic stocks that are nearly genetically identical, differing only at one (or a very few) chromosomal segments. NIL series are produced by repeated backcrossing and selection (either phenotypic or genotypic). This brings a range of loci from one or more sources (donors) into a common genetic background (recurrent parent). A chromosome segment substitution library is a set of NILs that together carries most or

all of a donor genotype in the genetic background of the recurrent parent. Each line

carries a distinct chromosomal segment, and when ordered through molecular marker

analysis, the final set of NILs represents a "tiling path" of introgressions that cover the

genome. Each member is called a chromosome segment substitution line (CSSL).

NILs are useful genetic stocks for investigating gene function and regulation.

NILs remove the genetic background effect and allow the effect of the introgressed QTL

allele to be precisely analyzed. With a uniform genetic background, causal introgressions

can be identified and easily characterized. NILs also permit fine mapping of QTL. In the

Nelson lab, NILs have been used to discover disease resistance QTL (Chung, 2008). The

purpose of this study was to utilize an existing NIL series for teosinte / maize to discover

disease resistance QTL from teosinte. A set of maize teosinte introgression lines derived

from Z. *parviglumis* in the background of B73, an elite maize inbred, have been

developed and provided by Dr. Sherry Flint-Garcia of the USDA unit at Columbia

Missouri. Five Z. *parviglumis* accessions were used as parental donor lines. These lines

are documented by the North Central Plant Introduction Station, Ames, Iowa, and are a

small sample of the available teosinte genetic variation.

Four accessions (Ames 21889, Ames 21785, Ames 21786, and Ames 21789) were

used to develop populations of BC_4S_2 (**B**acrossed four times **S**elfed twice) lines (Flint-

Garcia S. personal communication, 2008). At this level of backcross introgression, each

line should contain approximately 3% teosinte alleles and 97% maize alleles, which

should represent a random sample of the genome of the donor accession for that given

line. The fifth population is a BC_4 population from teosinte accession PI 384071, which

has gone through a doubled haploid (DH) system to produce completely homozygous lines. These lines are expected to be segregating for many traits, such as flowering time, tiller number, and disease resistance. The double haploid population is expected to be similar to the BC_4S_2 populations, except with increased homozygosity. In the future, these CSSL lines will be developed into formal NILs after two or three more generations of backcrossing in order to reach near-isogenic status that can be used for further study. Molecular marker analysis will be used to identify the specific introgression(s) in each line (Flint-Garcia S. personal communication, 2008). These intogressions will be compared with the location of known resistance QTL. In the present study, a sample (four populations; n=50 per population) of the available pre-NILs have been evaluated for resistance to NLB. This allowed preliminary assessment of the hypothesis that teosinte carries a novel genetic variation (relative to cultivated maize) for resistance to NLB.

Objectives

The general objectives of this project were to identify chromosomal segments introgressed from teosinte that affect the level of disease resistance when compared to B73. This was be done by phenotypically comparing the teosinte introgression lines to the recurrent parent, B73. Lines with significantly different phenotypic disease ratings were interpreted to be the result of introgressions carrying a QTL from teosinte. By comparing the disease responses of a set of CSSLs lines carrying different *Z. mays* ssp. *parviglumis* introgressions into the genetic background of the recurrent parent B73, we inferred that changes in disease resistance resulted from the introgression of teosinte alleles at quantitative and/or qualitative resistance loci.

Methods and Materials

Plant Resources

The teosinte introgression lines were provided by Dr. Sherry Flint-Garcia of the USDA of Columbia Missouri and were evaluated during the summer of 2008 at the Cornell Musgrave research farm at Aurora, NY. Fifty lines of each of the five populations were evaluated. The experimental material was planted on May 14th and laid out in an augmented incomplete block design with two replications. The two replications of a given population were planted together or continuously in order to minimize field variation within a population. Within each replication, there were five incomplete blocks. Individual lines of the populations were randomized within the incomplete blocks of 10 with two rows of B73 per block as control (see field map in appendix). Lines were planted in two meter rows with 12 seeds per row.

Pathogen Materials

The maize/teosinte lines were artificially inoculated with the NLB pathogen *Exserohilum turcicum,* isolate EtNY001, which was originally collected in 1983 in Freeville, New York. The inoculum consisted of both a liquid spore suspension and a dry inoculum preparation. The spore suspension was prepared from cultured media plates of lactic acid agar grown for three weeks. The plates were washed with autoclaved distilled water to remove the spores. Spores were diluted to a concentration of 4000 spores per ml, and 0.5 ml of this spore suspension was then applied to the whorl of the plant. The dry inoculum was prepared using sorghum grains. The sorghum was produced by autoclaving 1200 ml of sorghum and 1000 ml of distilled water together in 1-gallon plastic jugs. This

autoclaved sorghum was then inoculated with *Exserohilum turcicum* isolate EtNY001,

which was grown for about three weeks until there was an even layer of mycelium on all

of the sorghum in the jug. Two to three ml of this dried infected sorghum was then placed

in the whorl of each plant to provide an additional source of inoculum. Plants were

inocluated at the five to seven leaf stage on the 27th of June 2008.

Data Collection

Data were collected throughout the season. The following phenotypes were

recorded: (1) incubation period (IP; number of days after inoculation until 50% of plants

in a row showed disease symptoms), (2) diseased leaf area (DLA is measured on a 0-

100% scale with one percent increments, (3) days to anthesis (number of days until 50%

of plants in row had shed pollen) (4) plant height, (5) ear height, (6) tiller score (0-3

depending on number of tillers) (7) number of plants in a row (stand count) and (8)

number of plants in a row that had lodged at the end of the season.

IP was scored starting 15 days after inoculation, the day that B73 typically

showed 50% lesions in previous year's experiments. Rows found to have lesions on day

15 were marked; unmarked rows were then scored and marked until all of the rows in the

experiment had IP scores. Individuals that did not have credible IP scores, which may

have been skipped during inoculation, may still have had DLA or AUDPC estimates as

they were infected by surrounding inoculum.

Diseased leaf area measurements were taken at three time points in the season at 10 day

intervals corresponding to August 5th, 15th and 25th. DLA was rated using a continuous

scale with 1% increments considering the entire leaf area of each plant. From these three

DLA measurement the area under disease progress curve (AUDPC) was calculated. AUDPC is a measurement that requires the use of repeated disease assessments that are taken during the growth season. This measurement gives a better understanding of the extent of disease accumulation on a genotype at different growth stages, as well as the overall disease progression on the individual genotypes and populations through the whole season. AUDPC was calculated by using the below formula, where t_{i+1}-t_i is the number of days between the *i* rating and the *i+1* rating and T the number of days between the first and last DLA ratings .

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{\text{DLA}_{i+1}+\text{DLA}_i}{2}\right)\left(\frac{t_{i+1}-t_i}{T}\right)$$

A qualitative disease assessment was made by examination of lesion types. It has been observed that NLB lesions on different maize genotypes can differ in their color, shape, average size, and other characteristics that may or may not affect fecundity of the plant or the pathogen. Variations were noted when comparing the normal B73 lesion type with several of the introgression lines, and were visually assessed. Infected leaves with lesions from these diverse materials, and material that was identified as more resistant in an early analysis of the DLA scores were collected, documented and contributed to the Cornell Plant Pathology Herbarium.

Statistical Analysis

The field data set was analyzed using a mixed linear model in ASReml. ASReml is a statistical software package, (ASReml version 2.0, VSN International, Hemel Hempstead, UK) that is used for fitting linear mixed models using restricted maximum

likelihood, a technique commonly used in plant breeding and quantitative genetics.

Based on the field design used, replication was nested within population, and the

incomplete blocks were nested within replication and population. The model used was:

audpc (trait) WT ~ mu source !r rep.pblock block.rep.pblock
Where:
- audpc = AUDPC calculated using the three DLA ratings (other traits were substituted for audpc)
- WT= each trait weighted by stand count
- mu = intercept of model fixed effect
- source = individual genotypes
- !r = randomization of the following effects
- rep.pblock = replications nested within populations
- block.rep.pblock = blocks nested within reps within populations

This model was used to obtain least square (LS) mean estimates of the fixed

source effects. Each line was then compared to B73 and introgression lines that were two

standard deviations from the LS mean of B73 were selected (95% confidence interval

based on standard error for each line). These lines may harbor QTL that affect the amount

of disease resistance, and were identified for further analysis.

Genotype Analysis

These populations were genotyped with 1536 single nucleotide polymorphism

(SNPs) over the summer of 2008 by S. Flint-Garcia at the University of Missouri, The

location of putative QTL can be identified by comparing disease responses of lines carrying

different introgressions to the recurrent parent B73. This data provides us only a moderate

resolution and certainty to identify QTLs affecting disease resistance. The putatively

resistant NILs then must be used in subsequent validation experiments in order to

definitively identify the chromosomal segment(s) associated with resistance. Initial

genotype data were obtained on three lines of interest. Using these genotype data, we analyzed the teosinte/maize introgressions by comparing the locations of the chromosomal segments from teosinte to the known locations of QTL that have been identified in the maize Nested Association Mapping Population (NAM) and identified in a summary of prior QTL studies on disease resistance in maize (Wisser, 2006) .

The NAM population is a joint linkage and association mapping population consisting of 5000 recombinant inbreed lines (RILs) from 25 families, with 200 recombinant inbred lines (RILs) per family. These families were generated by crossing 25 diverse maize inbred lines that were chosen to maximize the diversity that was available in the *Z. mays ssp. mays* breeding pool, with B73 as a common parent. Additionally, the well-known intermated B73 x Mo17 (IBM) mapping population was included as a 26th family. The entire population of 26 families have been genotyped with 1536 SNP loci (1106 informative). This was the same set of SNPs that were used to genotype the populations of teosinte maize introgressions (Yu, 2007).

The NAM population has been screened for resistance to NLB by Jesse Poland of the Rebecca Nelson Lab (Poland, 2008). By comparison of the putative QTL from teosinte with those identified in the NAM it is hoped that novel loci may be distinguished from those already identified in the maize gene pool. Teosinte-derived QTL that are not in these regions may either be novel QTL not detectable in the NAM, or random introgressions that are not associated with enhanced disease resistance. These resistance loci and/or novel alleles at established loci then have the potential to be used in breeding

for resistance to NLB As noted above the comparisons made to date are preliminary

and not presented as definitive.

Results

The first trait of the season that was recorded was IP. This is a measurement of the

plants initial response to pathogen infection. Lines were first examined for IP at 15 days

after inoculation. After day 15, rows were checked every day for the remaining time until all

of the rows had 50% of the plants showing lesions. By rating for IP after day 15 we were

able to identify genotypes that were more resistant than B73. IP data ranged from 15 to 19

days after inoculation, when the last row was called. The average IP of B73 was 15.4 days.

Three genotypes were identified with an IP score of 18 or 19 days in both replications of the

experiment. From these three lines, four plants in each replicate that looked the least

diseased at flowering time were marked. The lines of interest include 07PR140101A,

07PR141901A, and 7PR172301A. These plants were both selfed and backcrossed to B73.

Backcrossing was done in an attempt to further clean up the background, to provide genetic

material for future confirmation of introgressed QTL, and for fine-mapping. From each of

these three lines, four plants were selected from each genotype as seed sources to be sent to

the winter nursery in Homestead Florida to self and to backcross to B73 in order to further

clean up the background.

The main trait of interest for this project is AUDPC, corresponding to the progress

of the disease throughout the growing season. The amount of diseased leaf area that a

genotype accumulates over the growing season is the best representation of the level of

disease resistance of that genotype. AUDPC ranged from 9.9 to 35.5 with the average of B73 being 20.8.

One of the traits of teosinte that is not present in cultivated maize is profuse tillering. Some lines showed a difference in tiller number relative to B73. The average tiller score for B73 was 0.094 with scores ranging from 0 to 3.04. It was also noted that there seemed to be an increased number of plants that had lodged in comparison to B73. This trait was scored with the number plants in the row that had lodged with the break point within three nodes of the primary ear. The average number of lodged plants for B73 was 1.02, with a range in the experiment from 0 to 4.78 this may be due to a wind storm that occurred the night before the lodging score was recorded.

Plant and ear height were also measured. The average B73 height was 241 cm and the average ear height 100 cm. No lines were found to have ears that were outside of the 95% confidence interval for B73. For plant height, plants were found to be both taller and shorter than B73, with heights ranging from 191 cm to 279 cm. Genotype 07PR173201A, with the average height of 191 cm, was also the genotype with the lowest ears; it was also one of the lines that was later in flowering. From an early stage, it was noted that this line had unique leaf morphology with very thin erect leaves with a somewhat silvery sheen. As they developed, the plants kept their phenotype, which may have reduced their overall fitness. This line also had a higher-than-average number of tillers but was not outside of the range for B73.

Table 1. Summary of all the traits measured and the results of the statistical analysis including the relative LS means, the corrected actual values with the μ added back in, and the standard error. B73 trait means are indicated and lines that were significantly different from B73 are also included.

TRAIT	GENOTYPE	LS MEAN	EFECT ESTIMATE	STANDARD ERROR
Plant Height (cm)	B73	241.6	-1.2	7.17
	Shorter			
	07PR173201A	191.0	-51.8	10.07
	AR70760_1	195.1	-47.7	12.39
	07PR146901A	210.4	-32.4	9.956
	07PR148901A	211.0	-31.7	10.08
	07PR141901A	213.8	-28.9	9.986
	Taller			
	AR70735_1	271.6	28.8	10.06
	07PR160901A	273.6	30.8	10.07
	07PR155801A	273.7	30.8	10.08
	07PR148801A	278.3	35.5	10.09
	07PR151701A	279.2	36.4	10.09
Ear Height (cm)	B73	100.8	-4.15	7.02
	Shorter			
	07PR173201A	70.4	-34.5	9.85
	AR70760_1	74.3	-30.6	12.0
	AR70751_1	80.5	-24.4	9.85
Incubation period (days after inoculation)	B73	15.4	0.49	0.57
	Longer			
	07PR140101A	17.9	3.0	0.79
	07PR141901A	17.5	2.6	0.80
	07PR141701A	17.8	2.9	0.99
	07PR172301A	17.4	2.4	0.80
AUDPC	B73	20.9	-1.0	0.51
	Less Disease			
	07PR144901A	9.1	10.7	4.03
	07PR140101A	11.0	8.8	2.08
	AR70760_1	11.3	8.5	3.60
	07PR172301A	12.2	8.3	2.28
	07PR143701A	12.7	7.6	2.36
	07PR169101A	13.5	7.1	2.14
	07PR150801A	13.9	6.3	2.02
	07PR162801A	14.1	5.9	2.50
	07PR170601A	14.2	5.7	2.43
	07PR141801A	14.3	5.6	2.20
	07PR146101A	14.5	5.5	2.22
	07PR147501A	14.5	5.4	2.35
	07PR168901A	14.5	5.3	2.34
	More Disease			
	07PR167101A	28.9	-9.1	2.42
	07PR159901A	31.2	-11.3	2.42
	07PR154301A	33.7	-13.8	3.33
Tiller score	B73	8.35E-02	9.40E-02	0.31
	More Tillers			
	07PR148901A	3.04E+00	3.0	0.43
	07PR151001A	2.02E+00	2.0	0.43
	07PR160901A	2.00E+00	2.0	0.43
	07PR139201A	1.99E+00	1.9	0.43
	AR70769_1	1.55E+00	1.5	0.43

Number Lodged	B73	1.02	0.6	0.92
	More lodging			
	07PR162001A	4.7	4.3	1.31
	07PR151001A	4.1	3.7	1.31
	07PR170901A	3.7	3.4	1.31
	07PR160101A	3.7	3.3	1.31
Days to anthesis (days after planting)	B73	79.4	-2.6	1.42
	Later Flowering			
	07PR159501A	91.9	10.0	2.43
	07PR143401A	91.6	10.0	1.97
	07PR155601A	89.4	7.4	1.98
	AR70776_1	89.0	7.0	1.99
	07PR148801A	87.7	5.7	1.99
	07PR153001A	87.5	5.5	1.99
	07PR174701A	87.4	5.4	1.99
	07PR159201A	86.6	4.6	2.43
	07PR141701A	85.7	3.7	2.42

The three lines discussed below had genotype data available. These lines were identified as the lines with the longest incubation period (days until lesions appearance) and were also genotypes that had reduced AUDPC values. One of the lines that stood, both in the analysis for AUDPC and IP was line 07PR172301A in the (B73×Ames 21789) BC4S2 population. 07PR172301A had an AUDPC score of 12.2 ± 2.28 (B73: 21.0 ± 0.52) and an average IP score of 17.4 ± 0.81 (B73: 15.4 ± 0.577). This line showed introgressions in several regions on several chromosomes, including chromosomes 1, 2, 4, 7, 8, and 10. An introgression of particular interest covers the region where *qEt8.06.2* (for *q*uantitative resistances to *E. turcicum* chromosome **8** bin *06* 2nd QTL) and qET8.06.1 have been found in the NAM. Locus *qET8.06.2* is the QTL with the largest effect on NLB resistance in the NAM population, and affects both AUDPC and IP. Other introgressions in this line that co-localize with identified resistance QTL are a single introgression that covers two QTL indentified on chromosome 1, and an introgression that ends 0.6 cM from a QTL that had been identified on chromosome 7. The possible QTL for this line may lie in the regions $8.06_{A221789}$, $1.06_{A221789}$ and $1.07_{A221789}$ and very close to region $7.02_{A221789}$.

The introgressions on chromosomes 2, 4, and 10 are not near any known QTL. Further work with segregation analysis is needed to confirm which of these introgressions affect disease resistance.

Another line that stood out, with an IP of 17.9 ±0.79 (B73: 21.0 ± 0.52) and AUDPC of 11.0 ± 2.08 (B73: 15.4± 0.577) was 07PR140101A. This line contained two introgressions, both on chromosome 1. The first introgression overlaps with the introgression on chromosome 1 in 07PR172301A. This first introgression also covers the region that includes *qEt1*.06, which contains the third largest QTL in the NAM. The second introgression lies 1.7 cM from the interval that contains *qEt1.08,* another QTL that was identified in the NAM.

The third line that stood out in the field was line 07PR141901A, with an IP score of 17.6 ± 0.79 B73: 21.0± 0.52) and an AUDPC of 9.18 ± 4.03 (B73: 15.43± 0.577). This line had three introgressions, on chromosomes 2, 5, and 7. The introgression on chromosome 2 corresponds to the area were *qEt2.02* is located. This QTL is the 8th largest QTL that had been identified in the NAM. The introgression on chromosome 5, located in bin 5, is not in the same interval as any resistance QTL that have been identified with the use of the NAM by our lab, but may correspond to a cluster of NLB resistance genes that have been identified through a syntheses of previous studies on chromosome 5 (Wisser, 2006). More work is needed to see if this introgression could contain beneficial QTL. The introgression on chromosome 7 is very large, covering 20 cM. This region includes the location of *qEt7.02*, a small-effect QTL in NAM. The *qET7.02* locus from teosinte is thus a candidate for further testing. Further work is needed

to determine if these introgressions contain novel alleles for an already identified locus

qET2.02 or *qET7.02*, or a another QTL on chromosome 5.

For each of these lines, eight individuals were selfed/and back-crossed to B73.

Tissue was collected from all of the plants in the row and will be genotyped to ascertain

which of the selfed/plants were homozygous for the teosinte introgression region.

Progeny from plants in these lines will be used for characterization using different

pathogen races. Tissue was also collected and bulked from the rows of several other lines

that were noted for their low AUDPC scores during initial data analysis and identified as

potential lines of interest.

Table .2 Summary of teosinte introgressions in the three lines that have been found in the same regions as QTL that have been identified in Nested Association Mapping population with subscript of teosinte germplasm source (Poland, 2008).

INTROGRESSION AREA WITH NOTATION OF GERMPLASM SOURCE	PRIORITY FOR FURTHER STUDY	RANK IN EFFECT IN NAM
$8.06.2_{A221789}$	1	1
1.06_{A21889}	3	3
2.02_{A21889}	4	8
1.08_{A21889}	5	9
$8.06.1_{A221789}$	6	11
$1.07_{A221789}$	7	20
$7.02_{A221789}$ and 7.02_{A21889}	2	23
5.05_{A21889}	8	Not indicated by NAM

Lesion Types

Several lines with lesion types distinct from those of B73 were noted. The typical

B73 lesion was a moderately sized cigar shaped lesion that was often surrounded by a

slightly purple border. This purple border (caused by a buildup of anthocyanin in the lesion

margin) varied from non-existent, to thin and light colored. The actual shade of the purple

also varied highly, from a red flushed color to a very deep purple with even greater extremes of these observations found in the introgression lines.

There was a large range of phenotypes in lesion types in the introgression lines. The categories that were scored included the overall size of the lesion. Relative length and width of lesions was also examined and the extent of coloring of the lesion margin. On some lines, lesions were of normal length but were narrower than the typical B73 lesion. By far the most variable lesion trait was the extent of the purple coloring. One of the most interesting lines, 07PR177401A (see picture bellow), showed large swathes of red/purple coloring that connected lesions at opposite ends of the leaf, thus causing almost the entire plant to appear purple.

Figure 1. Teosinte introgression line 07PR177401A, note large amount of red/purple streaking along leaf length.
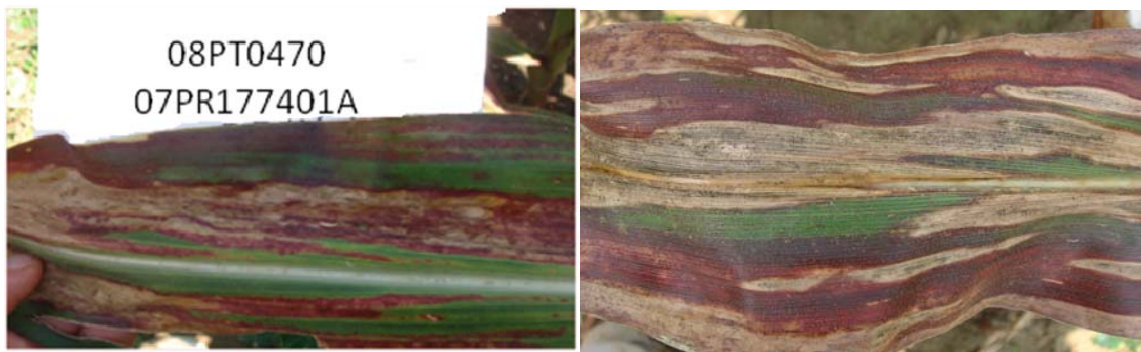
It will be interesting to determine whether there is any correlation with the visual phenotype of a lesion and the resistance QTL. Based on the preliminary analysis of the AUDPC, introgression lines that had means outside the 95% confidence interval of B73 were collected and mounted to examine the differential lesion type that may occur on genotypes that had lower DLA scores in the middle of the season. These selected samples

were dried, pressed and mounted on herbarium sheets for donation to the Cornell Plant

Pathology Herbarium. A photographic record of these sheets was also made so that a digital

copy can be used by interested parties and thus the specimens do not have to leave the

herbarium. Upon receiving additional genotypic data we can try to match up the lines that

had differential lesion types to introgressions to look at the different characteristics of the

lesions, and to examine the mode of action of single QTL.

Discussion

QTL Discovery

This study has identified teosinte-maize introgression lines that have enhanced

resistance to NLB when compared to the recurrent maize parent B73. Some of the more

resistant lines contain teosinte introgressions in locations that correspond to previously

identified NLB QTL. It will be important to confirm which of these chromosomal

segments are actually associated with resistance. It will be interesting to determine

whether introgressions at recognized QTL loci carry novel alleles. It is also possible that

novel QTL at loci that have not been previously identified are associated with resistance and

have been captured in the resistant introgression lines.

In addition to genetic studies to confirm the location of resistance QTL, next steps

include tests to determine to what extent, and how differently, the teosinte-derived alleles

affect disease progression when compared to those of maize. The region where the major

NLB resistance gene *Htn1* is located is covered by an introgression in the line

07PR172301A on chromosome 8. *Htn1* is unusual in that it causes a longer incubation

period rather than the more common hypersensitive response that is conditioned by most

other major genes. As noted above, *qEt8.06.2* maps in the same chromosomal region

as *Htn1*. A QTL in the same region, *qEt8.06$_{DK888}$,* shows race specificity (Chung, 2008).

Breeders may be reluctant to use these available resistance alleles the 8.*06* region in

breeding for NLB resistance due to race specificity. If a teosinte resistance QTL is

confirmed to reside in the same chromosomal region, it will be worthwhile to determine

its race specificity.

Lesion Types

It is hypothesized, but not confirmed, that the anthocyanin is produced in the

margins of a lesion may play a role in disease resistance. The syntheses of phenolic

compounds like anthocyanin are often important components of disease resistance in plants.

The accumulation of phenols around infection sites acts to restrict pathogen growth, perhaps

by protecting tissue from the accumulation of oxidative metabolites.   It has been found that

there is an up regulation of anthocyanin in maize lines resistant to *Biopolaris maydis,* the

causal pathogen of southern leaf blight, while anthocyanins were not found surrounding the

lesions of susceptible controls, and the "anthocyanin ring" was only present in healthy

tissue were the pathogen had not yet progressed (Hipskid, 1996). There were also no

anthocyanins present in non-inoculated control plants of either susceptible or resistant

varieties.

Future Work

The lines 07PR172301A, 07PR140101A, and 07PR141901A, which were

identified as more resistant than B73 in our analysis, were back-crossed to B73, the

recurrent parent, and are being selfed at the winter nursery in Homestead, Florida. The F_2

progeny will then be segregating at the introgression sites and with segregation analysis it can be determined which of the introgressed chromosomal segments are associated with of the differential disease resistance response. It will be interesting to see when genotypes are available for the other introgression lines, if there are introgressions in other regions of the genome that are associated with lesion type differences.

Further identification of both novel QTL and potentially novel QTL alleles at known loci will facilitate the use of marker assisted selection, in conjunction with phenotypic selection to enhance the breeding processes of disease resistance in elite maize. The localization of chromosomal segments from teosinte affecting disease resistance that are not found in maize, may then be used as a genetic resource for the development of disease resistance in modern maize, in order to gauge the true potential of teosinte for increasing the amount of disease resistance in yield-focused breeding programs. The development of NILs using subspecies, or species of teosinte that are even farther away from *Z. mays* ssp. *mays* on the phylogenetic tree, would be useful to further study to examine if more distant relatives would be a source of other novel disease resistance alleles.

Prior to this study, little work had been done looking at the resistance to foliar diseases in teosinte. By using maize-teosinte introgression lines, to gain insight into the resistance of teosinte, we have been able to tentatively localize chromosomal segments that may carry resistance QTL to NLB, and identify potentially novel resistance alleles in chromosomal locations that are similar to those already identified. This project gives us a starting point to further identify genetic resources for NLB resistance that are unexploited in teosinte for the breeding of NLB resistant varieties.

Acknowledgments

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Appendix

Field map of Teosinte Introgression lines (color coded by population)

145	146	147	148	437	438	439	440		
143	144	149	150	435	436	441	442		
141	142	151	152	433	434	443	444		
139	140	153	154	431	432	445	446		
137	138	155	156	429	430	447	448		
135	136	157	158	427	428	449	450		
133	134	159	160	425	426	451	452		
131	132	161	162	423	424	453	454		
129	130	163	164	421	422	455	456		
127	128	165	166	419	420	457	458		
125	126	167	168	417	418	459	460		
123	124	169	170	415	416	461	462		
121	122	171	172	413	414	463	464		
119	120	173	174	411	412	465	466		
117	118	175	176	409	410	467	468		
115	116	177	178	407	408	469	470		
113	114	179	180	405	406	471	472		
111	112	181	182	403	404	473	474		
109	110	183	184	401	402	475	476		
107	108	185	186	399	400	477	478		
105	106	187	188	397	398	479	480		
103	104	189	190	395	396	481	482		
101	102	191	192	393	394	483	484		
99	100	193	194	391	392	485	486		
97	98	195	196	389	390	487	488		
95	96	197	198	387	388	489	490		
93	94	199	200	385	386	491	492		
91	92	201	202	383	384	493	494		
89	90	203	204	381	382	495	496		
87	88	205	206	379	380	497	498		
85	86	207	208	377	378	499	500		
83	84	209	210	375	376	501	502		
81	82	211	212	373	374	503	504		
79	80	213	214	371	372	505	506		
77	78	215	216	369	370	507	508		
75	76	217	218	367	368	509	510		
73	74	219	220	365	366	511	512		
71	72	221	222	363	364	513	514		
69	70	223	224	361	362	515	516		
67	68	225	226	359	360	517	518		
65	66	227	228	357	358	519	520		
63	64	229	230	355	356	521	522		
61	62	231	232	353	354	523	524		
59	60	233	234	351	352	525	526		
57	58	235	236	349	350	527	528		
55	56	237	238	347	348	529	530		
53	54	239	240	345	346	531	532		
51	52	241	242	343	344	533	534		
49	50	243	244	341	342	535	536		
47	48	245	246	339	340	537	538		
45	46	247	248	337	338	539	540		
43	44	249	250	335	336	541	542		
41	42	251	252	333	334	543	544		
39	40	253	254	331	332	545	546		
37	38	255	256	329	330	547	548		
35	36	257	258	327	328	549	550		
33	34	259	260	325	326	551	552		
31	32	261	262	323	324	553	554		
29	30	263	264	321	322	555	556		
27	28	265	266	319	320	557	558		
25	26	267	268	317	318	559	560		
23	24	269	270	315	316	561	562		
21	22	271	272	313	314	563	564		
19	20	273	274	311	312	565	566		
17	18	275	276	309	310	567	568	08PN1	
15	16	277	278	307	308	569	570	599	600
13	14	279	280	305	306	571	572	597	598
11	12	281	282	303	304	573	574	595	596
9	10	283	284	301	302	575	576	593	594
7	8	285	286	299	300	577	578	591	592
5	6	287	288	297	298	579	580	589	590
3	4	289	290	295	296	581	582	587	588
08PT1	2	291	292	293	294	583	584	585	586