MAPPING THE OZONE TOLERANCE TRAIT IN *Arabidopsis thaliana*

By

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ABBREVIATIONS

O_3 ..... Ozone

NO..... Nitric oxide

H_2O_2 .....Hydrogen peroxide

RIL .....Recombinant inbred line

NIL .....Near isogenic lines

QTL..... Quantitative trait loci

SSLP ..... Simple sequence length polymorphism

LOD ..... Logarithm of odds ratio

bp-1 ..... Brevipedicellus

Ler .....Landsberg erecta

Ws ..... Wassilewskija

ozs1 ..... Ozone-sensitive1-1

vtc1.....Vitamin C deficient1

rcd1 .....Radical-induced cell-death1

eto1-1 ..... Ethylene-overproducing1-1

jar1 .....Jasmonic acid-signaling deficient

EM.....Expectation-maximization

JA .....Jasmonic acid

ABA .....Abscisic acid

ROS..... Reactive oxygen species

MAPK ..... Mitogen activated protein kinase

PP2C ..... Protein Phosphatase 2C

cM ..... Centimorgan

CHAPTER I

INTRODUCTION

Ozone (O_3) is a secondary pollutant in the troposphere, the lower most layer of atmosphere where plants, animals and human beings reside. It is a strong oxidant and one of its major source in the troposphere is nitrogen oxides (NOx) which originates from consumption of fossil fuels. It is formed in the atmosphere when oxygen reacts with the nitrogen oxides and hydrocarbons in the presence of sunlight (λ). Ozone stress is responsible for causing major crop losses worldwide. In United States alone crop loss due to O_3 damage is around 2.8 – 5.8 billion dollars (Grantz 2005). (Chameides WL 1994) predicted that if nitrogen oxides emission is not restricted, reduction due to O_3 pollution in food crop yields may triple by the year 2025 (Kim, Kwon et al. 2004).

As a pollutant ozone has harmful effects on plant growth and metabolism. Exposure to acute and chronic levels of ozone induces biochemical and physiological changes in plants (Schmitt and Sandermann 1990). Short-term acute exposure to high O_3 concentrations (>200 $nL.L^{-1}$) leads to visible injury such as necrosis and chlorosis of leaves, and long-term chronic exposure to lower concentrations (40 – 60 $nL.L^{-1}$) leads to reduction in growth as well as delayed visible leaf damage (Weber, Tingey et al. 1994). However, there are fluctuations in the atmospheric ozone such as episodic bursts in ozone concentration that occur especially during summer months. Growing seasons for many

economically important crop plants is in the summer months when the levels of ozone are usually high. It is because of this burst in ozone levels that the crops experience extensive damage and also a dramatic loss in yield.

Physiological and biochemical changes in plants due to ozone stress have been extensively analyzed. It has been found that ozone defense response is dependent on the interplay between various signaling pathways and phytohormones such as ethylene, abscisic acid, jasmonic acid and salicylic acid (Ludwikow and Sadowski 2008). Thus it is apparent from literature that ozone sensitivity is under the control of many different loci and hence QTL mapping will assist in identifying the genetic architecture of ozone defense mechanisms. Numerous studies have been conducted to identify the genetic basis of ozone resistance in several crops such as wheat (Rawlings and Cure 1985), soybean (Mulchi, Lee et al. 1988), rice (Sohn, Kwon et al. 1998), (Kuno 1994) and maize (Roose 1991). This study focuses on exposure of *A. thaliana* to acute ozone for a duration of six hours. Thus understanding the genetics of ozone resistance in the model plant Arabidopsis will aid in cloning the QTLs and thus possibly permit breeding of ozone-resistant cultivars of other major staple crops such as wheat and maize.

The characteristic features of Arabidopsis that make it a model system for QTL analysis include its relatively small and completely sequenced genome (2000), rapid life cycle, collection of knock-out mutants (Alonso, Stepanova et al. 2003) (Sessions, Burke et al. 2002), and availability of markers through convenient web-based search engines such as http://www.arabidopsis.org (Borevitz, Maloof et al. 2002). It is used for various studies such as linkage mapping, since large number of offspring can be raised under uniform conditions. Its relatively high recombination rate has enabled scientists to do fine

scale mapping. Its natural selfing ability has made possible construction and maintenance of recombinant inbred lines (RILs) and near-isogenic lines (NILs) (Borevitz and Nordborg 2003). Moreover, using an F_8 RIL population as opposed to an F_2 population has several advantages for QTL studies. Since RILs are permanent populations they can be indefinitely amplified. The new markers that are thus identified can be mapped and immediately integrated into their genetic map (Alonso-Blanco and Koornneef 2000). Replicates of the same genotype within and across conditions and experiments have become possible through the use of RIL population (Hoekenga, Vision et al. 2003). In selfing organisms such as Arabidopsis, each RIL is derived from an independently segregating individual from the F_2 generation, and after repeated generations of single-seed descent it is fixed for heterozygosity. Consequently, each RIL is homozygous at every locus, as they are unique mosaic of the paternal and maternal chromosome complements (Burr and Burr 1991).

A number of mutagenesis studies have been done in Arabidopsis in order to understand the genetic mechanism of ozone tolerance. These mutants include ozone-sensitive1-1 (*ozs1*; (Saji, Bathula et al. 2008)), ascorbate-deficient mutant (*vtc1*; (Conklin and Barth 2004)), radical-induced cell-death1 (*rcd1*; (Overmyer, Tuominen et al. 2000), and ethylene-overproducing mutant (*eto1-1*; (Tamaoki, Matsuyama et al. 2003). However, apart from induced mutations, extensive naturally occurring genetic variation in sensitivity to ozone exists in Arabidopsis. QTL mapping was first applied in maize (Edwards, Stuber et al. 1987) and tomato (Paterson, Lander et al. 1988). In the model system Arabidopsis, several mapping studies have been conducted which include QTL identification for photomorphogenesis (Borevitz, Maloof et al. 2002); salt tolerance

(Quesada, Garcia-Martinez et al. 2002); aluminum and cesium tolerance and accumulation (Kobayashi and Koyama 2002); (Hoekenga, Vision et al. 2003); (Payne, Bowen et al. 2004); root growth and architecture (Mouchel, Briggs et al. 2004); (Oliver, Virginie et al. 2005), control of seed dormancy (van Der Schaar, Alonso-Blanco et al. 1997), seed size (Alonso-Blanco, Blankestijn-de Vries et al. 1999), seed soluble oligosaccharides and storability (Bentsink, Alonso-Blanco et al. 2000), and flowering time (Kowalski, Lan et al. 1994); (Clarke, Mithen et al. 1995); (Alonso-Blanco, El-Assal et al. 1998); (Juenger, Purugganan et al. 2000). The *CRY2* gene, which encodes the blue-light receptor cryptochrome 2 (El-Din El-Assal, Alonso-Blanco et al. 2001) and response to ABA and Salt 1 (*RAS1*) QTL (Ren, Zheng et al. 2010) of Arabidopsis, have been successfully cloned using a QTL mapping approach. Several major QTLs have been cloned in Arabidopsis through positional cloning approaches. These QTLs include DOG1 for dormancy (Bentsink, Jowett et al. 2006), ED1/CRY2 (El-Din El-Assal, Alonso-Blanco et al. 2001) and FLW/FLM (Werner, Borevitz et al. 2005) for flowering time, ESM1 for content of glucosinolates (Zhang, Ober et al. 2006), GS-elong/MAM for structure of glucosinolates (Kroymann, Donnerhacke et al. 2003), BRX for root morphology (Mouchel, Briggs et al. 2004) and TEI/Erecta for transpiration efficiency (Masle, Gilmore et al. 2005).

For this study we used 147 RILs derived from a cross between ozone resistant, Landsberg erecta (Ler-0) and ozone sensitive Wassilewskija (Ws) to map the QTL responsible for O_3 tolerance. PCR based Simple Sequence Length Polymorphism (SSLP) markers were used for generating a map of Ler x Ws in order to identify the O_3 tolerance QTL. In this study we used 31 SSLP and 9 morphological markers to generate a linkage

map of the Arabidopsis genome. Practical application of QTL mapping of O_3 resistance may greatly facilitate prevention of O_3 stress in Arabidopsis.

CHAPTER II

MATERIALS AND METHODS

Plant materials

A mapping population of 147 F_8 recombinant inbred lines (RIL) was obtained from The Arabidopsis Stock Center (Stock #CS2225). This population was derived from a cross between Landsberg erecta (Ler-0), an ozone resistant ecotype of *A. thaliana* and Wassilewskija (Ws), an ozone sensitive ecotype. The seeds from the parents and the RILs population were cultivated in a soil mixture containing vermiculite, Rediearth and sand (ratio 12:3:1 respectively) in a growth room under a light source of 100μmol s^{-1} m^{-2}, with 10 hours of light and 14 hours of darkness for three-to-four weeks. Plants were maintained in 2-inch pots and each pot had one plant. Pots were placed on a plastic tray and watered at 3-5 day intervals. This RIL population is deficient in pyrimidine which is essential for its growth. Thus the plants were supplemented with 1mM pyrimidine in the irrigation water. The RILs were used along with the parents of the population (Ler-0 and Ws) to screen for resistance to ozone as described below.

Scoring for morphological traits

Three to four week old RILs were analyzed for differences in phenotypes for nine morphological traits. These traits include angustifolia (an-1) and apetela (ap1-1) on

chromosome 1, erecta (er-1) and pyrimidine requiring (py-201) on chromosome 2, long hypocotyls (hy2-1) and glabra (gl1-1) on chromosome 3, brevipedicellus (bp-1) and eceriferum (cer2-1) on chromosome 4 and finally transparent testa (tt3-1) on chromosome 5 (Suppl. Fig. 1). Table 1 details the phenotypic characteristics of these mutants.

Ozone treatment

Ozone was generated in a UV-based ozone generator (model 2000; Jelight Company, Irvine, CA, USA) which was connected to an oxygen tank via Tygon tubing (United States Plastic Corporation, Lima, OH, USA). The ozone levels in the growth chamber were measured using an ozone monitor (model 450; Advanced Pollution Instrumentation, Inc., Irvine, CA, USA) (Mahalingam, Jambunathan et al. 2006) (Suppl. Fig. 2). Three-to-four week old plants were exposed to 300 nL L^{-1} of ozone for six hours, between 10:00 AM and 4:00 PM in this O_3 chamber. Control plants were maintained in the growth room under ambient ozone conditions and identical lighting and temperature settings. Reactions of parents and RILs to O_3 stress were evaluated by visually examining leaf damage 24 h after the end of the treatment. This included examining individual leaves for any visible damage such as lesions, chlorosis, wilting, or total collapse. The screening of O_3 induced phenotypes was done on a scale of 1-5, with 1 resembling the resistant Ler-0 parent and 5 resembling the sensitive Ws parent. Three biological replicates of ozone treatment for RIL population were conducted and in each repeat 4-5 plants were analyzed for each RIL.

DNA isolations

Leaf tissue was homogenized in liquid nitrogen and DNA was extracted using phenol:chloroform:isopropanol as follows: tissue was ground in liquid nitrogen 3-4 times and suspended in 500µl of urea extraction buffer (For 400ml: 168g urea, 25ml 5M NaCl, 20ml 1M Tris-HCl pH 8, 16ml 0.5M EDTA pH8, 20ml 20% Sarkosine in distilled water). A phenol:choloroform extraction was performed with 200µl of phenol and 200µl of chloroform. The tube was centrifuged before the aqueous phase was transferred to a new eppendorf tube. DNA was precipitated with an equal volume of isopropanol. The tube was again centrifuged before the pellet was resuspended in 500µl TE, 100µl 4.4 M ammonium acetate (pH 5.2) and 700µl isopropanol. After another step of centrifugation the pellet was washed with 250µl ethanol and dissolved in 100µl of Tris-EDTA buffer. Working DNA solutions of 10ng/µl were obtained by dilution of stock DNA with ddH_2O.

DNA polymorphisms between the parental lines, Ler and Ws

We identified 50 SSLP markers from TAIR database between Ler and Ws. Primer pairs were designed (Suppl. Table 2) for each of these SSLPs, and Ler and Ws parental DNA was used for testing the polymorphisms. PCR analyses was conducted in a total volume of 10µl using 1µl of 10x PCR buffer (0.1M Tris pH 8.0, 0.5M KCl, 25mM $MgCl_2$), 1µl of 2.5mM dNTPs, 1µl each of 2.5 µM forward and reverse primer, 0.4µl of Taq polymerase, and 4.6µl of autoclaved distilled water. PCR was performed in a programmable thermal cycler using thermal cycling conditions of 94°C for 5min followed by 35 cycles of 94°C for 30s, 50°C for 45min, 72°C for 50s and a final extension at 72°C for 10min. PCR

products were analyzed on 1.7% agarose gels. The polymorphisms that were thus confirmed were subsequently tested on the RIL population as described above.

QTL analysis

All phenotype and genotype data were analyzed for main-effect QTLs using R/qtl (Broman, Wu et al. 2003) and implemented as an add-on for QTL analysis to the freely available statistical language/software, R (http://www.r-project.org) (Ihaka and Gentleman 1996). First, a calc.genoprob function of R/qtl was used to calculate the probabilities of the true underlying genotypes given the observed marker data. Then a genome-wide scan with a single-QTL model (i.e. a single-QTL per chromosome) was performed using standard interval mapping, expectation-maximization algorithm (EM), and Haley-Knott regression (Haley and Knott 1992) methods in order to identify chromosomal regions associated with ozone tolerance. LOD thresholds established using one thousand permutations were used to determine the significance of the QTLs. A LOD score of >2.15 was used to identify QTL in order to minimize type II error.

Genevestigator analysis of genes in the vicinity of bp-1

Using TAIR database we identified several genes in the vicinity of *bp-1* locus (~2 – 4cM) and analyzed them in Genevestigator analysis tool (https://www.genevestigator.com). We focused the analysis of these genes to a few abiotic and oxidative stressors such as oxidants: O_3, H_2O_2, methyl vialogen (MV) and hormones: abscisic acid (ABA), ethylene (ET), methyl jasmonate (MeJA), salicylic acid (SA).

CHAPTER III

RESULTS

Ozone response phenotype of Ler and Ws

The O_3 concentration used in this study was appropriate for differentiating the resistant genotypes from the susceptible genotypes by visual examination of leaf damage. The Ler-0 line was resistant to ozone and showed no visible damage while the Ws lines being sensitive to ozone displayed numerous necrotic lesions, discoloration of the leaves and cell death 24h after the end of a 6h ozone treatment (Fig 1).

Fig. 1 Example of O_3 induced phenotypes in the parents of the recombinant inbred line population, Ler = resistant (*left*) and Ws = sensitive (*right*).

Ozone response phenotype of Ler x Ws RIL population

The ozone response of the RIL population showed a spectrum of phenotypes with varying levels of damage (Fig 2 & 3). The RILs were divided into five groups by visual examination of leaf injury (Score 1-5) caused by ozone. Plants with a score 1 index displayed no visible symptoms, plants with score 2 displayed very few necrotic lesions. Plants with ozone injury score 3 showed many punctuate necrotic lesions and slight discoloration of leaves, plants with score 4 exhibited numerous necrotic lesions and patchy chlorosis of the leaves while those with a score of 5 displayed numerous patchy necrotic lesions, extensive chlorosis and some cell death, resembling the Ws parent which was assigned a score of 5. Interestingly, we also identified some RILs that were more sensitive to ozone than the sensitive parental line Ws and these were assigned an ozone damage score of 6. Of the 147 lines, 13 had score 1, 12 had score 2, 15 displayed score 3, 50 were score 4, 31 were score 5 and 23 were score 6 plants. Nearly 70% of the RILs screened suffered more than 50% leaf damage, 24 hrs after ozone treatment, i.e. the phenotypes of the RILs were more skewed towards the sensitive parent.

Fig. 2 Examples of Ozone induced phenotypes in the recombinant inbred lines (RILs) derived from Ler x Ws. The plants are arranged in order of their relative ozone sensitivities, from the most resistant to most sensitive. Visual scores for O_3 response phenotype, 1-6 scale, 1 = resistant resembling the resistant (Ler) parent, 5 = sensitive resembling the sensitive (Ws) parent, 6 = more sensitive than the Ws parent.

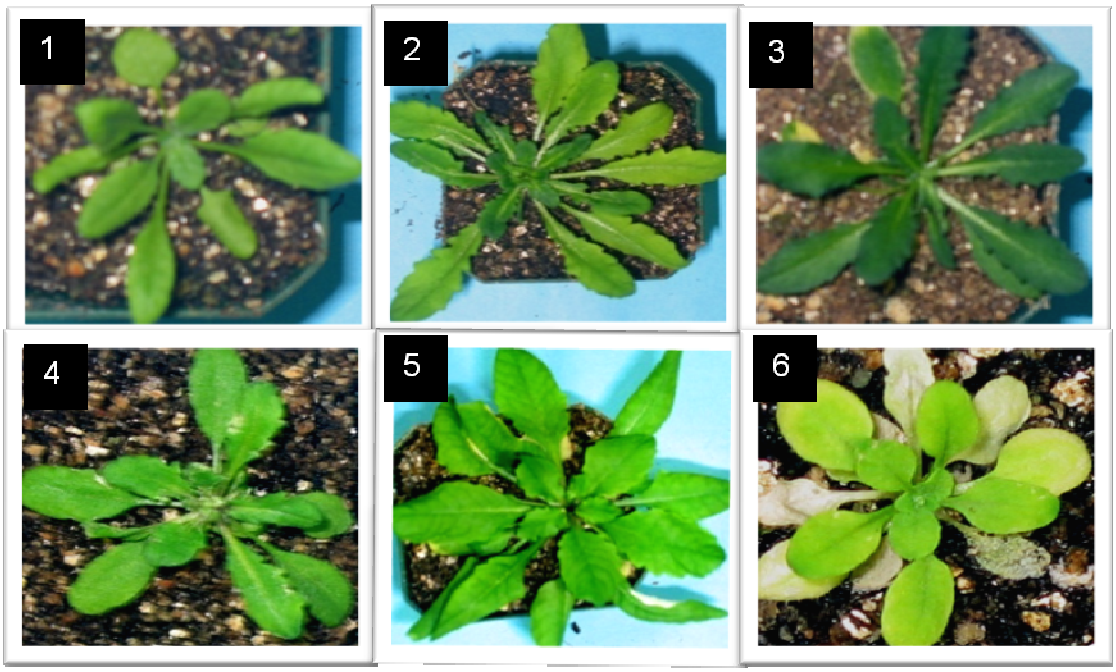
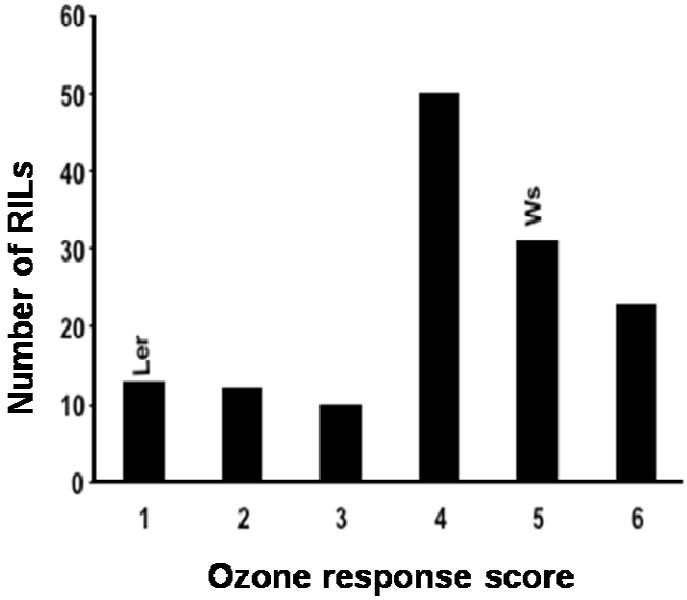


Fig. 3 Frequency distribution of ozone response phenotype in the 147 recombinant inbred population. Visual scores for O_3 response phenotype, x-axis: 1-6 scale, 1 = resistant, 5 = sensitive, 6 = more sensitive than the Ws parent. Spectrum of phenotypes is a continuous distribution implying ozone response is a quantitatively inherited trait. The RILs are more transgressive towards the sensitive parent.



Analysis of morphological markers in Ler x Ws RIL population

The RILs were analyzed for differences in nine morphological traits. These include an-1, ap1-1, er-1, py-201, hy2-1, gl1-1, bp-1, cer2-1, and tt3-1 and each of these traits have a very distinct phenotype (Suppl. Fig 1 & Suppl. Table 1). The descriptions of the nine morphological triats are described in Table 1. For each of these Mendelian traits the Ler parent bore the recessive allele while the Ws parent carried the wildtype allele. The segregation ratios of the RILs and the chi-squared analysis for the nine traits are displayed in Table 2. The chi-squared analysis for the morphological traits shows that the RIL population deviates from the Mendalian segregation as all of the markers except gl1-1 and an-1 have a $P > 0.05$ (Table 3). It was obvious from the segregation ratios that there are no heterozygotes.

Table 1 Phenotypic description for the nine morphological markers located on the five chromosomes of *Arabidopsis thaliana.*

gene symbol	name	Phenotype	chromosome location*
An-1	angustifolia	narrow leaves and slightly crinkled siliquae	1-0.0
Ap1-1	apetala	no petals or rudimentary petals	1-103.5
Er-1	erecta	compact inflorescence, fruits more blunt	2-15.9
Py-201	pyrimidine requiring	leaves except cotyledons white, thiamine restores to normal	2-22.0
hy2-1	long hypocotyl	elongated hypocotyl, more slender plant	3-0.0
gl1-1	glabra	trichomes (hairs) absent on leaf surface and stems	3-40.9
Bp-1	brevipedicellus	short pedicels, siliquae bent downwards, plant height reduced	4-10.8
cer2-1	eceriferum	Bright green stems and siliquae due to deviating wax layer	4-42.2
ms-1**	male sterile	no functional pollen, therefore no outgrowth of siliquae.	5-25.7
tt3-1	transparent testa	Yellow seeds and no anthocyanin in any part of the plant	5-61.2

*****Locations based on map (Koornneef, Hanhart et al. 1987).
******as the progeny of ms-1/ms-1 x Ms-1/ms-1

Table 2 The 31 SSLP markers used to construct the Ler x Ws genetic map. The markers are from the TAIR website (http://www.arabidopsis.org). The segregation ratios and X^2 analysis test values are displayed, and significance was determined at P = 0.05.

Marker	Chrom.	Ler (bp)	Ws (bp)	Segregation ratios	X^2	Probability
nF21M12	1	<200	215	57:78	4.12	0.0423
nga111	1	165	146	104:41	26.87	2.17 e-7
nga128	1	190	170	79:68	0.823	0.3643
nga59	1	115	83	71:72	0.176	0.6751
nga692	1	90	110	98:49	16.22	0.00
T518 (meg4)	1	298	268	66:78	1.08	0.2986
CER459346	2	124	141	85:60	4.285	0.0384
CER459358	2	130	160	88:52	9.19	0.0024
CHIB	3	74	82	117:28	53.57	0.00

ciw4	3	215	190	81:65	1.756	0.1851
ciw11	3	230	240	62:78	2.161	0.1415
nga6	3	123	131	97:50	14.92	0.0001
nga12	3	234	247	50:97	14.92	0.0001
DHS1	4	194	165	61:79	2.617	0.1057
CIW5	4	144	500	35:79	20.88	0.00
CIW7	4	123	150	102:45	21.95	0.00
JV30/31	4	165	190	89:56	7.418	0.0064
nga8	4	198	166	56:82	5.242	0.0220
TGSSLP1	4	750	861	72:67	0.716	0.3974
CER455033	5	100	130	101:44	22.01	0.00
F17C15-2ME	5	185	220	37:60	21.15	0.00
F8L15-1ME	5	190	240	74:55	4.87	0.0273
K18I23-2ME	5	154	185	66:78	1.08	0.2986
MHF15-3ME	5	190	210	62:84	3.29	0.0697
nga129	5	179	165	114:34	43.24	0.00
NGA151	5	120	102	72:73	0.067	0.795
T15N1	5	185	168	65:81	1.752	0.1856
T20L15-1ME	5	185	220	74:72	0.054	0.8162
T22D6-2ME	5	175	201	73:73	0.027	0.8694
T32M21-1ME	5	190	220	60:86	4.59	0.0321
T6I14-1	5	190	220	63:81	2.297	0.1296

Table 3 Segregation of Ler x Ws RIL for the nine morphological markers located on the five chromosomes of A.thaliana, with significance at P=0.05.

Morphological Marker	Chrom.	Segregation ratios (B:A)	X^2	Probability
an-1	1	83:64	2.44	0.1182
ap1-1	1	112:35	40.06	0.00
er-1	2	106:41	28.54	9.163e-8
py-201	2	107:40	30.34	3.63e-8
gl1-1	3	97:50	14.93	0.0001
hy2-1	3	84:63	2.98	0.0842
cer2-1	4	86:61	4.23	0.0397
bp-1	4	109:38	34.06	5.32e-9
tt3-1	5	122:25	51.95	0.00

SSLP analysis

In order to generate a genetic map of the Ler x Ws population Simple Sequence Length Polymorphism (SSLP) markers were identified that exhibited base pair polymorphisms between Ler-0 and Ws, using The Arabidopsis Information Resource (TAIR) database. SSLPs are PCR based markers that help in the identification of indels within the parental lines. These markers were first used in the identification of polymorphism between the parental lines and confirmed polymorphisms were subsequently tested in the RILs (Suppl. Fig 3).

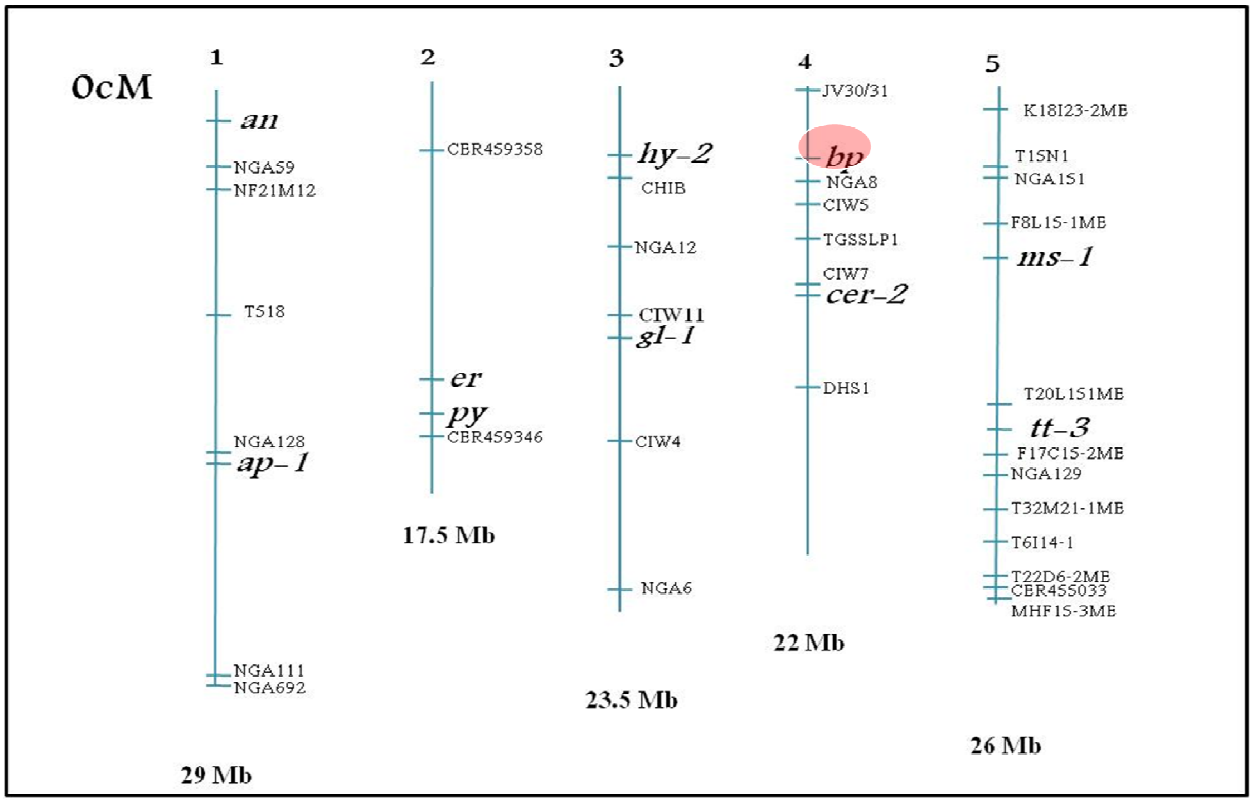
We identified 50 SSLPs between Ler-0 and Ws from the TAIR database. However, upon testing 31 out of 50 displayed the expected polymorphism. Among these 31 SSLPs, six were found on chromosome 1, two on chromosome 2, five on chromosome 3, six on chromosome 4 and twelve on chromosome 5 (Suppl. Table 2). These identified SSLP polymorphisms are very distinct in that they are small base pair differences (insertions or deletions) ranging between 8 – 50bp, displayed by 29 markers, with the

exception of two markers on chromosome 4, CIW5 and TGSSLP1 which demonstrated 360bp and 111bp polymorphisms, respectively, between the Ler-0 and Ws parental lines (Table 3). Chi-squared analysis for the segregation of these 31 molecular markers indicated fourteen with a probability >0.05. These fourteen markers include nga128, nga59 and T518 on chromosome 1, ciw4 and ciw11 on chromosome 3, DHS1 and TGSSLP1 on chromosome 4 and K18l23-2ME, MHF15-3ME, NGA151, T15N1, T20L15-1ME, T22D6-2ME, and T6l14-1 on chromosome 5 (Table 3).

Map of Ler x Ws

The 40 markers; nine morphological and 31 SSLPs, were used to generate a map of Ler x Ws RIL population (Fig 4). The coordinates of each of the SSLP markers were identified from the TAIR database by locating their BAC counter parts or contigs and then these were localized on the AGI map of Arabidopsis to generate the Ler x Ws map. Along with the SSLP markers we also incorporated the information of the nine morphological traits into this map. The map displays that the confirmed SSLP markers were distributed evenly across the entire genome with the exception of chromosome 2, which was sparsely populated. Chromosomes 1, 3, 4, and 5 had a good distribution of markers with chromosome 5 being the most densely populated chromosome.

Fig. 4 Genetic linkage map of Ler x Ws for the nine morphological traits and 31 simple sequence length polymorphism (SSLP) segregating in 147-F_8 Arabidopsis recombinant inbred lines. Morphological markers are represented in *bold, italicized, lowercase letters* and SSLP markers are represented in *uppercase letters*. Ozone resistance QTL is represented by an *oval shape.*

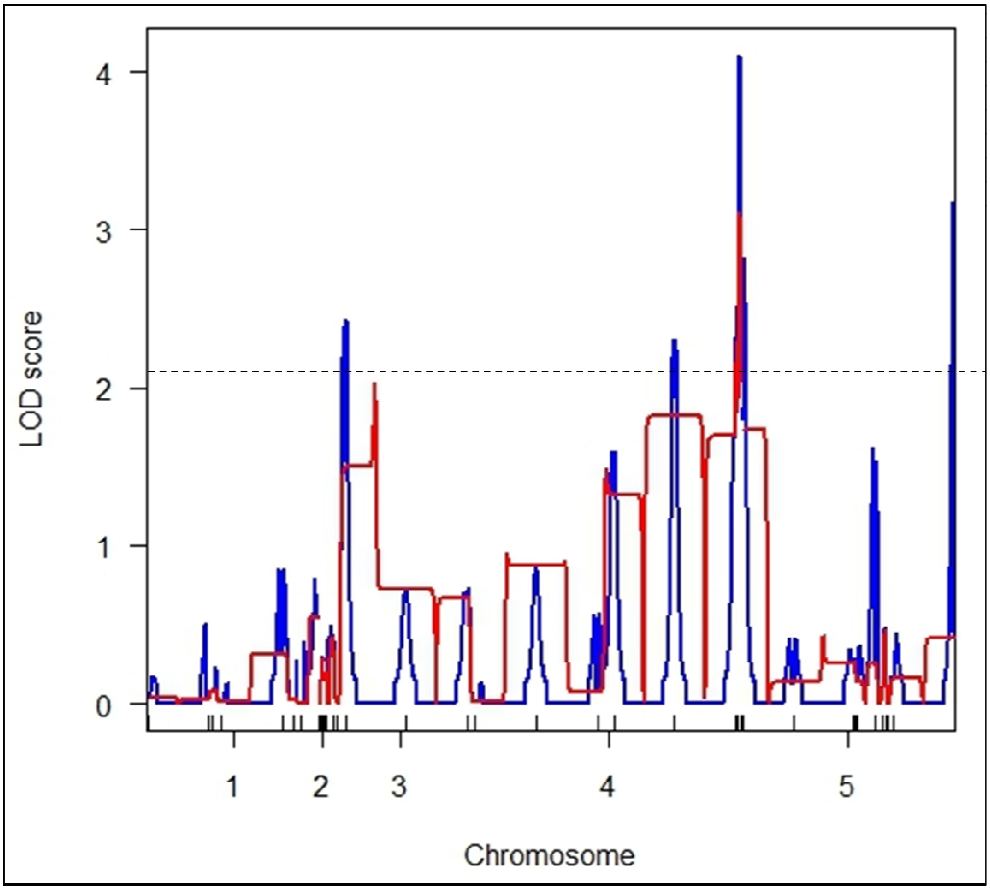


Localization of ozone response QTL on chromosome 4

The map data derived from the morphological markers and data from the phenotypic response was used for QTL determination using the R/QTL package. Results from the genome-wide QTL analysis of the total RIL population are displayed in (Fig 5). Empirical threshold significance value were established from the results of 1000 permutations of the total RIL data and was determined to be $P < 0.004$ with 95% confidence level. QTL were considered significant when LOD scores exceeded the calculated threshold value of 2.15. Two methods were employed in order to establish a significant QTL; the Haley-Knott regression method and standard interval mapping (EM

algorithm). Based on the set significant threshold value, the total RIL data set identified one significant QTL; on chromosome 4 (near *bp-1*, LOD = 4.11, Fig 4), using the Haley-Knott regression and standard interval mapping (EM algorithm) method. In order to get a numerical estimate of the QTL effect, fitqtl was performed. Using fitqtl the estimated effect of the chromosome 4 QTL was determined as 9% ($+0.089 \pm 0.028$).

Fig. 5 Genome-wide logarithm of the odds (LOD) plot of ozone resistance in the total Ler-0 x Ws derived RIL population (n=147) from R/qtl. Highly significant (LOD score > 2.15, dotted line) threshold was established by using Haley-Knott (red) and standard interval mapping (EM algorithm, blue).



CHAPTER IV

DISCUSSION

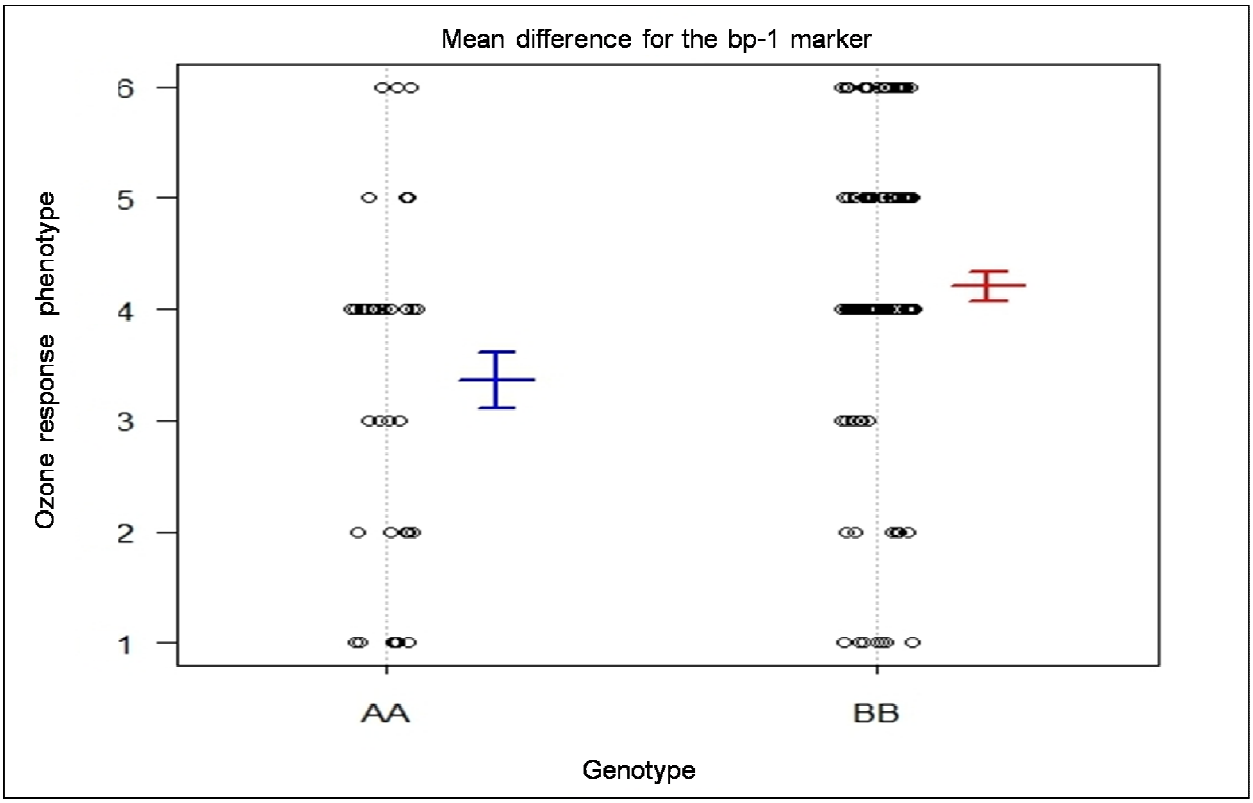
The ecotypes Ler and Ws show very distinct and opposite phenotypic responses to ozone that can be identified visually within one day after treatment (Fig 1). Ozone tolerance has been evaluated in many plant species such as rice (Kuno 1994), soybean (Mulchi, Lee et al. 1988), tobacco (Heggestad and Menser 1962), white clover (Heagle, Miller et al. 1994), snap bean (Reinert and Eason 2000), and sweet corn (Harris and Heath 1981). In this study we demonstrate for the first time the quantitative inheritance of ozone resistance in Arabidopsis (Fig 3). Genetic variation of ozone resistance has been established as a quantitatively inherited trait in several major crops such as potato (De Vos, Hill et al. 1982), maize (Roose 1991) and rice (Sohn, Kwon et al. 1998). These results indicate that ozone-induced stress is controlled by multiple genes. We first analyzed the phenotypic variation in 147 RILs in response to O_3. Judged from the segregation of O_3 phenotype in offspring obtained from Ler-0 and Ws crosses, it appears that O_3 tolerance in this population is controlled by multiple genes, thus confirming previous findings (Fig. 3). The phenotype of RILs to ozone stress deviates from normal distribution and shows skewness towards the sensitive parent. About 16% of the lines showed extreme symptoms to ozone stress compared to the wild type sensitive parent,

Ws, thus demonstrating transgressive segregation. The mechanism of transgressive segregation has been tied to increase in mutation rates, exposure of recessive alleles in segregating hybrid populations, epistasis and overdominance (Rieseberg, Archer et al. 1999). The appearance of transgressive segregants in this study can be explained by the mutant Ler line used to generate the RILs. The Ler ecotype (CS20/W100), Ler-0 (Koornneef, Hanhart et al. 1987) used in this study is different from the wildtype Ler sequenced by Monsanto (www.monsanto.com). Thus the polymorphisms described for the Ler are from the wildtype and not from the mutant line. Mutation rates in the RIL population derived from this particular Ler line could have potentially caused this population to skew towards sensitivity. Using a larger population size could help in identifying more extreme phenotypes and thus force the population to follow a normal distribution.

Using SSLP analysis, 19 polymorphic markers identified from the TAIR database was not confirmed with the RIL population used here. The derived Ler line may explain the discrepancies between the observed and expected polymorphisms. The information from the morphological data and genetic polymorphisms was used to identify correlation between them and ozone response phenotype. The confidence interval for the identified QTL included the *bp-1* locus which is located on chromosome 4q, at the proximal end of the centromere. Thus the presence of morphological marker, *bp-1* on chromosome 4 was quite efficient in identifying a tightly linked QTL controlling the given trait. The effect of this QTL for ozone stress accounted for ~9% of the phenotypic variation. This is due to the non-normal distribution or transgressive nature of the RIL population. Other QTL mapping studies done in rice and sorghum bicolor using RIL populations also

demonstrate less than 20% phenotypic variation for the QTLs identified. In rice using 164 RILs, (Kim, Kwon et al. 2004) located one major QTL for ozone resistance (qOZ-1-2) on chromosome 1 explaining 8.4% of phenotypic variations with a LOD score of 2.5. In addition, the resulting small amount of variation demonstrated in our study is due to the small mean difference between the AA homozygotes and BB homozygotes for the *bp-1* phenotype (Fig 6). The RILs were scored for the ozone response phenotype in three replicates and in each replicate the results were consistent, thus increasing our confidence in the identification of the QTL. Furthermore, with as few as 40 markers we were able to identify one ozone response QTL with a LOD score of 4.11, with $P<0.004$ and 95% confidence level.

Fig. 6 Mean difference in the genotypes for the ozone response phenotype of the *bp-1* marker.

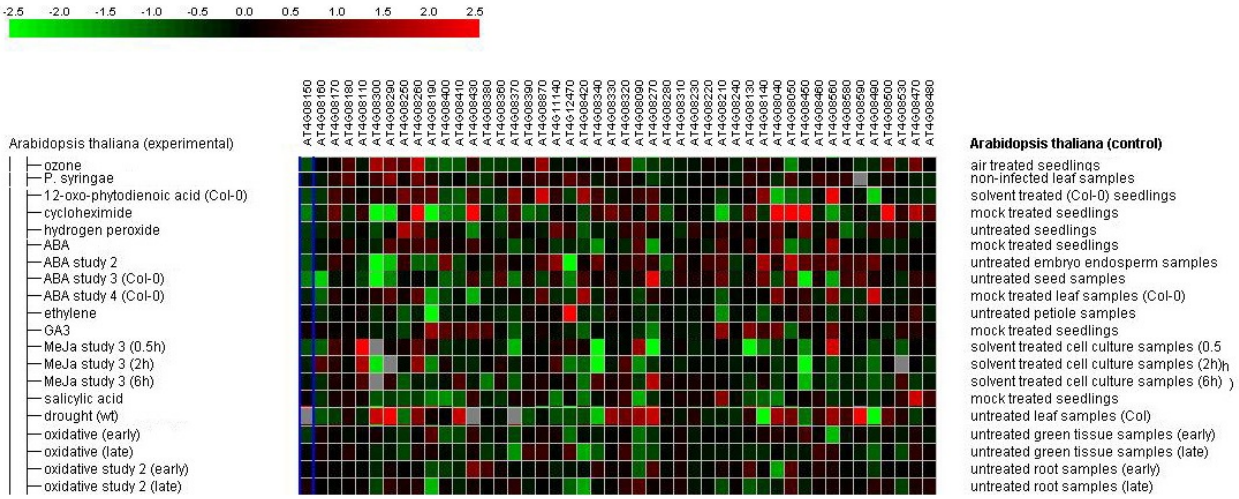


Mutagenesis studies in Arabidopsis have led to identification of several ozone sensitive mutants. These mutants include ozone-sensitive1-1 (*ozs1*; (Saji, Bathula et al. 2008) and radical-induced cell-death1 (*rcd1*; (Overmyer, Tuominen et al. 2000) located on chromosome 1, ascorbate-deficient mutant (*vtc1*; (Conklin and Barth 2004) and JA-signaling deficient 1 (*jar1*; (Rao, Koch et al. 2000) located on chromosome 2, and ethylene-overproducing mutant (*eto1-1*; (Tamaoki, Matsuyama et al. 2003) on chromosome 4. Of the five ozone sensitive mutants identified only one is located on chromosome 4; *eto1-1*. However, the gene for this mutantion is on the p-arm of chromosome 4 and thus is distant from the QTL identified in our study. Thus, we have identified a novel locus that has not been recognized in previous screens of ozone sensitive mutants.

In order to analyze the response profile of the genes around the *bp-1* locus using Genevestigator tool, we obtained 200 genes in its vicinity from TAIR database. It has been pointed out that a genetic distance of 1% recombination, in Arabidopsis corresponds to a physical distance of about 250kb on average (Lukowitz, Gillmor et al. 2000). This implies there are 50 genes within the genetic distance of 1 cM (1 gene ~ 5kb). The majority of the identified genes were from the distal end of the locus as the centromeric region was not very rich in genes. Of the identified 200 genes, 150 did not have probe sets in the Affymetrix chips. Nonetheless, the pattern of the 50 genes which had available probe sets was closely examined. From the Genevestigator analysis it was apparent that many genes involved in signal transduction cascades, metabolism, respiration and general pathogen and disease resistance pathways were in this chromosomal region. We specifically focused our analysis on ozone response profile even though the array data

was derived from different ozone treatment than used in our study (500nL L^{-1} for 6 hrs). The majority of the analyzed genes under ozone stress were repressed. Genes that were strikingly inhibited include those that encode for proline-rich extensin-like family protein (AT4G08400), transposable element gene; CACTA-like transposase family (Ptta/En/Spm; AT4G08090) and transposable element gene; and gypsy-like retrotransposon family (Athila; AT4G08050). In addition a few genes were observed to be induced in response to ozone and these include genes encoding arginase (AT4G08870), protein phosphatase 2C (AT4G08260), MAPK/ERK Kinase Kinase3 (AT4G08470), and tetratricopeptide repeat (TPR)-containing protein and nodulin MtN21 family protein (encoded by two genes; AT4G08300 and AT4G08290) (Fig 7).

Fig. 7 Genevestigator analysis of 50 genes in the vicinity of the *bp-1* locus; showing the expression profile of the genes in response to various abiotic and biotic stressors.



When comparing the profile of these genes under other abiotic and biotic stressers, a different profile of response for various stimuli was observed. The gene encoding proline-rich extensin-like family protein was repressed in response to 12-oxo-phosphodionic acid (oPA), cycloheximide, 0.5μM ABA (study 3), salicylic acid (SA) and

oxidative stress caused by methyl viologen (MV). However, in response to 20μM ABA (study 2) and 1μM gibberellic acid (GA) this gene was induced. Furthermore, the gene for protein phosphatase 2C was induced when plant tissue was treated with hydrogen peroxide (H_2O_2; 20mM, 1 hr), *P. syringae* (10e8 cfu/ml), 12-oxo-phosphodionic acid (75 μM, 4hr), cycloheximide (10μM, 3hr), ABA (study 4, 50μM), methyl jasmonate (MeJA; 0.5hr and 2hr) and drought (no irrigation for 10 days). On the other hand in the presence of ABA (study 2, 20μM) and SA (10μM, 3 hr) the protein was repressed. When comparing the above findings with the MAPK/ERK kinase kinase 3 gene; it was found that this gene was upregulated in response to cycloheximide, ABA (0.5μM, 2days), ET and SA and was down-regulated in presence of MeJA (6hrs) and drought. Additionally, arginase gene was induced in response to 12-oxo-phosphodionic acid and ABA (20μM) and was repressed in response to cycloheximide, and ABA (0.5μM and 50μM).

PP2C-type protein phosphatases have been categorized into 10 groups (A-J). Group A PP2C has been found to be associated with the regulation of a plant hormone, ABA signal transduction proteins *ABI1 and ABI2*. Through genetic analysis of *abi1* and *abi2* mutants it was demonstrated that Group A *Arabidopsis* PP2Cs negatively regulate ABA signaling (Sheen 1998) and (Merlot, Gosti et al. 2001). On the other hand Group B PP2C has been associated with the regulation of MAPK signal cascade (Schweighofer, Hirt et al. 2004). In addition it has also been speculated that PP2C enzymes are regulated by the cellular redox balance. Hydrogen peroxide (H_2O_2) is known to play a crucial role in the reversible inactivation of ABI1 and ABI2 PP2C proteins as H_2O_2 is involved in the oxidation of thiol residues of cysteine and glutathione. Thus H_2O_2 is involved in

modulation of ABA-dependent signal transduction process (Schweighofer, Hirt et al. 2004).

Plant being a sessile organism has efficiently evolved response mechanisms towards stress conditions in order to facilitate survival (Popko, Hansch et al. 2010). When a plant experiences damage (localized wounding) due to ozone or other environmental stress, this protein gets activated and in turn initiates large-scale changes in transcription coupled to growth arrest, allowing resource diversion for defense. Thus there is crosstalk between various pathways that ensures survival of the plant. One such crosstalk mechanism exists between methyl jasmonate (MeJA) and Arabidopsis arginase protein ARGAH2 (ARG: Altered Response to Gravity) as the transcripts of ARGAH2 are found to accumulate in response to MeJA (Brownfield, Todd et al. 2008). This implies that ARGAH2 is involved in reducing the stress induced by MeJA either by MeJA breakdown or by regulating other pathways that can regulate the levels of MeJA. In addition arginase has also been demonstrated to play a role in Nitric Oxide (NO) signaling which is in turn involved in stomatal conductance via the action of ABA (Flores, Todd et al. 2008), (Neill, Desikan et al. 2003). Therefore, we can speculate that upon sensing stress caused by ozone, plants respond by stomatal closure which in turn affects the levels of ABA, NO and auxin in the cells and eventually channel the resources towards development and survival.

CHAPTER V

CONCLUSION

In summary, we have identified one QTL linked to ozone resistance. However, the search for QTL associated with ozone resistance is not complete. These results indicate that understanding the genetic basis of natural variation in ozone resistance may require a broader search of traits related to ozone resistance. The *A. thaliana* genome needs to be assessed for additional molecular markers, particularly on chromosome 2 and flanking the *bp-1* locus on chromosome 4 in order to identify more relevant QTLs and also to determine the phenotypic variation of the identified QTL more accurately. In addition, transcriptomic approaches can be used to identify QTL regions through comparative expression analyses of RILs and parent lines that differ in O_3 tolerance (Frei, Tanaka et al. 2010). There has been recent advancement using whole genome tilling arrays (WGA) for QTL mapping. This innovative technology can be used to rapidly identify useful polymorphisms. In addition to enhancing our understanding of plant O_3 response, the results from these studies may ultimately be applicable for breeding plants with higher levels of O_3 tolerance, and will aid in the identification of genes whose eventual manipulation could improve plant environmental stress tolerance.

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APPENDICES

Fig. 1 Phenotypic representation of the morphological traits for the RIL population (Ler x Ws) compared to the wildtype.

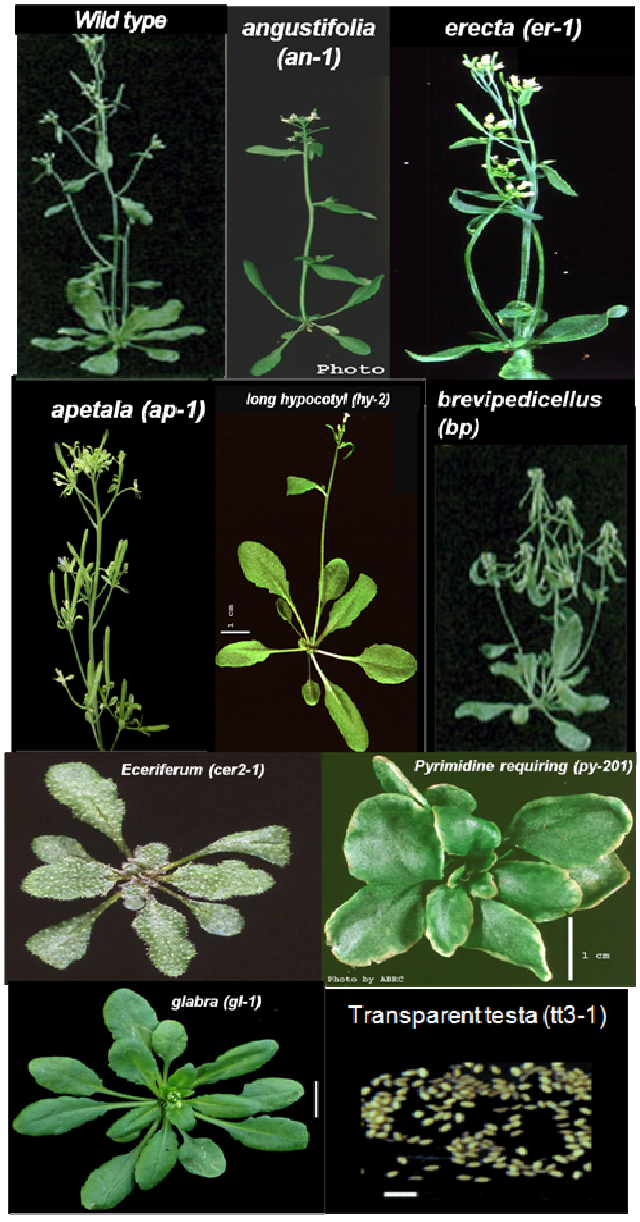

Fig. 2 Fumigation chamber used for treating plant samples with ozone. a) Pure oxygen source, b) gas regulator, c) ozone generator model no. 2000, Jelight Company, Inc., d) ozone monitor model 450, Advanced Pollution Instrumentation, Inc., e) Percival scientific growth chamber, f) ozone destruct unit, g) stainless steel adjustable valve, h) stainless steel “T”-section. The red lines indicate tygon tubing connecting the units.

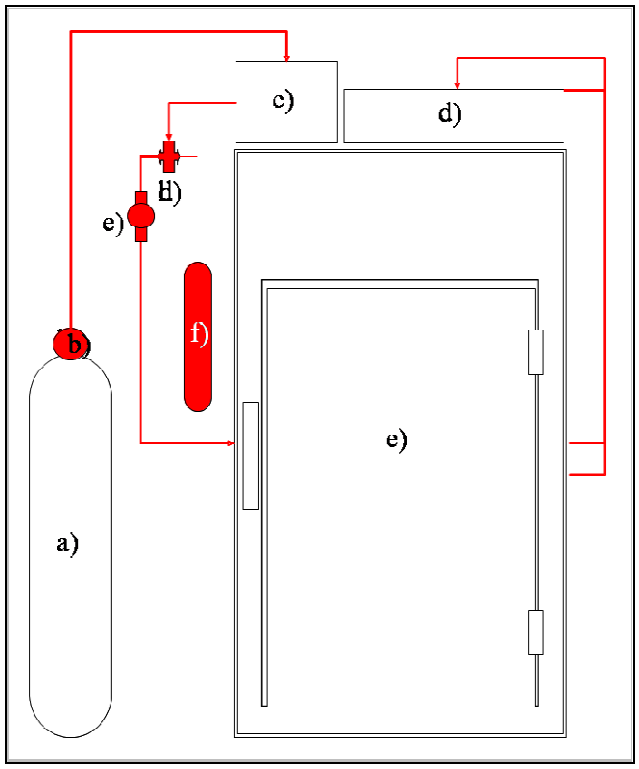

Fig. 3 Polymorphism of simple sequence-length polymorphism (SSLP) marker in Ler, Ws and the recombinant inbred lines (RILs) from Ler x Ws.

Table 1 Morphological traits in 147recombinant inbred population derived from a cross between ozone tolerant Ler and ozone susceptible Ws genotypes. The RILs were scored for 10 morphological traits, segregating ratios of the RILs for the nine traits is shown at the bottom of the table.

mapping line #	**tt3-1**	**gl1-1**	**an-1**	**hy2-1**	**cer2-1**	**er-1**	**bp-1**	**py-201**	**ap1-1**
CS2001 (1)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2002 (2)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	ap1-1
CS2005 (5)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2007 (7)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2009 (9)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2010 (10)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2013 (13)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2014 (14)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2015 (15)	tt3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2016 (16)	TT3-1	gl1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2017 (17)	TT3-1	gl1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2018 (18)	tt3-1	gl1-1	an-1	HY2-1	cer2-1	er-1	BP-1	PY-201	AP1-1
CS2021 (21)	tt3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	BP-1	PY-201	AP1-1
CS2022 (22)	tt3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2023 (23)	TT3-1	gl1-1	an-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	ap1-1
CS2025 (25)	TT3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2026 (26)	TT3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2028 (28)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2029 (29)	tt3-1	gl1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2030 (30)	tt3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	bp-1	py-201	AP1-1
CS2031 (31)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	bp-1	py-201	AP1-1
CS2033 (33)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	er-1	BP-1	PY-201	AP1-1
CS2034 (34)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2035 (35)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2036 (36)	tt3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2037 (37)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2039 (39)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2041 (41)	tt3-1	gl1-1	an-1	HY2-1	cer2-1	er-1	bp-1	PY-201	AP1-1
CS2042 (42)	tt3-1	gl1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2043 (43)	tt3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2047 (47)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	er-1	bp-1	PY-201	AP1-1
CS2049 (49)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2050 (50)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2051 (51)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2053 (53)	tt3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2055 (55)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2056 (56)	TT3-1	gl1-1	an-1	HY2-1	CER2-1	ER-1	bp-1	PY-201	AP1-1

CS2057 (57)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2058 (58)	TT3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2059 (59)	tt3-1	gl1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2065 (65)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	ap1-1
CS2066 (66)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2068 (68)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2069 (69)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2070 (70)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2071 (71)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	ER-1	bp-1	py-201	AP1-1
CS2072 (72)	tt3-1	gl1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2073 (73)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2074 (74)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2075 (75)	TT3-1	GL1-1	AN-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2076 (76)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	er-1	BP-1	PY-201	AP1-1
CS2077 (77)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2078 (78)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	BP-1	PY-201	ap1-1
CS2079 (79)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2081 (81)	tt3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2083 (83)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2084 (84)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2085 (85)	TT3-1	gl1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2087 (87)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2088 (88)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2089 (89)	tt3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2093 (93)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	er-1	bp-1	py-201	ap1-1
CS2094 (94)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2095 (95)	TT3-1	gl1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2096 (96)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2098 (98)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2100 (100)	TT3-1	GL1-1	AN-1	hy2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2101 (101)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2106 (106)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2107 (107)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2108 (108)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	bp-1	py-201	AP1-1
CS2109 (109)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2110 (110)	tt3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2111 (111)	tt3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2112 (112)	TT3-1	gl1-1	an-1	hy2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2114 (114)	tt3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2116 (116)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	er-1	BP-1	py-201	ap1-1
CS2118 (118)	TT3-1	gl1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2119 (119)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2120 (120)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	AP1-1
CS2121 (121)	tt3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1

CS2122 (122)	TT3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS124 (124)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2126 (126)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	PY-201	ap1-1
CS2127 (127)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2128 (128)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	bp-1	PY-201	ap1-1
CS2129 (129)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2130 (130)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2131 (131)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2132 (132)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2133 (133)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2134 (134)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2136 (136)	tt3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2137 (137)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2138 (138)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2139 (139)	TT3-1	GL1-1	an-1	HY2-1	cer2-1	ER-1	bp-1	PY-201	AP1-1
CS2140 (140)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2141 (141)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	bp-1	PY-201	ap1-1
CS2143 (143)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	PY-201	AP1-1
CS2144 (144)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	PY-201	AP1-1
CS2145 (145)	tt3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2146 (146)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2148 (148)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2149 (149)	TT3-1	gl1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2150 (150)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	py-201	AP1-1
CS2151 (151)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	er-1	BP-1	PY-201	ap1-1
CS2152 (152)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2153 (153)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2155 (155)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	bp-1	PY-201	AP1-1
CS2156 (156)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	py-201	AP1-1
CS2157 (157)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2159 (159)	tt3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2160 (160)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	py-201	AP1-1
CS2161 (161)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1
CS2163 (163)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2165 (165)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	py-201	ap1-1
CS2166 (166)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2167 (167)	TT3-1	gl1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2168 (168)	tt3-1	GL1-1	an-1	hy2-1	CER2-1	er-1	bp-1	py-201	AP1-1
CS2169 (169)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	BP-1	PY-201	ap1-1
CS2172 (172)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2174 (174)	TT3-1	GL1-1	an-1	hy2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2176 (176)	TT3-1	gl1-1	AN-1	hy2-1	cer2-1	er-1	bp-1	py-201	ap1-1
CS2179 (179)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2180 (180)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	ap1-1

CS2183 (183)	TT3-1	GL1-1	an-1	HY2-1	CER2-1	er-1	bp-1	py-201	AP1-1
CS2184 (184)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2185 (185)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2186 (186)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	ER-1	BP-1	py-201	AP1-1
CS2187 (187)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2188 (188)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	bp-1	py-201	ap1-1
CS2189 (189)	TT3-1	GL1-1	an-1	hy2-1	cer2-1	ER-1	bp-1	py-201	AP1-1
CS2190 (190)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	bp-1	py-201	ap1-1
CS2191 (191)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	er-1	BP-1	py-201	ap1-1
CS2195 (195)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	er-1	BP-1	py-201	AP1-1
CS2202 (202)	TT3-1	gl1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	py-201	ap1-1
CS2204 (204)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2205 (205)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	er-1	bp-1	py-201	AP1-1
CS2206 (206)	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2207 (207)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	PY-201	AP1-1
CS2208 (208)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	er-1	bp-1	PY-201	ap1-1
CS2210 (210)	TT3-1	GL1-1	AN-1	hy2-1	cer2-1	er-1	BP-1	py-201	AP1-1
CS2211 (211)	TT3-1	GL1-1	AN-1	HY2-1	cer2-1	ER-1	BP-1	py-201	ap1-1
CS2215 (215)	TT3-1	gl1-1	AN-1	HY2-1	CER2-1	er-1	BP-1	py-201	ap1-1
CS2217 (217)	TT3-1	GL1-1	AN-1	hy2-1	CER2-1	er-1	BP-1	py-201	AP1-1
CS2219 (219)	tt3-1	gl1-1	AN-1	HY2-1	CER2-1	ER-1	bp-1	PY-201	AP1-1
CS2222 (222)	tt3-1	GL1-1	an-1	hy2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
WS Mapping	TT3-1	GL1-1	AN-1	HY2-1	CER2-1	ER-1	BP-1	PY-201	AP1-1
CS2224 (W100)	tt3-1	gl1-1	an-1	hy2-1	cer2-1	er-1	bp-1	py-201	ap1-1
Segregations (B:A)	122:25	97:50	83:64	84:63	86:61	106:41	109:38	107:40	112:35
X^2	51.95	14.93	2.44	2.98	4.23	28.54	34.06	30.34	40.06
Probability	0.00	0.0001	0.1182	0.0842	0.0397	9.1629e-8	5.3208e-9	3.6294e-8	0.00
Chromosome	5	3	1	3	4	2	4	2	1

Table 2 The 31 SSLP markers used to construct the Ler x Ws genetic map. The markers are from the TAIR web site (http://www.arabidopsis.org). BAC/PI refers to the BAC and PI clone in which the marker is situated from the TAIR web site.

Marker	Chrom.	5'-3' Forward Primer	5'-3' Reverse Primer	Ler (bp)	Ws (bp)	Diff. (bp)*
nF21M12	1	TTACTTTTTGCCTCTTGTCATTG	GGCTTTCTCGAAATCTGTCC	<200	215	15
nga111	1	TGTTTTTTAGGACAAATGGCG	CTCCAGTTGGAAGCTAAAGGG	165	146	19
nga128	1	ATCTTGAAACCTTTAGGGAGGG	GGTCTGTTGATGTCGTAAGTCG	190	170	20
nga59	1	TTAATACATTAGCCCAGACCCG	GCATCTGTGTTCACTCGCC	115	83	32

nga692	1	AGCGTTTAGCTCAACCCTAGG	TTTAGAGAGAGAGAGCGCGG	90	110	20
T518 (meg4)	1	TTTGAGCTCTCTGTTTTTATCGAA	CTGGTTTCTTTGTTCTTTTCCA	298	268	30
CER459346	2	TTGAGGCTTCACTTGGTTACTTGA	GGCGGGAAAAGGATTTAGAACT	124	141	17
CER459358	2	AACTGGTTGGTTTAAGAATAA	AACCAACGATCCCCTTTTGA	130	160	30
CHIB	3	ATGAGAAGCTATAATTTTTTCAATA	CTCATATATACAAAGAACTACTATAC	74	82	8
ciw4	3	GTTCATTAAACTTGCGTGTGT	TACGGTCAGATTGAGTGATTC	215	190	25
ciw11	3	CCCCGAGTTGAGGTATT	GAAGAAATTCCTAAAGCATTC	230	240	10
nga6	3	ATGGAGAAGCTTACACTGATC	TGGATTTCTTCCTCTCTTCAC	123	131	8
nga12	3	TGATGCTCTCTGAAACAAGAGC	AATGTTGTCCTCCCCTCCTC	234	247	13
DHS1	4	GAGCTTTGTAAATCAACAACC	GATATTTTTCAGGCGACGTGGAAGC	194	165	29
CIW5	4	GGTTAAAAATTAGGGTTACGA	AGATTTACGTGGAAGCAAT	144	500	360
CIW7	4	AATTTGGAGATTAGCTGGAAT	CCATGTTGATGATAAGCACAA	123	150	27
JV30/31	4	CATTAAAATCACCGCCAAAAA	TTTTGTTACATCGAACCACACA	165	190	25
nga8	4	TGGCTTTCGTTTATAAACATCC	GAGGGCAAATCTTTATTTCGG	198	166	32
TGSSLP1	4	ACTGTTCGTCTCCTTCATCATG	TTGCTTGCCTGAAAAAAGTATG	750	861	111
CER455033	5	AGCTCATGCTTCCCTACACTG	AACAACTAGCATTAGCAACAATCA	100	130	30
F17C15-2ME	5	CTCAGAAATGGAAAGAGATTGTGATG	ATCGACGCCGTTTAATATTGTTTTAT	185	220	35
F8L15-1ME	5	TAAAGGTAAAAATCAGCATTGTTGTG	ACGCGCTTGACTCCGGTGTTGA	190	240	50
K18I23-2ME	5	AGTGGCAAGGCCGGAGATTC	GGCCCATGCTTTGGCTGTAAT	154	185	35
MHF15-3ME	5	GTCGGAGAAAATACCTTGAAACCTAC	TAAGCCTTAATGAAAAATCTATCTAT	190	210	20
nga129	5	CACACTGAAGATGGTCTTGAGG	TCAGGAGGAACTAAAGTGAGGG	179	165	14
NGA151	5	CAGTCTAAAAGCGAGAGTATGATG	GTTTTGGGAAGTTTTGCTGG	120	102	18

T15N1	5	GTTCCAATGTGTTCCCAGAGCTTG	CAAAGAATCTCAGAGGATAATAATG	185	168	17
T20L15-1ME	5	CTACTTTTGCGTCATCAATCATACTA	TGTCGGCATCGTAGGTCTAATA	185	220	35
T22D6-2ME	5	ATTGCGACTTGTTCTAGGTTCTACGA	CGCCCGCTCCCCAGTTA	175	201	26
T32M21-1ME	5	AAACGTTAAATTTTAGTCGGTGAGT	TCTCCGTTGCTTAGAACATTTG	190	220	30
T6I14-1	5	GGTTTCTTCTATTAAGGACCAGCG	AACCCTAAAATCACTCACTGCCTC	190	220	30

*Difference in bp between Ler and Ws PCR amplified fragment

VITA

Nazia Tabassum

Candidate for the Degree of

Master of Science

Thesis: MAPPING THE OZONE RESISTANCE TRAIT IN *Arabidopsis thaliana*

Major Field: Biochemistry and Molecular Biology

Biographical:

Education:
Received Bachelors of Science in Biochemistry and Molecular Biology from Oklahoma State University, Stillwater, Oklahoma in 2007.
Completed the requirements for the Master of Science in Biochemistry and Molecular Biology at Oklahoma State University, Stillwater, Oklahoma in December, 2010.

Experience:
Worked as a Research Assistant at Oklahoma State Univeristy – Center for Health Sciences (OSU-CHS) at Tulsa, Oklahoma in 2008. Employed by OSU as a Graduate Research Assistant in the Department of Biochemistry and Molecular Biology from 2008 – present.

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Name: Nazia Tabassum Date of Degree: December, 2010*

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: MAPPING THE OZONE RESISTANCE TRAIT IN *Arabidopsis thaliana*

Pages in Study: 41 Candidate for the Degree of Master of Science

Major Field: Biochemistry and Molecular Biology

Scope and Method of Study: Ozone is a secondary pollutant in the troposphere where plants, animals and human beings reside. Ozone has harmful effects on plant growth and metabolism. In sensitive plant species it causes visible injury such as necrosis and chlorosis of leaves leading to reduction in photosynthesis and that ultimately manifests as yield losses. The major goal of this study is to identify Quantitative Trait Loci (QTL) responsible for ozone resistance in *Arabidopsis thaliana*. For this study, we used a Recombinant Inbred Line (RIL) population of 150 lines derived from a cross between ecotypes Landsberg erecta (ozone resistant parent) and Wassilewskija (ozone sensitive parent). This project involves physiology, genetics, molecular biology and statistical genomics. The physiological analysis involved scoring the RIL population for the ozone response phenotype. A genetic linkage map for the Ler x Ws cross was constructed using nine polymorphic morphological traits and Simple Sequence Length Polymorphic (SSLPs) markers. R/QTL statistical software was used for identifying ozone resistance QTLs.

Findings and Conclusions: A single QTL for ozone resistance (LOD score: 4.11) was identified on the long-arm of chromosome 4 around the *bp-1*. This QTL explained 10% of phenotypic variation for the ozone resistance trait in this RIL population. Identification of more polymorphic markers in the vicinity of *bp-1* locus will facilitate fine-mapping and ultimately the positional cloning of this ozone resistance QTL.

ADVISER'S APPROVAL: Dr. Ramamurthy Mahalingam