# QTL ANALYSIS FOR GENES CONFERRING TOLERANCE TO DROUGHT STRESS AND DAMAGE FROM UV-B RADIATION

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ABSTRACT

A mapping experiment has been carried out on the IMB94 recombinant inbred maize population seeking loci that confer tolerance to drought stress, UV-B stress, and the combination of both. The effects of drought, UV-B radiation, and the combination of both on four general traits in maize seedlings were measured under greenhouse conditions and analyzed using QTL Network and Chromoscan software. Trait measurements indicated a seasonal affect on growth which was adjusted for by standardizing trait data by the median of corresponding parental 'check plants'. Non-adjusted and adjusted QTL Network analyses identified 19 QTLs with 5 epistatic interactions and 6 QTLs with 2 epistatic interactions, respectively while Chromoscan analysis identified 123 single locus QTLs. Results from these analyses suggest that QTLs for drought and UV-B tolerance exist across the entire genome and are somewhat more common than QTLs for tolerance to the combination of both stresses.

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# INTRODUCTION

## Abiotic Stress

Abiotic stresses are significant factors affecting grain yield in many agronomically important cereal crops. Drought is one of the most important of these environmentally-induced stresses (Langridge et al., 2006). In *Zea mays* (maize), drought stress can cause a variety of problems that ultimately result in reduced grain yield. Disruption in the synchronization pattern of male & female floral organ development occurs in drought-stressed fields of maize, resulting in vast numbers of ears with little or no seed set (Herrero and Johnson, 1981). Traditional selection and breeding techniques have been ineffective at generating more drought-tolerant lines due to the low heritability of the trait, coupled with decreases in yield under drought conditions (Blum, 1988 as cited in Hai et. al., 2003).

Excessive exposure to UV radiation is another abiotic factor that negatively affects the growth of important food crops. Solar radiation with wavelengths in the range of 280nm-320nm, or UV-B, is particularly damaging to plant DNA, resulting in the formation of cyclopyrimidine dimers (CPDs) and (6,4) pyrimidone dimers (Jordan, 2002). Damage to the plant's DNA that goes un-repaired can result in growth inhibition or long term alterations in morphology due to mutations in protein coding or other important regions of the genome (Teramura and Sullivan, 1994). Although maize has robust ways to protect against damage caused by UV-B radiation such as efficient repair mechanisms and UV-B absorbing pigments like flavonoids (Stapleton and Walbot, 1994), excessive irradiation will ultimately have a negative impact on final yield since the plant is diverting energy from grain production to UV-defense (Kalbin et al., 2001).

## QTL Analysis

Developing varieties of maize that have the innate ability to resist abiotic stresses such as drought & excessive UV-B radiation is a very desirable proposition for agronomists, farmers, and the general public. Understanding and identifying the genetic factors that underlie a plant's response to various stresses may allow for the selection of those genes or groups of genes that confer favorable attributes under the environmental conditions to which the plant will be subjected in the field. One method that can be used to identify the genes involved with a particular trait or set of related traits is called Quantitative Trait Loci (QTL) analysis. Resistances to drought stress or excessive UV exposure are both traits that can be measured quantitatively, such as on a scale of one to ten, and are thus called quantitative traits. Quantitative traits are characterized by having continuous variation and being under the control of many genes while qualitative traits, such as color or dwarfism, are characterized by discrete variation and are usually controlled by one or two genes (Lynch and Walsh, 1998). The complexity of quantitative traits and their heritability results from their being influenced by many genes. QTL analysis therefore uses specially designed populations and analysis methods to analyze and realistically link traits to particular chromosomal regions.

In order to conduct a QTL analysis for a trait or traits of interest, several factors must be considered. First, a population that is segregating for the trait of interest is desirable. For example, a population of maize plants that is either resistant, or susceptible to a given level of drought stress. A segregating or mapping population could be derived from a cross between two parents that have very different phenotypes for the quantitative trait of interest (Lynch and Walsh, 1998). However, parents of the population do not necessarily have to have different phenotypes for the trait of interest. It is also possible for a population to be segregating for a quantitative trait of interest without the parents being different, a process called transgressive

segregation (Rieseberg et al, 1999). Transgressive segregation is a phenomenon observed in hybrids where offspring have extreme phenotypes relative to either parental line (Rieseberg et al, 2003). Next, a defined set of molecular markers must be established throughout the genome of interest so that chromosomal regions in the analyzed individuals within the experimental population can be attributed to one of the parents (e.g., either the drought resistant parent or the drought susceptible parent). Fortunately, *Zea mays* mapping populations that already have defined markers are publicly available, such as the *IBM* population, which is derived from a cross between two well characterized inbred lines (Lee et al., 2002). Finally, a sufficiently large population of 100-300 individuals must be analyzed to rule out the variation in phenotype due to natural variation alone (Lynch and Walsh, 1998). In fact, if resources permit, larger populations are recommended (Beavis, 1994). If all of these requirements are met, a QTL analysis can be conducted and desirable phenotypes can be statistically linked to distinct chromosomal regions (QTLs). Over time, the desired QTLs can be selected for in breeding programs to generate superior crops through a method called Marker Assisted Selection (MAS, Stuber et al., 1999). This is a process by which traits are selected for, indirectly, by screening for molecular markers linked with the trait, rather than the trait itself. This process involves extensive breeding and is therefore time consuming and labor intensive.

IBM Population

The experimental plants studied in this experiment were recombinant inbred lines (RILs) of *Zea mays* from the IBM94 population. This population consists of 94 distinct lines (individuals) that all share portions of the parental backgrounds, B73 or Mo17. Recombinant inbred lines are developed by crossing the two parents to produce an F1 generation then allowing this F1 generation to cross-pollinate for four generations. Then these F5 seeds are planted,

grown to maturity, and self pollinated under tightly controlled conditions that ensure no cross pollination. This is achieved by keeping the developing ears on F5 plants covered with paper bags at all times except pollination time. Once ready, the F6 seed is harvested and one seed from each F6 ear is planted and self pollinated as was just described. This process is repeated at least six times in order to achieve consistent homozygosity at all marker loci.

Molecular markers are a highly useful feature of RILs that facilitate QTL studies. To identify these markers, both parental lines, as well as the RIL offspring, must be thoroughly genotyped and, in the case of the IBM94 maize population, 4751 molecular markers that are distinct between the parental lines have been identified. Due to crossing over and recombination during gamete formation, all of the F1 progeny are a mosaic of the parental genotypes at these molecular markers. As mentioned, after the six rounds of self pollination, the RILs are homozygotic at all the marker loci and the parental origin of each marker can be identified (Lee et al., 2002). Therefore, each RIL in the population has a unique genotype that is already mapped and may be compared to all other RILs in the population, greatly facilitating the isolation of useful QTLs, such as those associated with drought tolerance.

Tuberosa et al. (1998) have investigated drought tolerance in maize & report a significant association between drought stress and accumulation of abscisic acid (ABA) in leaf tissue. They have identified several QTLs linked to this trait. In a field experiment, stomatal conductance in maize leaves was sensitive to the ABA concentration in the xylem sap and was correlated strongly with the time of day (Tardieu and Davies, 1991). In response to water deficit, leaf ABA concentrations increase, causing stomata to close and thereby enabling the plant to conserve water by decreasing transpiration. This trait may be of great interest to certain breeders since selecting for plants with the “ABA QTL” in a breeding program could potentially lead to the

development of drought-tolerant varieties of maize, or better yet, the introgression of the trait into already established varieties that are popular with farmers. Drought stress causes an increase in reactive oxygen species (ROS) which can be detrimental to plant growth (Grzesiak et al., 2006). Although maize can increase production of anti-oxidative enzymes as a defense, chloroplasts are highly sensitive to singlet oxygen and thus, photosynthesis can still be hindered by drought stress (Grzesiak et al., 2006).

UV-B tolerance has been investigated extensively in *Oryza sativa* (rice) and a QTL for UV-B resistance has been identified. This QTL is on chromosome 10, and is designated *qUVR-10* (Sato et al., 2003; Ueda et al., 2004). This QTL was identified by analyzing a large (1850 plants) $F_2$ population derived from a cross between a UV-B-resistant variety and a UV-B-susceptible variety and it encodes a cyclobutane pyrimidine dimer (CPD) photolyase that repairs damage caused by UV-B radiation (Ueda et al., 2005).

### Objectives

The primary goal of this research is to identify QTLs associated with tolerance to drought, UV, and a simultaneous combination of the two so that subsequent research may further characterize and identify specific genes responsible for a tolerant phenotype.

## MATERIALS AND METHODS

### Greenhouse Facility and Experimental Space

The experimental maize populations were grown in the Kresge Greenhouse located on the UNCW campus. The seeds for these experiments were obtained by Dr. Ann Stapleton and provided to me. Two aluminum tables with surfaces 0.61 meters (2 feet) above the ground were used as the growing surface for the plants. Each table was divided into two equally sized spaces

by hanging a vertical partition above each table using sheets of mylar. The mylar partitions served to prevent any UV from reaching over to the other side of the table.

Two 4' fluorescent fixtures (Lithonia Lighting - model C24012ES) were suspended 0.61 meters (2 feet) above each treatment area and spaced equally apart. Two UV-313 fluorescent bulbs (Q-Panel Lab Products - Cleveland, OH) were installed in each fixture to provide UVB to the plants. The fluorescent fixtures above the control area and the drought treatment area were encased with mylar to filter the UV and prevent it from reaching the plants while the fixtures above the UV and UV + drought treatment areas were encased with cellulose acetate to allow the UV-B to pass to the plants. UV dose was eight hours per day for three days. Spectral graphs obtained using Ocean Optics Spectral Suite software (Dunedin, FL USA) show the effectiveness of mylar in blocking UV transmission and the passiveness of cellulose acetate for allowing UV transmission (Figures 1A and 1B, respectively), while Figure 1C shows the spectrum with no plastic in place. The very small peaks at 327nm and 365 nm on Figure 1A illustrate the blocking effect of mylar against UV while the peaks at 315nm in Figures 1B and 1C clearly show that these bulbs emit primarily in the UV-B range. No UV-C is emitted but some UV-A is transmitted, at 365nm.

Pilot Experiments

Prior to initiating the primary mapping experiments described in this paper, two pilot experiments were conducted in March and April of 2007 to assess the effect of varying intensities of drought and supplemental UV on maize seedlings. One of the parental lines of the IBM94 maize population, B73, was used in both of these pilot experiments.

The first pilot experiment, conducted in March 2007, assessed the effects of varying intensities of drought and supplemental UV, individually, on four traits in the maize seedlings: root and shoot dry weight, change in height, and change in leaf length (leaf #3). The interval between initial and final measurements for the latter two traits was ten days in all experiments to allow time for treatment effects to manifest themselves in a measurable way. The drought intensities ranged from 200mL of water per day down to 0mL of water per day, decreasing in 50mL increments. The UV durations analyzed were: 4 hours per day, 8 hours per day, and 10 hours per day for a treatment period of 3 days.

The second pilot experiment, conducted in April 2007, assessed the effects of varying levels of drought and supplemental UV, in combination, on the four measured traits. Based on the results of the first pilot experiment, only combinations of 8 or 10 hours of UV per day and 50mL or 0mL of water per day were assessed. Measurement and data collection procedures were the same as just described for the first pilot experiment. The exact parameters of the pilot experiments (soil, plant age, measurement techniques, etc) were the same as those in the primary mapping experiment which is described in the section that follows.

## Experimental Design

Due to non-viability of some seeds, 92 RILs from the IBM94 population were evaluated in this series of experiments. In addition, "check plants" were added for comparison to detect any effect that time of year may have had since the experiments took place over a four-month period with differing temperature and humidity conditions. Two check plants were used: a parental line of the IBM population, B73 (ID P-95), and a random member of the IBM population, O-302, and these check plants were planted alongside the experimental plants in each

treatment group in random locations. Check plants were planted in replicates of 4 and measured in the exact same way as the experimental plants. If no significant differences in trait measurements of check plants were detected across the 4-month time period it was assumed that there was also no significant time effect in the experimental plants. Each replicate (n=4) of the experiment consisted of four treatment groups: control, drought, UV and UV + drought. The primary purpose of the single stress groups (UV and drought) was for comparison against the UV + drought group to rule out the possibility of attributing an observed phenotype to the combined stress that was actually being caused by one of the single stresses.

Each experiment consisted of four groups of 100 plants each repeated four times over a four month period during the summer of 2007. Experiment 1 was conducted in May, experiment 2 in June, experiment 3 in July and experiment 4 in August. Plants were grown in 11.4cm (4.5") plastic pots (LandMark Plastic – Akron, OH) from seed stock obtained by Dr. Ann Stapleton. Pots were filled, by hand, with Metromix400 (Sun Gro Horticulture – Bellevue, WA) and seeds were germinated directly in the soil. Throughout the experiments, the greenhouse water supply was the irrigation source for all of the plants.

Plants were allowed to grow for ten days prior to beginning experimental treatment. Plants were irrigated every other day from sowing thru the eighth day post- planting for a total of five irrigations. The first four irrigations were light, approximately three seconds with the hose on each pot, followed by a soaking for the final irrigation the evening prior to beginning treatment. The purpose of this soaking was that all pots would be at a fairly consistent weight prior to treatment. This weight was measured the following morning after no more water was dripping from the bottom of the pots and recorded as the pot's full water weight (FWT). Two pots with soil but no plants were used for comparison in each experiment. One pot was filled

with soil and not watered while another was filled with soil and watered like the others. The pot with only soil provided us a good estimate of pot weight under extreme drought conditions, while the pot with soil and water allowed the evaporation rate of the water to be observed. Pots with soil but no plants as just described were used in each of the 4 experiments. Plants in the drought and UV + drought treatment groups were not watered again until three-day treatment was over while plants in the control and UV treatment groups continued to be watered on schedule every day. Pot weights were recorded again on the first day post-treatment in order to gauge the drought intensity on the drought treatment groups relative to the control group. These weights were measured using a Chefmate® kitchen scale to ± 1 g.

During the treatment period the UV lamps were turned on at 9am and remained on until 5pm to give the plants eight hours of UVB exposure. To ensure consistency, automatic timers (Intermatic USA) were used to control this light regimen.

On the first day, before treatment began, several plant measurements were taken. First, the weight of each individual pot was recorded so that it could be compared to the final weight at the end of the treatment period. In addition, the height of each plant and the length of each plant's third leaf was measured and recorded so they could also be compared to post-treatment measurements to determine the amount of growth over the experimental period. Height measurements were taken with a meter stick and measured from soil surface to plant canopy. The leaf measurements were taken with a clear, plastic, metric ruler and measured from ligule to leaf tip. Both measurements were recorded in centimeters. These data were recorded beginning at approximately 6:45am and were recorded from the UV and UV + drought treatment groups first to ensure these plants received the full eight hours of UVB exposure under the lamps.

Final height and leaf length measurements were recorded seven days post treatment, for a total of ten days between initial and final measurements. These measurements were used to gauge general plant health and were compared within and among treatment groups. The purpose of waiting seven days post treatment to take the final measurements was to allow time for the treatment effects to manifest themselves in a measurable way. Since these traits were both measuring growth, taking measurements immediately after treatment would not likely result in detection of a measurable effect on a slow process like leaf growth or height change. I tested my measuring precision by taking five separate measurements of height and leaf length on a random plant over the course of an hour and determined that my precision was approximately ± 0.2cm.

In addition to the height and leaf growth measurements, dry weights of root and shoot tissue from all plants were measured and recorded. Shoot tissue was harvested and dried for two days at 60°C in an oven. The root tissue from each plant was left to dry for two days in the greenhouse in its respective pot. Roots were not dried at 60°C in an oven due to their bulk and the small size of the oven but 48 hours in the greenhouse was sufficient to dry them completely. Dry weights were measured using a scientific scale (Sartorius – B31OP-OUR) to ±0.01 g. All data were compiled in Excel spreadsheets to be formatted for statistical analysis.

## Statistical Analyses

Several types of statistical analyses and software packages were utilized in the analysis of experimental data. First, all ANOVAs performed on the check plant trait data across time were calculated using JMP IN version 4 (© 2007 SAS institute Cary, NC). Assumptions of ANOVA were met by verifying that the data was normally distributed via QQ plot and was homogenous in its variance.

QTL Network version 2.0 (Yang and Williams, 2007) was used to detect QTLs in the experimental population. This software package is capable of conducting both 1-dimensional and 2-dimensional genome scans, as well as detecting epistatic interactions between QTLs. An epistatic interaction is an instance where neither QTL is significantly associated with the trait by itself but together, they interact in a way that is significant. In order to use this program, two files representing the experimental population being examined were required. The first file was called the 'map file' which contains all of the marker data – each molecular marker and its relative position on its designated chromosome. The second file (the data file) contained the unique marker data for each RIL in the population as well as the experimental data collected on those RILs during the course of the experiment. These files were imported into QTL Network and two types of analyses were performed, including a 1-dimensional genome scan with the option to map epistasis and a 2-dimensional genome scan to detect epistatic interactions with or without single-locus effects.

A SAS (SAS Institute Inc. 100 SAS Campus Drive - Cary, NC 27513-2414 USA) general linear model (GLM) program written by Dr. Jim Blum of the UNCW statistics department was adapted to analyze the data in much the same way as the QTL Network 1-dimensional genome scan. Basically, independent ANOVAs were conducted on every possible marker in the dataset to scan for significant QTLs with single locus effects. P-values for genetic difference and for interaction with treatments were then compiled into input files for further analysis in Chromoscan. Chromoscan, which was developed at the University of Michigan's School of Public Health Kardia Research Lab, uses a scan-statistic to detect QTLs that are statistically significant for a measured trait. The scan statistic utilizes a compound Poisson process to account for the complex distribution of genome variation (Sun, Y.V. et al., 2006). This program

produced results in a tabular format indicating the chromosomal regions where the various QTLs were detected as well as associated p-values, interval sizes, etc. Confidence limits are not assigned but the sizes of the QTL regions are indicative of confidence. A QTL that covers a very large chromosomal region would give less confidence of exact location due to the large amount of DNA encompassed by the QTL. Alternately, a QTL that covers a very small chromosomal region would give more confidence of exact location since there is less DNA encompassed by the QTL.

## RESULTS

### Pilot Experiments

The effects of increasing drought levels on root and shoot dry weights from the first pilot experiment are shown in Figure 2A; neither roots nor shoots exhibited a significant change in any of the drought treatments ($p = 0.856$ and 0.596, respectively). P-values that assessed the significance of treatment effects in both of the pilot experiments were generated by ANOVAs that modeled trait values against treatment regimens rather than a dose response model test. Next, the effects of increasing time under supplemental UV are shown in Figure 2B. Overall, increasing UV tended to reduce root dry weight, but the reduction was not significant ($p=0.353$). Shoot dry weight exhibited little variation with increasing UV ($p=0.593$). Increasing drought had no significant effect ($p=0.445$) on change in plant height over time (Fig.2C). The lowest mean change in plant height of 6.4± 2.5cm was observed in the 200mL per day group, while the highest overall mean of 11.4± 3.5cm occurred in the 150mL per day group, but due to high within-treatment variability these differences were not significant. Increasing time under supplemental UV had no significant effect ($p=0.171$) on change in plant height over time (Fig.

2D). The 8 hour regimen produced the lowest mean change in plant height of 5.4± 0.9cm while the 10 hour regimen resulted in the highest overall mean of 9.9± 1.9cm.

Leaf growth was highly variable in response to increasing drought (Fig. 2E). With the exception of the 100mL per day treatment, leaf growth seemed to be reduced with any reduction in watering. The highest mean of 2.2± 2.1cm occurred in the control group while the lowest mean of 0.2± 0.2cm occurred in the 0mL per day group, but due to the high among-treatment variability there was no significant treatment effect ($p=0.484$). The effects of increasing time under supplemental UV on leaf growth are shown in Figure 2F. For leaf growth, the highly-variable control group had the highest overall mean change in leaf length of 2.2± 1.2cm, while the lowest mean of 0.1± 0.05cm was detected in the 8 hours per day regimen (Figure 2F). Like drought, there was no significant among-treatment UV effect on leaf growth ($p=0.434$).

The effects on root and shoot dry weights of increasing time under supplemental UV coupled with a regimen of 0mL of water per day from the second pilot experiment are illustrated in Figure 3A. For root dry weight, the control group had the highest overall mean of 2.46± 0.19g while both of the 8 and 10hrs UV + 0mL groups had significantly-reduced root dry weights of 1.75± 0.09g and 1.56± 0.21g, respectively ($p=0.007$), but were not significantly different from each other as indicated by the 'As' and 'Bs' on the figure. For shoot dry weight the same trend is apparent with significant reduction ($p<0.001$). The control group had the highest overall mean of 1.1± 0.05g while both combo-treatment groups (8hrs UV + 0mL and 10hrs UV + 0mL) had nearly equal overall means of 0.73± 0.03g and 0.78± 0.06g, respectively. The mean root and shoot dry weights in the 0mL single-stress drought group from Figure 2A (1.24± 0.24g and 0.6± 0.08g, respectively) were not significantly different from the root and shoot dry weights from each combo-treatment group in Figure 3A. (1.75± 0.09g and 1.56± 0.21g for the root dry weights

and 0.73± 0.03g and 0.78± 0.06g respectively for the shoot dry weights). However, the 8hrs UV and 10hrs UV treatment regimens from Figure 2B are quite different from the corresponding values on Figure 3A, perhaps suggesting that UV had a more pronounced effect alone than when combined with drought. However, there is also the likelihood of a time effect between the first and second pilot experiments as evidenced by the large difference in means between the control groups in the two runs. The mean root dry weights for 8hrs UV and 10hrs UV were 0.7± 0.13g and 0.76± 0.09g, respectively and the mean shoot dry weights for each group was 0.55± 0.02g and 0.52± 0.02g, respectively.

In Figure 3B, the effects of the increasing stress regimens on change in plant height over time are shown and were statistically significant for the 8hrs UV + 0mL treatment group ($p=0.003$). These results show the control group and the highest stress level group (10hrs UV + 0mL) had similar growth of 18.2± 1.1cm and 17.6± 2cm, respectively. The lowest growth was observed in the 8hrs UV + 0mL of water regimen with a value of 9.6± 1.5cm. Comparing growth for the two combination-stress treatments from Figure 3B to their corresponding single-stress groups (0ml $H_20$, 8 and 10 hrs UV) from Figures 2C and 2D shows that the change in height for the 8hrs UV + 0mL H2O group (9.6± 1.5cm) was not very different from the growth values for the 0mL H2O single stress group from Figure 2C (11.0± 2.4cm). There is a marked difference, however, in the 10hrs UV + 0mL H2O combo group (17.6±s.e.cm) relative to the 0mL H2O group (11.0± 2.4cm). Again, UV stress alone seemed to have a more pronounced effect than drought combined with UV when Figure 3B is compared to Figure 2D. But the time effect between the first and second pilot experiments must also be acknowledged as a possible source of this observation since the temperatures and light levels are lower in March relative to April. The values for change in height for the two combo stress groups were 9.6± 1.5cm and

17.6± 2cm, respectively, compared to values of 5.4± 0.9cm and 9.9± 1.9cm for each single stress group, respectively.

The significant effects (p=0.054) of these combination-stress regimens on leaf growth are shown in Figure 3C. A clear trend is shown where the control group exhibited no change in leaf size while the 8 and10 hours of UV coupled with 0mL of water regimens showed decreases in leaf length of -1.4± 0.6cm and -2.6± 1cm, respectively. The reason for the absence of growth in the control and the negative growth in both treatments was likely due to leaf #3 having already reached its full size prior to initial measurement and subsequent decay of the mature tissue due to treatment or senescence. If the mean values for change in leaf length from the combination-stress treatments in Figure 3C are compared to the mean values from the corresponding single stress treatments in Figures 2E and 2F it seems that the combined stress had a more dramatic effect on leaf growth. Both combination treatment groups exhibited a loss of leaf tissue while the 0mL H2O single stress group showed minor mean leaf growth of 0.2± 0.2cm and the 8hrs UV and 10 hrs UV groups showed mean leaf growths of 0.1± 0.05cm and 0.67± 0.4cm, respectively.

Based on these pilot experiment results, the drought and UV intensities selected for the primary mapping experiments were 0mL of water per day and 8 hours of UV per day for a treatment period of 3 days. A treatment period of 5 days was also considered, but the risk of killing the plants and not getting any data resulted in selection of a 3 day treatment period.

Summary of check data

The primary mapping experiments described in this paper took place over a period of four months from May, 2007 to August, 2007. The differences in temperature and humidity, among other things, between May and August can be quite dramatic. To determine whether these environmental differences significantly affected the traits being measured in this experiment, a series of 'check plants' were grown alongside the experimental plants in each treatment group. In this case, the 'check plants' were genotype B73 and one member of the experimental population, genotype O-302, and were grown in replicates of four in each treatment group. These check plants were scattered within each treatment group to insure randomness. At the conclusion of the experiments, trait data from the check plants was compiled and multiple comparison ANOVAs were performed that modeled each set of mean trait values, individually, against treatment and time as well as the interaction between treatment and time. When significant differences ($p<0.05$) in mean trait values were detected, a Tukey's HSD test was conducted to categorize the different groups. Significant differences in the traits across different treatments were indicative of sufficient treatment effect. Significant differences in the traits across time suggested that the trait measurements should be standardized from the RILs to the check plants.

For the B73 check plants (ID P-95), treatment effects were significant on mean root dry weight ($p=0.004$) and shoot dry weight ($p<0.001$), but not on change in height or change in leaf length (Table 1). However, time had a significant effect on all four traits in the P-95 check with p values of <0.001, 0.003, 0.001 and <0.001 for root dry weight, shoot dry weight, change in height and change in leaf length, respectively (Table 1). UV seemed to have the significant effect on root dry weight while drought significantly affected shoot dry weight, as indicated by the Tukey groupings of A or B (Table 1). Groupings with the same letter are not significantly

different. The temporal effects on root dry weight, change in height and change in leaf length were all similar, with the month of May being significantly different than June, July and August in each of these three traits. Shoot dry weight, however, was similar in May, July and August and significantly different in June as indicated by the groupings (Table 1).

In the traits root dry weight and change in leaf length, a significant interaction between treatment and time was detected with $p=0.004$ and $p=0.023$, respectively (Table 1.). In these two cases, the table of groupings that resulted from the Tukey's HSD test was large and complex and each trait was therefore given its own 'sub-table' just below the P-95 portion of Table 1 that I have just described. In these sub-tables, groups that share any single letter are not significantly different from each other.

First considering each sub-table in terms of treatment group (columns), the effects of the interaction between treatment and time on root dry weight were isolated to the control and UV groups. The May-control group designated 'A' by Tukey's HSD was significantly different from the July-control group designated 'BC', but not significantly different from June-control or August-control, both designated 'ABC'. The July-UV group designated 'C' was significantly different from the May-UV group 'AB', but not significantly different from the June-UV or August-UV groups, both designated 'BC'. The effects of the interaction between treatment and time on change in leaf length were significant in all but the drought group. The May-control group 'AB' was significantly different from the other three control groups, all designated 'CD'. The May-UV group 'A' was also significantly different from the other three UV groups, all designated 'D'. Finally, the May-UVD group 'ABC' was significantly different from the June-UVD group 'D', but not the July-UVD or August-UVD groups, designated 'BCD'.

Next considering each sub-table in terms of time (rows), the effects of the interaction between treatment and time on root dry weight were isolated to the months of May and July. The May-control group 'A' was significantly different from the May-drought group 'BC', but not the May-UV or May-UVD groups. The July-UV group 'C' was significantly different from the July-drought group 'AB', but not significantly different from the July-control ('BC') or July-UVD ('BC') groups. The effects of the interaction between treatment and time on change in leaf length were significant only in the month of May. The May-UV group 'A' was significantly different from the May-drought group 'BCD' but not the May-control ('AB') or May-UVD ('ABC') groups.

For the O-302 check plant, treatment effects were significant on mean shoot dry weight ($p=0.001$) and change in leaf length ($p=0.049$) but not on root dry weight or change in height (Table 1). Time had a significant effect on shoot dry weight ($p=0.002$) and change in height ($p=0.044$), but not root dry weight or change in leaf length (Table 1). Similar to the P-95 check, drought seemed to significantly effect shoot dry weight in the O-302 check while the other three groups had similar means, as indicated by their Tukey groupings (Table 1). Change in leaf length was significantly effected by UV, while the other three groups were not significantly different under UV treatment (Table 1). No significant interaction between treatment and time was detected for any of the traits for check O-302.

Since O-302 was also a member of the experimental RIL population, it is worth noting that the mean trait values for check plants O-302 in each treatment were quite similar to the corresponding mean trait values for the O-302 RIL in the experimental population with the exception of the change in height over time trait which was higher in the check plant population, although not significantly so (checks = 11.5cm, experimental = 9.5cm).

Check Plant Drought Levels

As described in the Methods section, pot-weights of all the check plants in the control, drought, and UV + drought treatment groups were recorded just prior to treatment initiation and again at treatment termination. The purpose of recording these pot weights was to have a way of assessing the level of drought experienced by the different treatment groups since the well-watered plants in the control group will be heavier than the non-watered plants in the drought treatment groups. In addition, this pot weight data was analyzed using multiple comparison ANOVAs to check for significant effects of treatment, time, and the interaction between treatment and time on pot weight.

As expected, the mean pot weight of check P-95 was significantly effected by both drought treatments as indicated by the Tukey groupings (Table 2). Pot weights for check P-95 were consistent across all four months except for August, which was significantly different from the other three months (Table 2). The effects of the interaction between treatment and time were also significant ($p=0.014$). Considering the sub-table for check P-95 in terms of time (columns), the control group seems to have been the only one significantly affected by time. The May, June and July control groups were all designated 'A' while the August control group was significantly different and designated 'B' (Table 2). Both drought and UVD groups shared at least one letter in each month, indicating they did not differ significantly in their mean pot weights (Table 2). Considering the sub-table for check P-95 in terms of treatment (rows) indicates the expected effects of treatments on pot weight, with both drought treatment groups differing significantly from the control, regardless of the month (Table 2).

As expected, the mean pot weight for check O-302 was also significantly effected by both drought treatments, as indicated by their Tukey groupings (Table 2). Pot weights for May and August were not significantly different from each other and pot weights for June and July were not significantly different from each other, but May and August were significantly different from June and July (Table 2). The effects of the interaction between treatment and time were also significant ($p=0.047$). Considering the sub-table for check O-302 in terms of time (columns), it is again only the control group that seems to have been affected by time, with August significantly differing from the other three months (Table 2). Considering the sub-table for check O-302 in terms of treatment (rows) again indicates the expected effects of treatments on pot weight, with both drought treatment groups differing significantly from the control, regardless of the month (Table 2).

Non-Adjusted QTL Network Results

The first type of analysis conducted on the experimental data was a '1-dimensional genome scan' that simply identifies significant QTLs with single-locus effects. Four measurable traits were used in this QTL experiment: root dry weight, shoot dry weight, change in height over time, and change in leaf length over time.

For root dry weight, the 1-dimensional scan identified 2 QTLs that significantly contributed to this trait (Figure 4). The first QTL detected for this trait, on chromosome 9, was located 101 centi-Morgans (cM) from the "top" of the chromosome and was approximately 4.9 cM +/- in size (Figure 4, Table 3). Intuitively, small marker intervals indicate higher confidence in the exact location of the QTL than large intervals do because the amount of DNA

encompassed by the QTL is smaller. Finally, the QTL at position 101 had a fairly low heritability value of 0.0166. In this context, heritability reflects the amount of variation in the measured trait that can be attributed an individual's genotype. Low heritability indicates that the trait is influenced more by environmental factors than by genetic factors while a high heritability value indicates the opposite.

The next QTL for root dry weight was at position 424.3 on chromosome 10 and had a size of 3.6cM +/- (Figure 4, Table 3). The heritability value calculated for this was 0.0241, indicating that this trait was not highly influenced by genetic factors.

Overall, the amount of variation in root dry weight that could be attributed to genotype was 5%, while the amount of variation attributable to environmental factors was 3%. The amount of variation explainable by genotype/environment interaction was 1%, leaving 'other factors' to account for 89% of the observed variation in this trait.

For shoot dry weight, QTL Network again detected 2 significant QTLs (Figure 4, Table 3). The first QTL was located at position 410.8 on chromosome 4 and had an interval size of 3.3cM +/-, while the second QTL, at position 274.4 on chromosome 10, had an interval size of 1.8cM +/-. These QTLs had fairly low heritability values of 0.0189 and 0.0303, respectively.

Overall, the amount of observed variation in shoot dry weight that could be attributed to genotype was about 6% while the amount attributable to environment was about 7%. The amount of variation attributable to the interaction between genotype and environment was 0.3%, leaving 'other factors' to explain the remaining 86.7%.

For change in height over time, QTL Network detected only one QTL. Located at position 255.2 on chromosome 9, this QTL had an interval size of 13.8cM +/-, and the heritability value calculated for this QTL was 0.02 (Figure 4, Table 3).

Overall, the calculated amount of variation in the change height over time that could be attributed to an individual's genotype was 2%, while the amount attributable to environment was 1%. The variation explainable by genotype/environment interaction was 0.3%, leaving 'other factors' to account for the remaining 94.7%.

Finally, for change in leaf length over time, QTL Network detected 8 QTLs that were significantly associated with this trait. The first QTL, at position 229.6 on chromosome 1, had an interval size of 2.2 cM +/-and a heritability value of 0.0374 (Figure 4, Table 3). On chromosme 2, the QTLs at position 348 and 647.2 had interval sizes of 1.5cM +/- and 7.3cM +/-, respectively (Figure 4, Table 3). The QTL at position 83.8 on chromosome 3 had an interval size of 6.3cM +/- and a heritability of 0.0272 while the QTL at position 147.2 on chromosome 4 was 3.8cM +/- in size with a heritability of 0.0190 (Figure 4, Table 3). The QTL at position 481.2 on chromosome 5 had an interval size of 5.7cM +/- and possessed a heritability value of 0.0251 (Figure 4, Table 3). The last 2 QTLs for change in leaf length over time were on chromosome 9 and were located at positions 399.8 and 535.5 with respective interval sizes of 2.2cM +/-and 8.2cM +/-, and respective heritability values of 0.0626 and 0.0310 (Figure 4, Table 3).

Overall, the amount of variation in change in leaf length over time that could be attributed to genotype was 37%, while the amount attributable to environment was only 3%. The variation explainable by the interaction of genotype with environment was only 1%, leaving the remainder of 59% to be accounted for by 'other factors'. In all the traits discussed, the high percentage of unexplained variance could be due to undetected epistatic relationships between groups of genes, as well as genotype by genotype and genotype by environment interactions. This level of unexplained variation coupled with QTLs that only explain small percentages of

phenotypic variation has been observed in maize, *Drosophila* and mice and is likely due to undetected high-order epistasis between multiple, small effect QTLs (Holland, 2007).

The next type of analysis conducted in QTL Network was the '2-dimensional genome scan' that identified QTLs with single-locus effects as well as QTLs that share an epistatic interaction. This analysis was much more time-consuming than the 1-dimensional scan but revealed several additional epistatic QTLs (Figure 4, Table 3). It should be mentioned that all of the single-locus effect QTLs detected in the 1D scan were also detected in this 2D scan but will not be mentioned again to avoid redundancy.

For root dry weight, one epistatic interaction was detected between the QTLs at position 159 on chromosome 3 and position 426.4 on chromosome 6. (Figure 4, Table 3). These QTLs had interval sizes of 6.2cM +/- and 2.5cM +/-, respectively and the heritability of this epistatic QTL was 0.0101.

For shoot dry weight, an epistatic relationship was identified between the QTL at position 384.4 on chromosome 7 and and the QTL at position 223.1 on chromosome 9. Interval sizes for these QTLs were 1.3cM +/- and 2.1cM +/-, respectively and the heritability of the epistatic interaction was 0.0127 (Figure 4, Table 3).

No epistatic relationships were detected for change in height over time, but 3 epistatic relationships were detected for change in leaf length over time (Figure 4). The first epistatic QTLs detected for this trait were at position 348 on chromosome 2 and position 535.5 on chromosme 9, each possessing a respective interval size of 1.5cM +/- and 8.2cM +/-. The next epistatic relationship was between the QTLs at position 147.2 on chromosme 4 and position 481.2 on chromosome 5 and the interval size for each was 3.8cM +/- and 5.7cM +/-, respectively. The last QTLs that shared an epistatic relationship for change in leaf length over

time were at position 586.3 on chromosome 2 and position 389.1 on chromosome6. These QTLs had approximate interval sizes of 6.0cM +/- and 2.4cM +/-, respectively and the heritability values of these epistatic QTLs were all low, with values of 0.0137, 0.0182, and 0.0113, respectively.

Adjusted QTL Network Results

2 dimensional analysis of the temporally adjusted dataset yielded two single-locus QTLs as well as two epistatic interactions (Table 5, Figure 5).

For shoot dry weight, one single-locus QTL was detected on chromosome 5 at position 514.5 (Table 5, Figure 5). This QTL had an interval size of 3cM+/- and a heritability value of 0.0262. In addition, both epistatic interactions were detected in shoot dry weight. The first was detected between the QTL at position 97.4 on chromosome 2 and the QTL at position 237.8 on chromosome 4 (Table 5, Figure 5). The second epistatic interaction was detected between the QTL at position 244.7 on chromosome 3 and the QTL at position 399.4 on chromosome 3 (Table 5, Figure 5). These epistatic QTLs for shoot dry weight had heritability values of 0.0485 and 0.0198, respectively. In shoot dry weight, the amounts of variation explainable by genotype, environment and the interaction between them were 9%, 0.9% and 0.6%, respectively, leaving other factors to account for the remaining 89%.

For change in height over time, one single-locus QTL was detected on chromosome 9 at position 255.2 (Table 5, Figure 5). This QTL had an interval size of 3.6cM +/- and a heritability value of 0.0240. This QTL was also detected in the analysis of the non-adjusted adjusted data but the interval sizes differed by 10.2 cM. The interval size from the non-adjusted analysis was 13.8cM+/- while the size from the adjusted analysis was 3.6cM+/-. The amounts of variation

explainable by genotype, environment, and the interaction between the two were 2.4%, 2.4% and 0.3%, respectively, leaving other factors to account for the remaining 95%.

Chromoscan Results

After running a SAS (SAS Institute Inc. 100 SAS Campus Drive - Cary, NC 27513-2414 USA) general linear model (GLM) program written by Dr. Jim Blum of the UNCW statistics department on the trait data from the mapping experiments along with the IBM94 marker data, the resulting list of marker associated p-values was compiled into input files to be analyzed using the Chromoscan software described in the Materials and Methods section (Sun, Y.V. et al., 2006). This analysis proved to be much more sensitive than the QTL Network analyses described in the previous section, detecting a total of 123 QTLs across the entire maize genome (Table 5, Figure 6). Due to the large number of QTLs detected, only those that were detected in multiple traits and or treatments will be described in the following paragraphs.

Chromosome 1 had three QTLs detected in multiple traits or treatments. The first one was detected at position 290.1, was found in the drought treatment group and was significant in two traits, root dry weight and change in height (Table 5, Figure 6). A QTL at position 653.4 was significant in two traits and two treatments; root dry weight and shoot dry weight, and UV as well as the combination treatments, respectively (Table 5, Figure 6). A QTL at position 898.7 was detected in two traits in the combination UV + drought treatment group, shoot dry weight and change in height (Table 5, Figure 6).

Chromosome 2, which contained the most QTLs of any other, had 7 QTLs that were significant in either two traits or two treatments. The QTL at position 97.38 was significant in both the UV treatment group and the combination treatment group for the change in height trait

(Table 5, Figure 6). The QTLs at positions 186.67, 321, and 343.35 were all significant in both the root dry weight and change in height traits in the drought treatment group (Table 5, Figure 6). The QTL at position 273.67 was detected in both the drought and the UV treatment groups for the change in height trait. QTLs at positions 378.9 and 645.75 were each detected in two traits (Table 5, Figure 6). The QTL at position 378.9 was found in the combination treatment group for both root dry weight and change in leaf length while the QTL at position 645.75 was found in the drought group for shoot dry weight and change in leaf length (Table 5, Figure 6).

Chromosome 3 had 1 QTL in the UV group that was significant in 2 traits, shoot dry weight and change in height, at position 426.45 as well as 1 QTL for change in leaf length that was significant in both the drought and UV treatment groups at position 596.15 (Table 5, Figure 6).

Chromosome 4 had 2 QTLs in the combination treatment group that were significant for two traits. The first QTL at position 487.7 was found in both root dry weight and shoot dry weight while the second QTL at position 536.3 was found in both root dry weight and change in leaf length (Table 5, Figure 6).

Chromosome 5 had 2 QTLs that were found in two traits and 1 QTL that was found in 2 treatment groups. The QTL at position 23.3 was found in both root dry weight and change in leaf length and the QTL at position 376.4 was found in both root dry weight and shoot dry weight, each of these in the UV treatment group (Table 5, Figure 6). The QTL at position 90.81 was detected in the drought treatment group as well as the combination treatment group (Table 5, Figure 6).

Chromosome 6 had 1 QTL at position 228.88 that was found in two traits, both shoot dry weight and change in height, as well as 2 treatment groups, drought and the combination treatment group (Table 5, Figure 6).

Chromosme 7 had 1 QTL at position 0 that was significant in both root and shoot dry weights as well as the drought and combination treatment groups (Table 5, Figure 6). Also, a QTL at position 494.27 in the combination treatment group was significant for 3 traits, root and shoot dry weight as well as change in height (Table 5, Figure 6).

Chromosome 8 had a QTL at position 342 that was detected in both root and shoot dry weight and a QTL at position 515 that was found in both shoot dry weight and change in leaf length as well as the drought and combination treatment groups (Table 5, Figure 6).

Chromosome 9 had a QTL at position 240.5 that was found in both root dry weight and change in height among the drought treatment group. The QTL at position 361.4 was found among the UV treatment group in both root dry weight and shoot dry weight while the QTL at position 477.2 was found in shoot dry weight among both UV and drought treatment groups (Table 5, Figure 6).

Finally, Chromosome 10 had 1 QTL at position 194.32 that was significant in both root dry weight and change in height among the combination treatment groups (Table 5, Figure 6). For more detail on all of these QTLs, including those not discussed, Figure 6 provides a complete map of all 123 QTLs detected by Chromoscan and Table 5 provides more details about these QTLs that could not fit on the map.

## DISCUSSION

I have identified chromosomal regions that are significantly linked to either drought stress tolerance, UV-B tolerance, or the combined stress in maize. Although prior work has been done in both of the single-stress areas, an analysis of the effects of drought and UV stress combined makes this research novel.

Pilot Experiment Results

The first pilot experiment, conducted in March 2007, had the purpose of determining appropriate levels of drought and UV stress that should be applied singly to experimental plants to elicit a measurable response. The varying drought intensities did not have a significant effect on any of the 4 measured traits although there was a distinct downward trend in change in leaf length over time as drought intensity increased. Similarly, UV stress alone did not evoke significant responses in the plants for the measures traits, although downward trends are evident in root and shoot dry weight as well as change in leaf length as UV stress was increased. The ineffectiveness of the drought and UV stresses in eliciting a response could be attributable to insufficient drought or UV intensity, treatment period, or to the small sample size ($n = 3$) used in the pilot. Also, it is possible that these stresses, applied alone, at these intensities are insufficient to cause a response but the same stresses, applied in combination would be sufficient.

Based on the ineffectiveness of the stress treatments of the first pilot experiment, only the most intense drought treatment (0mL of water per day) and the two most intense UV treatments (8 and 10 hours per day) were chosen for the second pilot experiment, conducted in April 2007. Although no significant effect was detected under these stresses alone, it was thought that their simultaneous application under otherwise identical conditions would be sufficient to have a significant effect on the seedlings. This prediction seems to have been supported by the results

of the second pilot experiment. Both root and shoot dry weights were significantly affected by the simultaneous application of drought and UV stress. Change in leaf length was also significantly impacted by these combination stress treatments as well as change in height. The negative change in leaf growth in the second pilot experiment could be attributable to the combination of drought and UV stress, but also may be attributable to the leaf having already reached maturity at the first measurement and was senescing prior to the second measurement.

Treatment and Temporal Effects on Check Plants

Overall, treatment effects were significant on half of the measured traits in each check plant. Drought significantly effected shoot dry weight in both checks, while UV significantly affected root dry weight in check P-95 and change in leaf length in check O-302. Interestingly, in all cases where there was a significant effect of either single stress on a measured trait, the combination treatment (UVD) was not significantly different from the control group which may suggest a confounding effect between drought and UV. For example, in check P-95 for root dry weight, neither the drought treatment nor the combination treatment (UVD) was significantly different from the control but the UV treatment group was. Shoot dry weight also showed a similar response in both checks but in these cases it was the drought treatment that had the significant effect and both the UV and UVD treatments were not significantly different from the control. Confounding effects between drought and UV have also been observed in soybean on traits related to photosynthesis and growth (Sullivan, 1990). Although the treatments administered did not have a significant effect on all measured traits in both checks, it is still reasonable to expect the treatments to have significant effects on many of the experimental RILs due to transgressive segregation (Rieseberg, 1999 and 2003). This phenomenon, described

earlier, is characterized by offspring from two inbred lined expressing very different, sometimes extreme, phenotypes relative to their parents. Since this RIL population was derived in such a way, adequate genetic variation for the measured traits was still expected within the population.

Overall, temporal effects were significant on all traits for check P-95 and half the traits for check O-302. In check P-95, all three traits except shoot dry weight were significantly different in May while June, July and August had similar trait values. This suggests changes in outside environmental conditions from May into the summer months were adequate to impact trait values in spite of the environmental control systems inside the greenhouse. Oddly, shoot dry weight in check P-95 was different in June than every other month. The pattern in the O-302 check is less clear with shoot dry weight appearing to have been significantly effected by the change in conditions from May into the summer and change in height being significantly different in August than the other three months.

The interaction between treatment and time significantly effected root dry weight and change in leaf length in check P-95. For root dry weight, the control and UV treatment groups were significantly different between the months of May and July but neither month was significantly different from June or August. The pattern was somewhat more clear for change in leaf length with the May control and UV groups being significantly different from the remaining three control groups. The May UVD group was also significantly different from June but not July or August.

The pot weights of the check plants were significantly affected by both treatment and time in these experiments. The effect of treatment was expected due to the stark difference in watering between control and drought treatment groups. The effect of time, however, was somewhat unexpected but confined to the control groups in both check plants. When the

interaction between treatment and time was considered for check P-95, the August control group pot weights were significantly different from the other three control groups. A similar but less abrupt difference was observed in check O-302. The August control group pot weights were significantly different from both May and June but not from July, which was not significantly different from either May or June.

While some of the patterns were unclear, the fact that time had a significant impact on the measured traits in this experiment warranted adjustment of the trait data to standardize for time effects. This standardization was carried out by dividing the trait values from each experiment by the median of the corresponding check P-95 trait values. Consequently, the QTL Network analysis discussed in the next section was conducted on both the original and the adjusted datasets. Check P-95 was chosen because it was a B73 parental genotype and could be expected to have a milder phenotype relative to the experimental RILs and would therefore be less susceptible to temporal effects.

2-Dimensional Genome Scan QTLs from Non-Adjusted Data

The 2-dimensional genome scan conducted using QTL Network detected a total of 19 significant QTLs and 5 epistatic interactions. Most of the QTLs detected, 10 out of 19, and 3 of the 5 epistatic interactions, were for change in leaf length. Chromosome 2 contains 3 significant QTLs, all for change in leaf length, and 2 of these are involved in epistatic interactions, indicating that chromosome 2 contains several regions that participate in leaf development under stressful conditions. Chromosome 9 also contains 2 QTLs for change in leaf length that are in relatively close proximity (~135cM apart). The large proportion of change in leaf length QTLs detected may indicate the sensitivity of this trait, in seedlings, to UV and drought stress. It is

also possible that the inherent variability of this trait in 10-day old seedlings induced additional variation to what would be induced by the stress treatments alone.

Root dry weight and shoot dry weight were similar in that each trait had 4 significant QTLs, 2 of which were involved in an epistatic interaction. On chromosome 3, the QTL at position 159 for root dry weight, which is involved in an epistatic interaction with the QTL at position 426.4 on chromosome 6, is of note because it was the only QTL detected by QTL Network that was significant under a particular stress treatment in addition to being significant overall. The QTL at position 159 was significant under drought stress, suggesting that this region participated in root development under drought stress conditions.

One QTL was detected for change in height on chromosome 9 at position 255.2. Chromosome 9 also seemed to be the most active in terms of number of QTLs detected, with 5 total QTLs and at least one for each trait represented.

As previously mentioned, the heritability values of all the QTLs detected using this analysis technique were quite low, indicating that environmental factors played a more important role in inducing the expression of these traits than genetic factors did. In addition, the percentage of variability in all traits, except change in leaf length, attributable to genotype, environment, or interaction between genotype and environment, was very low. In root dry weight, shoot dry weight, and change in height, the percentage of variability attributable to other factors was 0.90, 0.86, and 0.94, respectively. Change in leaf length was better but still high with 58% of variability attributable to other factors. High unexplained variation in these traits could be attributable to undetected epistasis between groups of genes or interactions between genotype and environment (Rieseberg, 1999 and 2003). In addition, the 5 epistatic interactions that were

detected in the QTL Network analysis are indicative of the complex nature of quantitative traits like stress tolerance.

2-Dimensional Genome Scan QTLs from Adjusted Data

The number of QTLs detected by 2-D analysis of the temporally adjusted dataset yielded far fewer QTLs than the non-adjusted dataset. The analysis of the non-adjusted data yielded a total of 19 QTLs while analysis of the adjusted data yielded only 6 QTLs. This suggests that temporal effects had quite an impact on the measured traits across the course of the four experiments. QTLs that are temporally unstable have also been observed in studies conducted on Chestnut trees (Casasoli et. al, 2004). While experiments conducted outside over the course of three years may expect temporally variable QTLs it was thought that the experiments described in this paper, conducted inside of a greenhouse over the course of four months, would not be as susceptible to temporal effects. The apparent temporal instability of many of the QTLs detected in the two datasets indicates indoor greenhouse conditions were still significantly affected by the outside conditions. While parameters like temperature and, to a lesser degree, humidity can be controlled fairly well by greenhouse equipment while other factors like day length and light levels are less easily controlled. Retractable shade cloths that can filter or block light are commercially available but not present in Kresge greenhouse at UNCW where these experiments were conducted. However, one QTL was detected in the analyses of both datasets which suggests some degree of temporal stability. This QTL was for change in height and was located on chromosome 9 at position 255.2. Also, the interval size for this QTL in the adjusted dataset was 10.2cM smaller than the same QTL from the non-adjusted dataset, suggesting that this QTL was still affected by environmental changes over the course of these experiments.

Adjustment of the data for temporal effects resulted in detection of a smaller marker interval which increased confidence in the precise location of the QTL.

Chromoscan QTLs

The Chromoscan analysis of the marker regions associated with the raw p-values produced by the SAS general linear model was much more sensitive than the QTL Network analysis and resulted in the detection of 123 significant QTLs across the maize genome (Figure 5, Table 5a). The distribution of these QTLs across the 10 chromosomes was much more even than in the QTL Network results, although chromosomes 2 and 9 were densely packed with QTLs relative to the remainder of the genome. Also, many of the Chromoscan analysis QTLs exhibited quite a bit of overlap between treatments as well as traits, with some QTLs overlapping entirely.

Across the genome, 44 QTLs associated with the drought treatment were detected, 38 of them associated with drought alone, 3 with drought and UV alone, and 3 with drought in addition to the combination treatment. QTLs detected under both drought and UV stress alone (chromosome 2 position 273.67, chromosome 3 position 596.15, and chromosome 9 position 473.2) but not under the combination treatment may suggest a masking effect of the response to one stress over the other when the stresses are applied simultaneously. Alternately, QTLs detected under both drought and combination treatments (chromosome 5 position 90.81, chromosome 6 position 228.88, and chromosome 8 position 515) may suggest the lack of any masking effect of the UV response over the drought response when the stresses are applied together. These drought QTLs were also detected in a variety of traits and 9 of them in a

combination of traits. Those QTLs detected in more than one trait suggest that stress tolerance is being expressed in multiple physiological ways (leaf growth, biomass, etc) (Gao et al., 2007).

Forty-three QTLs associated with the UV treatment were detected in the Chromoscan analysis. Three of these QTLs were detected under both UV stress and the combination treatment (chromosome 1 position 653.4, chromosome 2 position 97.38, and chromosome 7 position 0). QTLs detected under both UV stress alone and the combination treatment indicate that drought provides no shielding effect over the UV response when both stresses are applied simultaneously. These UV associated QTLs were also detected in a variety of traits and in 6 cases, in a combination of 2 traits.

36 QTLs associated with the combination drought + UV treatment group were detected by the Chromoscan analysis. Again these QTLs were detected in a variety of traits and in 7 cases, in a combination of traits. Notably, the QTL at position 494.27 on chromosome 7 was detected in 3 traits (root Dwt, shoot Dwt, and change in height) under the combination drought + UV treatment.

Overall, the large number of QTLs detected using this analysis coupled with their apparently random distribution across the genome among the various traits and treatments suggest that the complexity of these stress resistance traits in maize is high (Cushman and Bonhert, 2000). Quantitative traits are inherently complex and rarely controlled by one or two discreet chromosomal regions which limits our ability exploit their true potential in terms of improving agriculture (Sinha, 2006). Additionally, the fact that most QTLs detected were related to either single stress (drought or UV) rather than to the combination of drought and UV together suggests the maize genome possesses significant genomic versatility or plasticity to adapt to singular environmental factors. Similar genomic plasticity has also been observed in wheat

which has also maintained genomic diversity in spite of heavy domestication influence (Dubcovsky et al., 2007). If stress tolerance QTLs were lumped together in large groups, the ability of the organism to respond to one single factor would not be there and many unnecessary genes may be turned on.

Comparison of QTL Network and Chromoscan analyses

There were clear differences between the 2 analyses techniques that were utilized in this experiment. QTL Network was less sensitive than Chromoscan, detecting only 19 QTLs upon analysis of the unadjusted data and only 6 QTLs upon analysis of the adjusted data compared to the 123 detected by Chromocscan. However, QTL Network provided more detail on the QTLs it detected than did Chromoscan. QTL Network calculated variance components for each measured trait in addition to calculating the relative effect of various treatments on detected QTLs. In all traits, the majority of the variance was largely attributable to error, which in QTL studies, usually indicates undetected epistasis between groups of genes as well as between groups of genes and their environment (Rieseberg et al., 1999 and Rieseberg et al., 2003). However, the 2 most useful features of QTL Network were its ability to detect epistasis between loci and the calculation of heritability values for detected QTLs, which indicate whether a trait is more influenced by genotype or environment. Heritability values of all QTLs were quite low, indicating that environmental factors play a larger role in influencing the measure traits than does genotype.

Chromoscan analysis required significantly less time than QTL Network analysis (minutes compared to weeks), and provided a higher level of sensitivity for detecting QTLs. Chromoscan analysis also allowed for detection of overlapping QTLs among different treatments

and traits. As previously mentioned, 3 sets of 3 QTLs were detected in multiple treatments (Table 5a), suggesting presence or absence of masking or confounding effects. Although the probability of detecting 3 completely overlapping QTLs by random chance was fairly high, 0.26 (Communication from Susan Simmons, 2008), a relationship between them may have been detected.

In spite of the differences between the two methods, 3 QTLs were detected in extremely close proximity to each other (less than 5cM) in both analyses. On chromosome 2, QTLs at position 348 and 647.2 in the unadjusted QTL Network results are within QTLs at position 345.2 and 645.75 in the Chromoscan results. In addition, the QTL at position 535.5 on chromosome 9 in the unadjusted QTL Network results is within the QTL at position 535.95 on chromosome 9 in the Chromoscan results.

When comparing the temporally adjusted QTL Network results with the Chromoscan results, 2 QTLs were detected within 3cM of each other. On chromosome 2, the QTL at position 97.4 in the adjusted QTL Network results was within the Chromoscan QTL at position 97.38. On chromosome 3, the QTL at position 399.4 from the QTL Network adjusted results was within the Chromoscan QTL at position 394.8.

Detection of QTLs is such close proximity by 2 differing analysis techniques increases the confidence that these QTLs are playing an important role in stress response. In future studies focused on drought and UV resistance, these QTLs that were detected in both analyses would be a logical place to begin investigating. In addition, the QTL on chromosome 9 that was detected in both the adjusted and unadjusted QTL Network analyses may be an interesting place to study due to its stability in detection across variable time periods.

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Appendix. Tables and Figures:

| | Check P-95 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Effect: | | | | | Time Effect | | | | | Treatment x Time Interaction Effect |
| | p-value | Control | Drought | UV | UVD | p-value | May | June | July | August | p-value |
| mean root Dwt | <0.004 | A | A | B | A | <0.001 | B | A | A | A | 0.004* |
| mean shoot Dwt | 0.001 | A | B | A | A | 0.003 | A | B | A | A | 0.803 |
| mean Δ height | 0.297 | | | | | 0.001 | B | A | A | A | 0.461 |
| mean Δ leaf length | 0.126 | | | | | <0.001 | B | A | A | A | 0.023* |

| Root Dwt | Treatment x Time Interaction Effects | | | |
|---|---|---|---|---|
| | Control | Drought | UV | UVD |
| May | A | BC | AB | AB |
| June | ABC | ABC | BC | BC |
| July | BC | AB | C | BC |
| August | ABC | BC | BC | ABC |

| Δ Leaf Length | Treatment x Time Interaction Effect | | | |
|---|---|---|---|---|
| | Control | Drought | UV | UVD |
| May | AB | BCD | A | ABC |
| June | CD | D | D | D |
| July | CD | CD | D | BCD |
| August | CD | D | D | BCD |

| | Check O-302 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Effect: | | | | | Time Effect | | | | | Treatment x Time Interaction Effect |
| | p-value | Control | Drought | UV | UVD | p-value | May | June | July | August | p-value |
| mean root Dwt | 0.485 | | | | | 0.281 | | | | | 0.422 |
| mean shoot Dwt | 0.001 | A | B | A | A | 0.002 | B | A | A | A | 0.069 |
| mean Δ height | 0.527 | | | | | 0.044 | A | A | A | B | 0.941 |
| mean Δ leaf length | 0.049 | A | A | B | A | 0.815 | | | | | 0.287 |

***Table 1.*** P-values associated with treatment, time and treatment x time interaction effects on mean trait values in each check plant. Significant p-values ($p<0.05$) required a Tukey's HSD test, the groupings of which are shown to the right of significant p-values. Groups with significantly different mean trait values are assigned different Tukey groupings (e.g. A or B). The two small tables below the check P-95 main table are the results of the Tukey's HSD test on the two traits that were significantly affected by treatment x time interaction. Any groups that share a single Tukey grouping letter are not significantly different.

| | Check P-95 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Effect: | | | | Time Effect | | | | | Treatment x Time Interaction Effect |
| | p-value | Control | Drought | UVD | p-value | May | June | July | August | p-value |
| mean pot weight | < 0.001 | A | B | B | < 0.001 | A | A | A | B | 0.014* |

| Check P-95 | Treatment x Time Interaction Effect | | |
|---|---|---|---|
| Pot Weight | Control | Drought | UVD |
| May | A | CD | CD |
| June | A | CD | C |
| July | A | CD | CD |
| August | B | D | D |

| | Check O-302 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Effect: | | | | Time Effect | | | | | Treatment x Time Interaction Effect |
| | p-value | Control | Drought | UVD | p-value | May | June | July | August | p-value |
| mean pot weight | < 0.001 | A | B | B | 0.0026 | A | B | B | A | 0.047* |

| Check O-302 | Treatment x Time Interaction Effect | | |
|---|---|---|---|
| Pot Weight | Control | Drought | UVD |
| May | A | C | C |
| June | A | C | C |
| July | AB | C | C |
| August | B | C | C |

***Table 2.*** P-values associated with treatment, time and treatment x time interaction effects on mean trait values in each check plant. Significant p-values ($p<0.05$) required a Tukey's HSD test, the groupings of which are shown to the right of significant p-values. Groups with significantly different mean trait values are assigned different Tukey groupings (e.g. A or B). Significant treatment x time interaction was detected in both checks and the resultant Tukey groupings are shown below the main table for each check. Any groups that share a single Tukey grouping letter are not significantly different.

| Chromosome | QTL position | Marker Begin | Marker End | Interval Size | Trait | Heritability |
|---|---|---|---|---|---|---|
| 1 | 229.6 | AY110052 | GPM559 | 2.2 | Δ Leaf Length | 0.0374 |
| 2 | 348 | GPM653A | IDP723 | 1.5 | Δ Leaf Length | 0.0582 |
| 2 | 586.3 | UMC1516 | IDP1450 | 6 | Δ Leaf Length | 0.0133 |
| 2 | 647.2 | GPM466B | BNLG469B | 7.3 | Δ Leaf Length | 0.0663 |
| 3 | 83.8 | GPM783 | GPM854 | 6.3 | Δ Leaf Length | 0.0272 |
| 3 | 159 | UMC1030 | GPM810A | 6.2 | Root Dwt | 0.0101 |
| 4 | 147.2 | ADH2 | IDP2387 | 3.8 | Δ Leaf Length | 0.019 |
| 4 | 410.8 | MMP147 | UMC2038 | 3.3 | Shoot Dwt | 0.0189 |
| 5 | 481.2 | IDP163 | NFD104A | 5.7 | Δ Leaf Length | 0.0251 |
| 6 | 389.1 | GPM795 | UMC1859 | 1.4 | Δ Leaf Length | 0.0113 |
| 6 | 426.4 | AY105728 | AY105785 | 2.5 | Root Dwt | 0.0101 |
| 7 | 384.4 | UMC2329 | UMC1112 | 1.3 | Shoot Dwt | 0.0127 |
| 9 | 101 | UMC1588 | UMC1967 | 4.9 | Root Dwt | 0.0166 |
| 9 | 223.1 | UFG71 | GPM94 | 2.1 | Shoot Dwt | 0.0127 |
| 9 | 255.2 | MMP2 | UMC2340 | 13.8 | Δ Height | 0.0201 |
| 9 | 399.8 | UMC2341 | CSU634 | 2.2 | Δ Leaf Length | 0.0626 |
| 9 | 535.5 | GPM379C | AY109819 | 8.2 | Δ Leaf Length | 0.031 |

***Table 3.*** A list of QTLs detected using the QTL Network software as represented in Figure 4, with additional details. QTL pairs highlighted with the same colors indicate results from 2-D analysis and epistasis. These QTLs are from the non-adjusted data.

| Chromosome | QTL position | Marker Begin | Marker End | Interval Size | Trait | Heritability |
|---|---|---|---|---|---|---|
| 2 | 97.4 | GPM398C | IDP2388 | 6 | Shoot Dwt | 0.0485 |
| 3 | 244.7 | AY110297 | GPM848A | 1.6 | Shoot Dwt | 0.0198 |
| 3 | 399.4 | UMC1730 | UMC1027 | 6.4 | Shoot Dwt | 0.0198 |
| 4 | 237.8 | IDP2588 | SDG108A | 12.4 | Shoot Dwt | 0.0485 |
| 5 | 514.5 | GPM893 | IDP36 | 3 | Shoot Dwt | 0.0262 |
| 9 | 255.2 | MMP2 | UMC2340 | 3.6 | Δ Height | 0.024 |

***Table 4.*** A list of QTLs detected using QTL Network software as represented in Figure 5, with additional details. QTL pairs highlighted in the same colors indicate epistasis between loci. These QTLs are from the temporally adjusted data.

| Chromosome | QTL position | Marker Begin | Marker End | Interval Size (cM) | p-value | Trait(s) | Treatment(s) |
|---|---|---|---|---|---|---|---|
| 1 | 8.99 | gpm113b | gpm27 | 2.19 | 0.004544741 | Δ Height | UV + Drought |
| 1 | 31.1 | AY110314 | mmp49 | 26.7 | 0.009677851 | Δ Leaf length | UV |
| 1 | 82.2 | bnlg1014 | IDP847 | 20.4 | 1.59334E-05 | Δ Leaf length | UV + Drought |
| 1 | 151.6 | lim504 | bnlg1953 | 18.4 | 0.001322834 | Δ Height | UV |
| 1 | 290.1 | bnlg1866 | IDP1489 | 41.86 | 2.56E-05 | Root Dwt and Δ Height | Drought |
| 1 | 348.94 | IDP182 | gpm933 | 19.28 | 0.008187316 | Δ Leaf length | Drought |
| 1 | 369.2 | ufg13b | umc2228 | 22.6 | 2.99577E-05 | Root Dwt | UV + Drought |
| 1 | 451.01 | gpm519 | IDP386 | 35.99 | 1.81493E-07 | Root Dwt | UV + Drought |
| 1 | 508.18 | IDP741 | asg58 | 17.52 | 2.43E-04 | Δ Height | UV |
| 1 | 518.9 | AY110566 | umc2234 | 10.1 | 0.006079494 | Δ Leaf length | Drought |
| 1 | 548.3 | bnlg1057 | umc2235 | 1.7 | 0.003087219 | Root Dwt | UV |
| 1 | 587 | IDP438 | php20644 | 2.6 | 0.006283403 | Δ Leaf length | Drought |
| 1 | 653.4 | umc1358 | umc23a | 16.8 | 0.008452633 | Root Dwt and Shoot Dwt | UV and UV + Drought |
| 1 | 656.7 | AY111834 | gpm420b | 4.62 | 0.00976796 | Shoot Dwt | Drought |
| 1 | 898.7 | umc2149 | IDP527 | 3.15 | 0.004672744 | Shoot Dwt and Δ Height | UV + Drought |
| 1 | 930.5 | lim78 | IDP2450 | 29.23 | 0.000651247 | Shoot Dwt | Drought |
| 1 | 1010.2 | IDP3856 | ufg61 | 60.84 | 1.16952E-07 | Shoot Dwt | UV |
| 2 | 47.4 | IDP1702 | gpm156a | 4.11 | 0.007107806 | Δ Leaf length | UV + Drought |
| 2 | 55.6 | npi254a | umc1261a | 99 | 1.33227E-15 | Δ Height | UV + Drought |
| 2 | 93.3 | mmc0111 | eks1 | 29.1 | 0.004187013 | Δ Leaf length | Drought |
| 2 | 97.38 | gpm575a | umc6a | 89.29 | 2.13148E-09 | Δ Height | UV and UV + Drought |
| 2 | 141.6 | gpm786 | umc1261a | 13 | 0.005186871 | Shoot Dwt | UV + Drought |
| 2 | 172.39 | IDP3802 | gpm651b | 16.88 | 0.008174411 | Δ Leaf length | UV |
| 2 | 186.67 | gpm328b | gpm268a | 69.93 | 1.0105E-11 | Root Dwt and Δ Height | Drought |
| 2 | 197.15 | IDP668 | IDP1453 | 53.95 | 4.90726E-09 | Δ Leaf length | UV |
| 2 | 262.6 | AY110266 | mmp122 | 13 | 0.000898491 | Shoot Dwt | UV + Drought |
| 2 | 273.67 | hag103a | umc2251 | 22.13 | 2.45671E-08 | Δ Height | Drought and UV |
| 2 | 319.3 | bnlg121 | umc1454 | 20 | 0.00281937 | Shoot Dwt | UV + Drought |
| 2 | 321 | php10012 | AY107012 | 25.47 | 0 | Root Dwt and Δ Height | Drought |
| 2 | 343.35 | IDP488 | gpm835c | 8.55 | 3.33067E-16 | Root Dwt and Δ Height | Drought |
| 2 | 345.2 | umc1922 | gpm91 | 16.04 | 4.40248E-12 | Δ Leaf length | UV + Drought |
| 2 | 378.9 | agp1 | gpm482 | 7.2 | 0.002607059 | Root Dwt and Δ Leaf length | UV + Drought |
| 2 | 401.5 | umc1108 | gpm89b | 24.47 | 0.002769956 | Δ Leaf length | UV |
| 2 | 459.18 | gpm565a | IDP3864 | 23.02 | 3.91628E-05 | Δ Height | UV |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 466.08 | IDP3824 | IDP3864 | 16.12 | 0.001268924 | Δ Leaf length | UV + Drought |
| 2 | 515.8 | AI668346 | gpm922f | 77.4 | 6.66134E-16 | Root Dwt | Drought |
| 2 | 577.6 | bnlg1940 | IDP143 | 15.66 | 8.66221E-05 | Root Dwt | Drought |
| 2 | 645.75 | gpm383c | AY106674 | 79.56 | 7.71827E-13 | Shoot Dwt and Δ Leaf length | Drought |
| 2 | 654.8 | bnlg1893 | gpm281a | 7.6 | 0.009993029 | Δ Height | UV |
| 3 | 0 | gpm788b | bnl8.15 | 10.8 | 0.000731498 | Shoot Dwt | UV |
| 3 | 7.55 | gpm257s | umc1780 | 3.65 | 0.008120457 | Δ Leaf length | Drought |
| 3 | 33.63 | IDP3785 | IDP3906 | 38.44 | 0.000200014 | Shoot Dwt | Drought |
| 3 | 72.07 | IDP3906 | gpm423a | 76.27 | 0.009316217 | Δ Leaf length | Drought |
| 3 | 124.8 | lim66 | gpm810a | 35.3 | 0.001310934 | Δ Height | Drought |
| 3 | 318.2 | mmc0022 | gpm789a | 32.76 | 2.10034E-07 | Shoot Dwt | UV + Drought |
| 3 | 394.8 | umc1311 | IDP854 | 30.37 | 0.001432634 | Δ Leaf length | Drought |
| 3 | 426.45 | gpm799 | gpm753d | 39.11 | 0.000179224 | Shoot Dwt and Δ Height | UV |
| 3 | 447.3 | IDP434 | gpm588a | 13.85 | 9.06018E-08 | Δ Leaf length | Drought |
| 3 | 485.2 | gpm34b | AI770795 | 27.5 | 1.32169E-06 | Shoot Dwt | UV |
| 3 | 491.41 | gpm211 | AY109828 | 75.09 | 3.33067E-16 | Δ Height | UV + Drought |
| 3 | 596.15 | IDP3846 | gpm173 | 22.49 | 0.006340892 | Δ Leaf length | Drought and UV |
| 4 | 250.78 | gpm888 | umc2282 | 4.12 | 0.008223722 | Δ Height | Drought |
| 4 | 279.79 | chr112a | jpsb67 | 12.61 | 0.008304637 | Δ Height | UV |
| 4 | 397.37 | gsy289b | bnlg2244 | 69.73 | 0 | Root Dwt | UV |
| 4 | 437.5 | asg33 | ssu1 | 84.6 | 2.22045E-16 | Root Dwt | UV + Drought |
| 4 | 487.7 | AY112127 | umc15a | 38.1 | 4.15538E-10 | Root Dwt and Shoot Dwt | UV + Drought |
| 4 | 536.3 | AY105971 | gpm611 | 52.39 | 4.32987E-15 | Root Dwt and Δ Leaf length | UV + Drought |
| 4 | 581.8 | AY111962 | umc1803 | 20.4 | 4.77396E-14 | Δ Leaf length | UV + Drought |
| 4 | 621.36 | mbd116 | umc1532 | 50.54 | 1.05025E-06 | Shoot Dwt | UV |
| 5 | 23.3 | IDP1658 | umc1423 | 6.7 | 0.008488428 | Root Dwt and Δ Leaf length | UV |
| 5 | 90.81 | IDP2588 | gpm394b | 43.61 | 0.000257253 | Δ Height | Drought and UV + Drought |
| 5 | 226.76 | gpm261 | mmp180 | 3.14 | 0.001417364 | Δ Leaf length | UV + Drought |
| 5 | 257.81 | gpm564 | IDP101 | 28.89 | 2.81641E-12 | Δ Height | Drought |
| 5 | 264.36 | gpm753h | isu61e | 24.34 | 1.02257E-09 | Root Dwt | UV |
| 5 | 296.84 | gpm177 | rz476b | 34.96 | 9.99201E-16 | Root Dwt | UV |
| 5 | 318.9 | umc1990 | IDP285 | 27.6 | 1.0536E-12 | Root Dwt | UV |
| 5 | 323.1 | bnlg1208 | IDP41 | 14.88 | 0.000915781 | Δ Height | Drought |
| 5 | 324.3 | lim4 | IDP1467 | 23.04 | 0.000247863 | Shoot Dwt | Drought |
| 5 | 376.4 | incw1 | bnl5.71a | 10.6 | 0.006040539 | Root Dwt and Shoot Dwt | UV |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | 470.1 | AY109938 | gpm363b | 42.14 | 3.99203E-05 | Δ Leaf length | Drought |
| 5 | 494.2 | php20531 | mmp169 | 22.1 | 0.000266626 | Shoot Dwt | UV + Drought |
| 6 | 75.13 | IDP360 | mmp76 | 28.67 | 9.99201E-16 | Δ Height | UV + Drought |
| 6 | 90.29 | gpm320a | gpm703 | 22.09 | 9.9016E-05 | Shoot Dwt | UV |
| 6 | 96 | uck1 | IDP301 | 25.12 | 1.11022E-16 | Δ Height | UV + Drought |
| 6 | 112.45 | IDP1430 | IDP350 | 9.56 | 8.98507E-07 | Δ Height | UV + Drought |
| 6 | 113.03 | gpm468a | rz242a | 14.77 | 1.30691E-05 | Shoot Dwt | UV |
| 6 | 139.24 | sdg102c | sbp3 | 14.46 | 0.000101503 | Shoot Dwt | UV |
| 6 | 228.88 | umc2318 | ufg11 | 19.22 | 2.59195E-06 | Shoot Dwt and Δ Height | Drought and UV + Drought |
| 6 | 362 | uaz400 | IDP1427 | 16.4 | 0.008143458 | Δ Leaf length | UV |
| 6 | 420.4 | lim379 | IDP2001 | 12.85 | 0.007608317 | Shoot Dwt | UV + Drought |
| 6 | 433.25 | IDP2001 | mmp50 | 15.25 | 0.006335636 | Δ Leaf length | UV + Drought |
| 6 | 541.53 | chr118 | cdo345c | 4.27 | 0.009076157 | Shoot Dwt | Drought |
| 7 | 0 | umc2177 | bnlg2132 | 52.4 | 3.73528E-07 | Root Dwt and Shoot Dwt | UV and UV + Drought |
| 7 | 255.92 | IDP2411 | gpm100a | 41.46 | 7.31342E-07 | Δ Height | Drought |
| 7 | 277.31 | gpm793 | mmp127 | 12.89 | 0.004342393 | Δ Leaf length | UV |
| 7 | 291.78 | gpm30b | bnl15.21 | 18.12 | 0.000153207 | Root Dwt | UV |
| 7 | 393.1 | gpm392 | asg32 | 23.4 | 0.002376166 | Root Dwt | Drought |
| 7 | 494.27 | gpm257p | mmp67 | 46.53 | 0.000506315 | Root Dwt and Shoot Dwt and Δ Height | UV + Drought |
| 8 | 46.7 | IDP3877 | IDP235 | 48.99 | 0.006355039 | Δ Height | UV |
| 8 | 55.05 | IDP397 | gpm152 | 77.36 | 0.009313293 | Δ Leaf length | UV + Drought |
| 8 | 128.62 | gpm534 | gpm926e | 35.35 | 2.59704E-07 | Δ Height | UV |
| 8 | 191 | mmp120 | npi260b | 20 | 1.89123E-08 | Δ Leaf length | UV + Drought |
| 8 | 194.1 | mmp72 | gpm746c | 89.73 | 1.20881E-12 | Root Dwt | UV + Drought |
| 8 | 327.31 | sdg105a | gpm674b | 19.45 | 0.001625826 | Δ Height | UV |
| 8 | 342 | AY104566 | gpm116a | 15.94 | 0.001441038 | Root Dwt and Shoot Dwt | UV + Drought |
| 8 | 515 | mmp64 | AY110053 | 35.4 | 2.40013E-06 | Shoot Dwt and Δ Leaf length | Drought and UV + Drought |
| 8 | 564.3 | npi112b | AY109853 | 67.7 | 4.5505E-11 | Δ Leaf length | Drought |
| 8 | 608.1 | umc1663 | bnlg1131 | 20.1 | 0.007014806 | Root Dwt | UV |
| 9 | 21.3 | bnlg2122 | IDP760 | 24.9 | 2.31382E-07 | Δ Leaf length | Drought |
| 9 | 55.59 | gpm493b | umc1967 | 28.71 | 0.000447315 | Shoot Dwt | Drought |
| 9 | 199.7 | umc1586 | mmp2 | 35.8 | 3.141E-11 | Δ Leaf length | UV |
| 9 | 204.4 | ufg71 | umc1191 | 28.4 | 0 | Δ Height | Drought |
| 9 | 238.4 | IDP717 | umc1921 | 11.2 | 0.006034757 | Shoot Dwt | UV + Drought |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9 | 240.5 | umc1271 | gpm68 | 14.79 | 0.002016515 | Root Dwt and Δ Height | Drought |
| 9 | 290.1 | gta101c | isu41a | 14.8 | 0.009381652 | Shoot Dwt | UV + Drought |
| 9 | 314.3 | mmp37 | IDP1640 | 30.5 | 0.009503592 | Δ Leaf length | Drought |
| 9 | 320.6 | gpm901 | mmp41 | 23.1 | 8.72873E-05 | Root Dwt | Drought |
| 9 | 361.4 | ufg67 | npi427a | 25.4 | 7.14198E-05 | Shoot Dwt and Δ Leaf length | UV |
| 9 | 374.44 | IDP708 | IDP192 | 25.58 | 0.003470976 | Root Dwt and Shoot Dwt | Drought |
| 9 | 477.2 | ufg75c | umc1675 | 64.2 | 4.40924E-09 | Shoot Dwt | Drought and UV |
| 9 | 535.95 | gpm705b | umc1675 | 5.45 | 0.009448417 | Shoot Dwt | UV |
| 9 | 556.5 | dupssr29 | gpm557a | 35.58 | 1.65566E-06 | Shoot Dwt | UV |
| 9 | 636.2 | mmp53 | IDP103 | 36.6 | 0.000887902 | Root Dwt | UV + Drought |
| 9 | 637.1 | AI901738 | IDP258 | 59.2 | 0.000912173 | Shoot Dwt | UV |
| 10 | 140.97 | IDP2434 | phi059 | 2.53 | 0.004963331 | Δ Height | UV |
| 10 | 155.9 | IDP1458 | gpm391 | 17.83 | 6.75685E-06 | Δ Leaf length | Drought |
| 10 | 194.32 | gpm777 | bnlg1079 | 18.78 | 0.000317623 | Root Dwt and Δ Height | UV + Drought |
| 10 | 245.9 | IDP1429 | AY109876 | 13.5 | 1.71282E-05 | Shoot Dwt | UV + Drought |
| 10 | 247.32 | gpm468b | AY110514 | 7.18 | 0.003720567 | Δ Height | UV |
| 10 | 253.5 | umc1077 | mzetc34 | 14.6 | 0.008376062 | Δ Leaf length | Drought |
| 10 | 299.8 | IDP3961 | IDP1682 | 3.53 | 0.007662632 | Δ Leaf length | UV |
| 10 | 328.98 | gpm763 | IDP263 | 70.57 | 1.33727E-11 | Δ Leaf length | UV |
| 10 | 381.48 | IDP377 | umc1084 | 64.22 | 2.52518E-09 | Root Dwt | Drought |

***Table 5.*** A list of QTLs detected using Chromoscan analysis as represented in Figure 5 with additional details. Traits highlighted in yellow indicate QTLs that were detected in multiple traits. Treatments highlighted in various colors represent QTLs detected under the multiple treatment conditions indicated.

Figure 1A

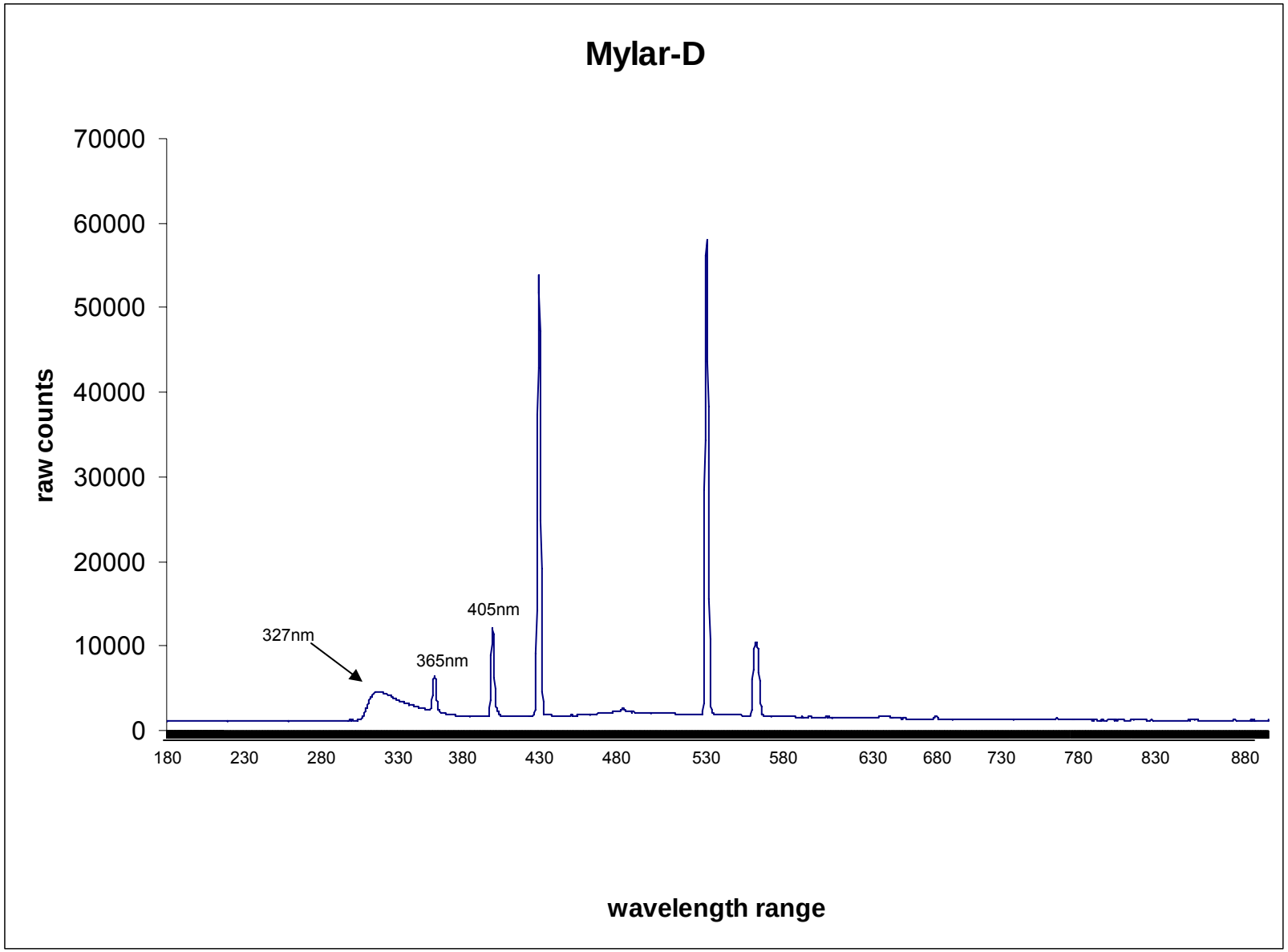

Mylar-D
70000
60000
50000
40000
30000
20000
10000
0
raw counts
327nm
365nm
405nm
180
230
280
330
380
430
480
530
580
630
680
730
780
830
880
wavelength range

Figure 1B

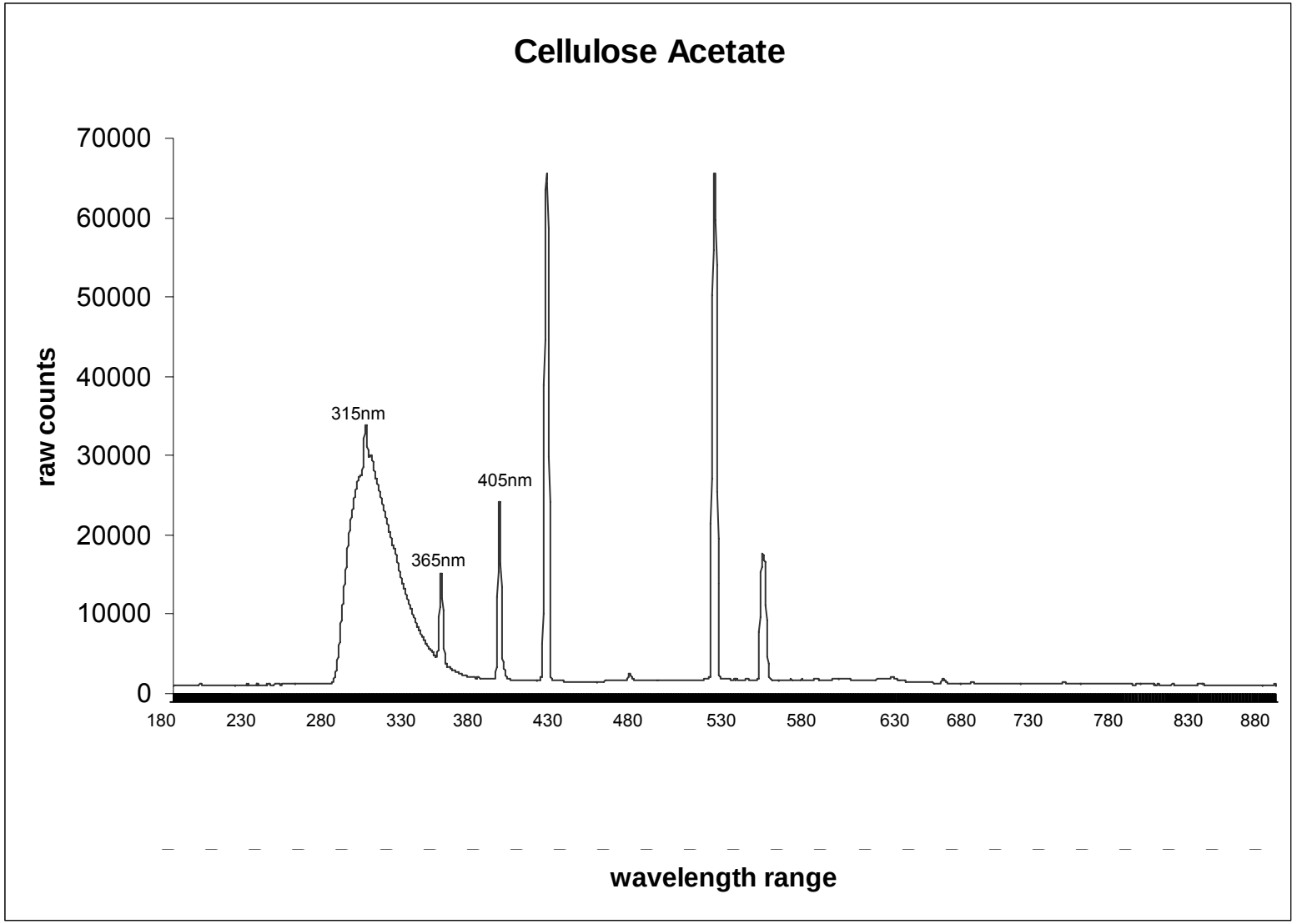
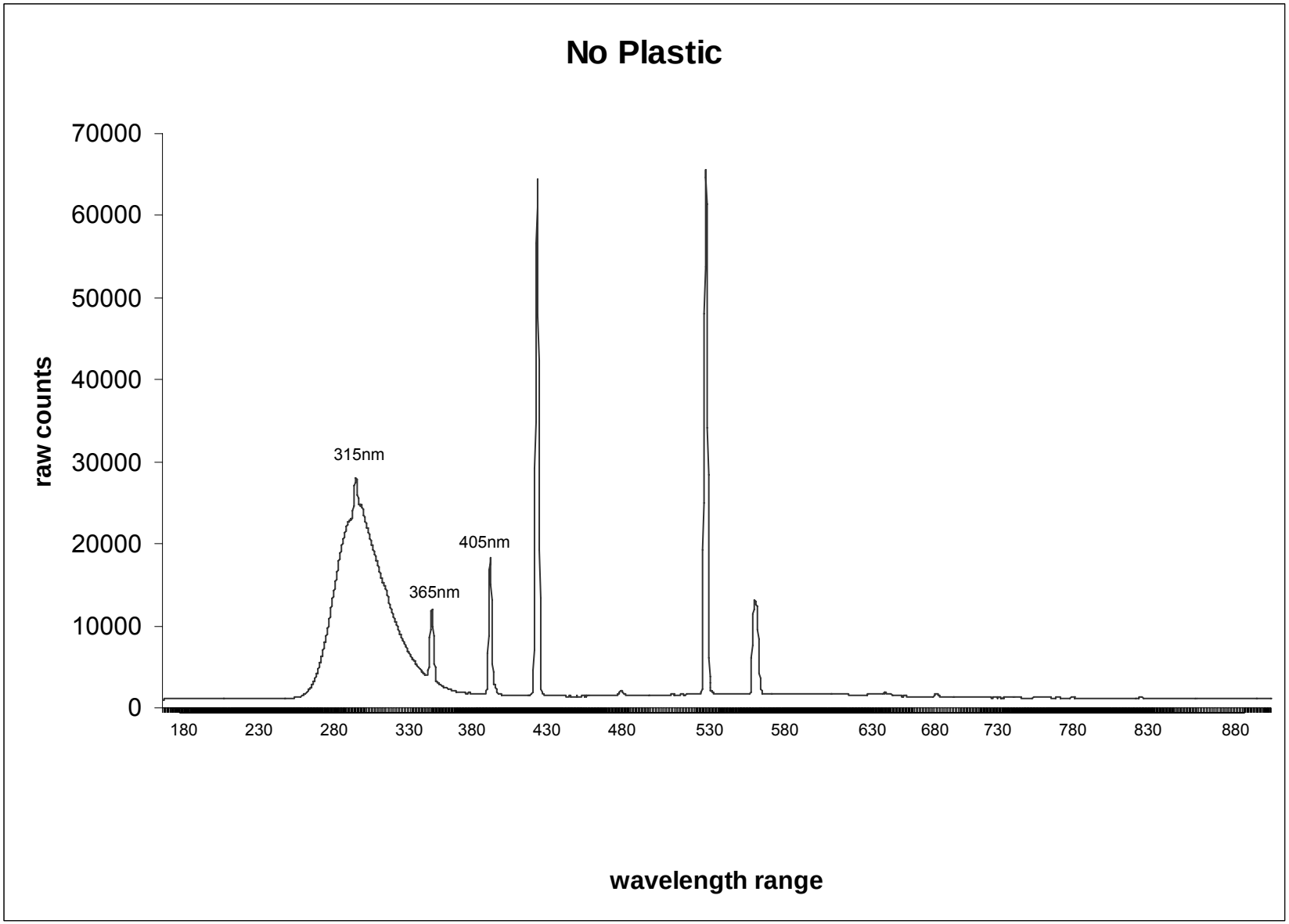

Cellulose Acetate
raw counts
70000
60000
50000
40000
30000
20000
10000
0
315nm
365nm
405nm
180
230
280
330
380
430
480
530
580
630
680
730
780
830
880
wavelength range

Figure 1C


No Plastic
raw counts
70000
60000
50000
40000
30000
20000
10000
0
315nm
365nm
405nm
180
230
280
330
380
430
480
530
580
630
680
730
780
830
880
wavelength range

***Figures 1A-1C.*** Spectral graphs of UV-315 bulb output when filtered with Mylar-D, cellulose acetate, and no plastic. 1A clearly shows a reduction in UV-B (315nm) transmission relative to the cellulose acetate filter in 1B. 1C is quite similar to 1B, demonstrating the ability of cellulose acetate to allow transmission of UV radiation.

Figure 2A

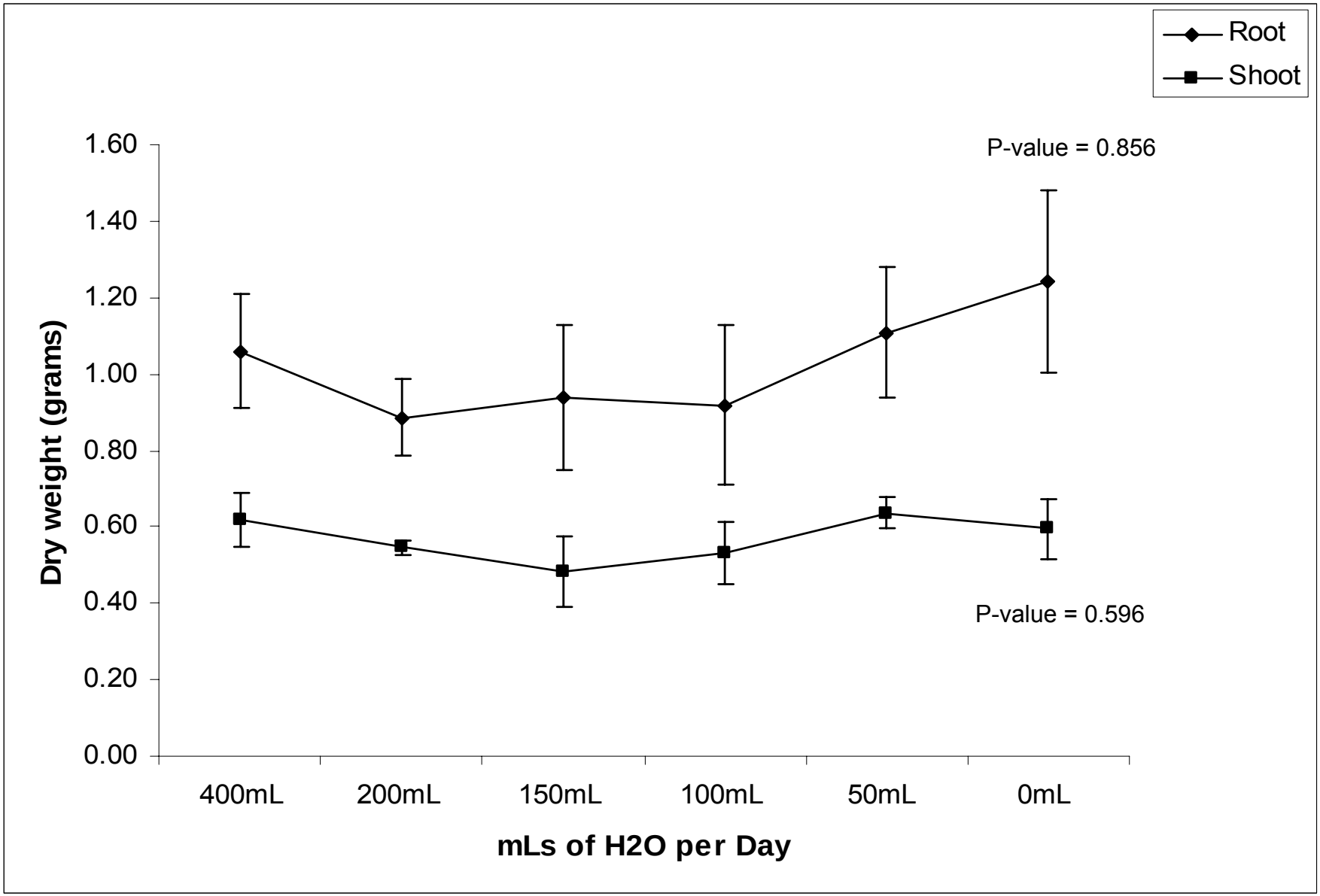

Root
Shoot
P-value = 0.856
P-value = 0.596
Dry weight (grams)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
400mL
200mL
150mL
100mL
50mL
0mL
mLs of H2O per Day

Figure 2B

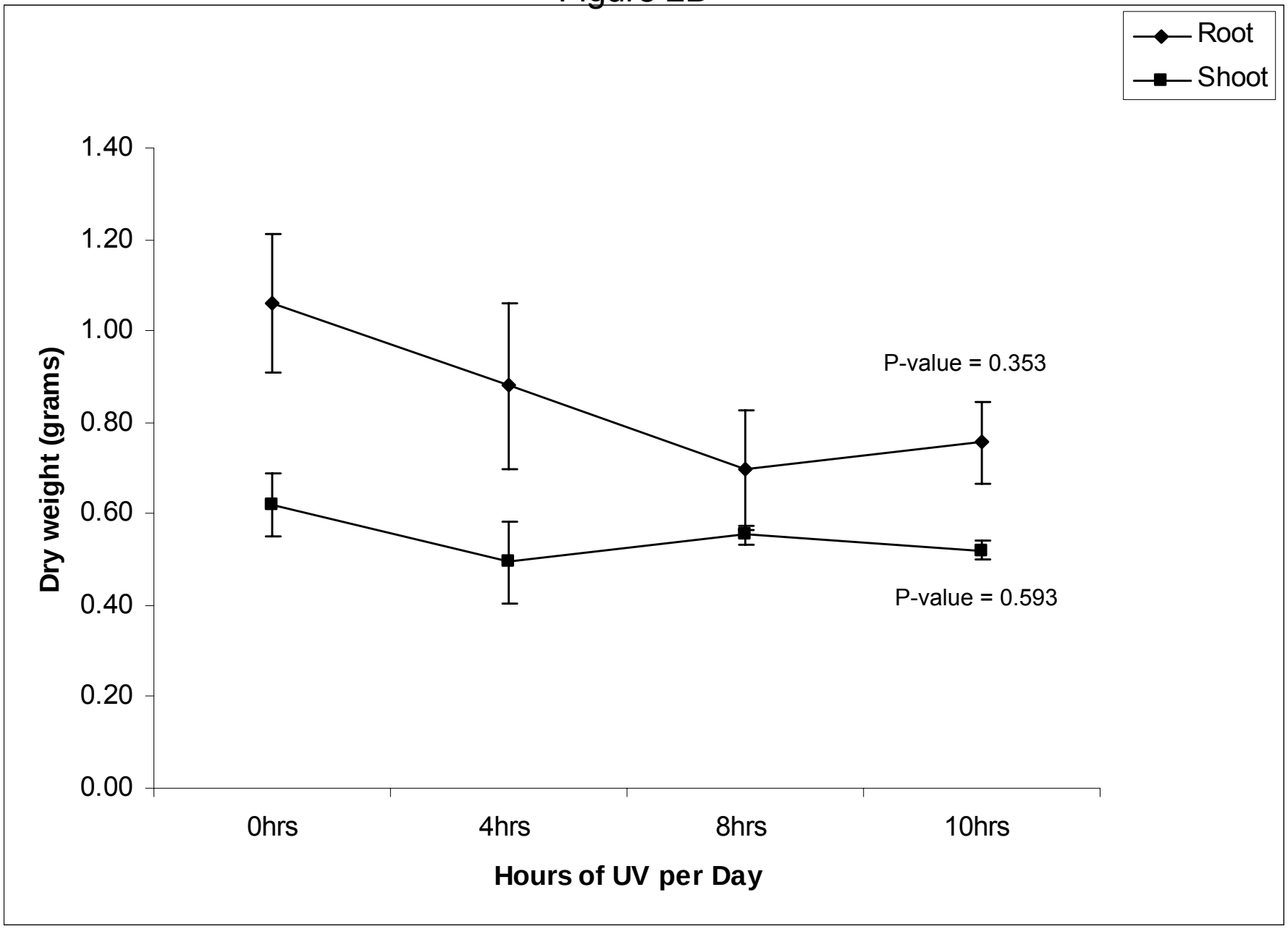

Root
Shoot
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Dry weight (grams)
P-value = 0.353
P-value = 0.593
0hrs
4hrs
8hrs
10hrs
Hours of UV per Day

Figure 2C

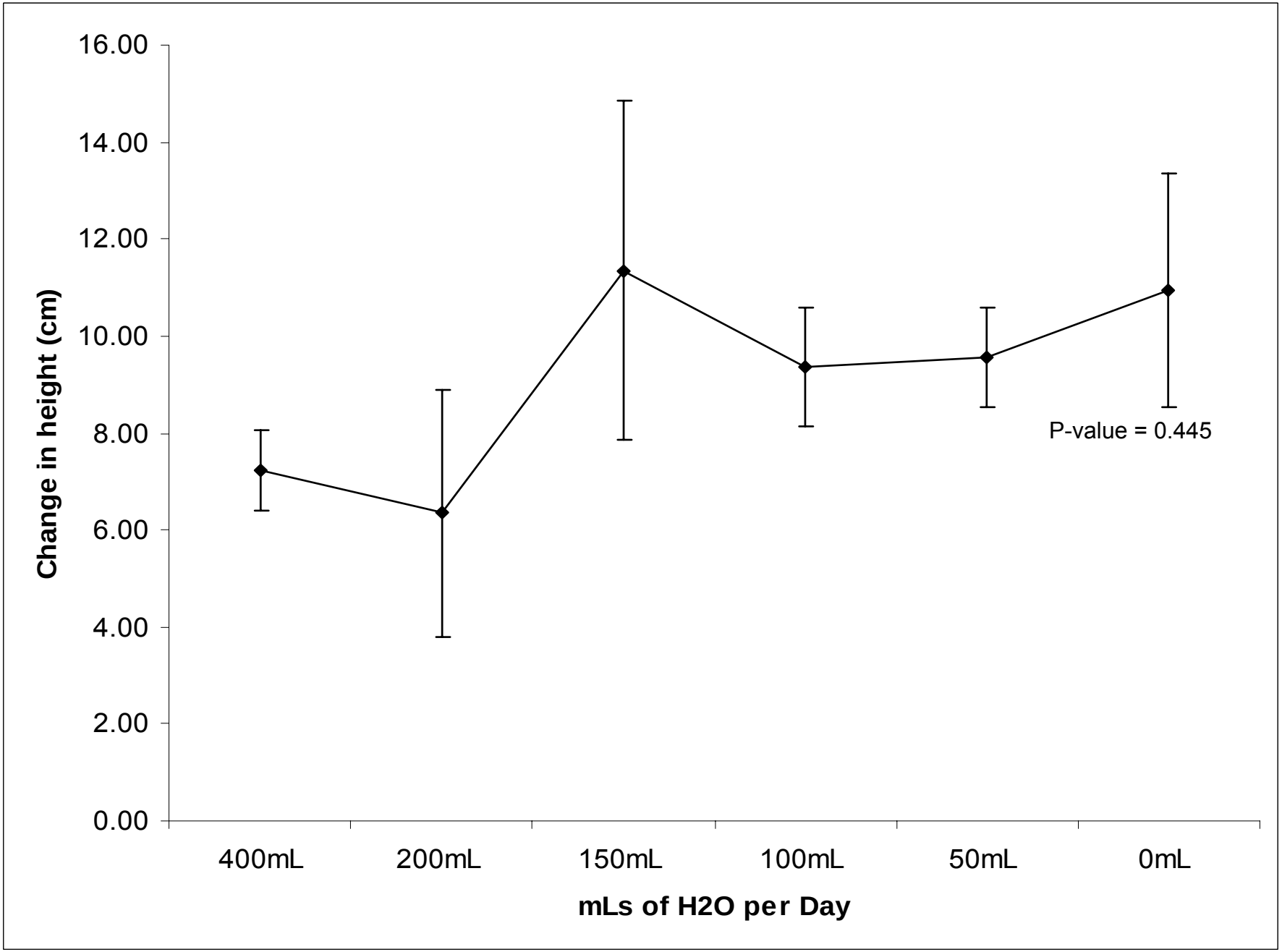

16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Change in height (cm)
P-value = 0.445
400mL
200mL
150mL
100mL
50mL
0mL
mLs of H2O per Day

Figure 2D

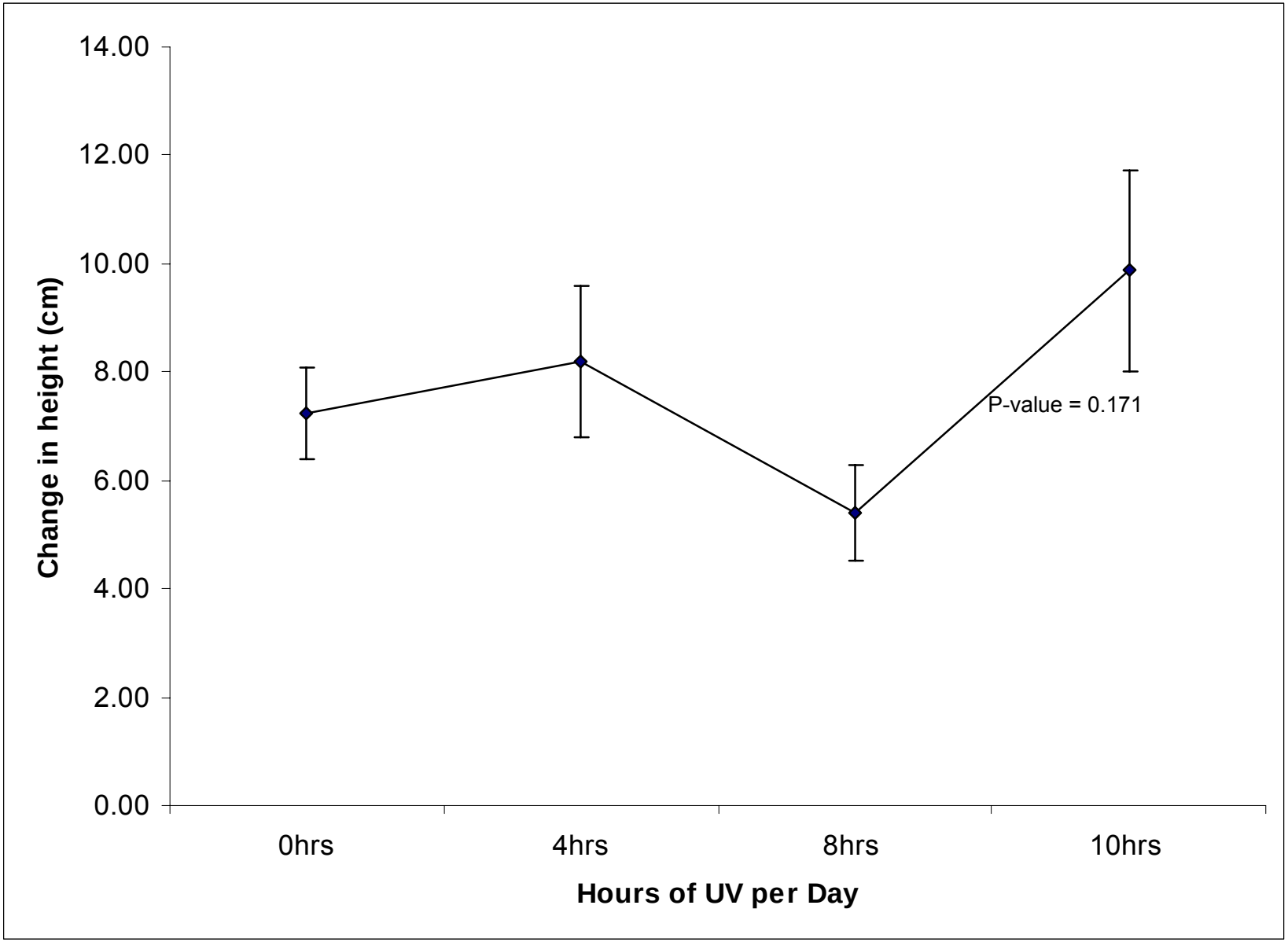

14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Change in height (cm)
P-value = 0.171
0hrs
4hrs
8hrs
10hrs
Hours of UV per Day

Figure 2E

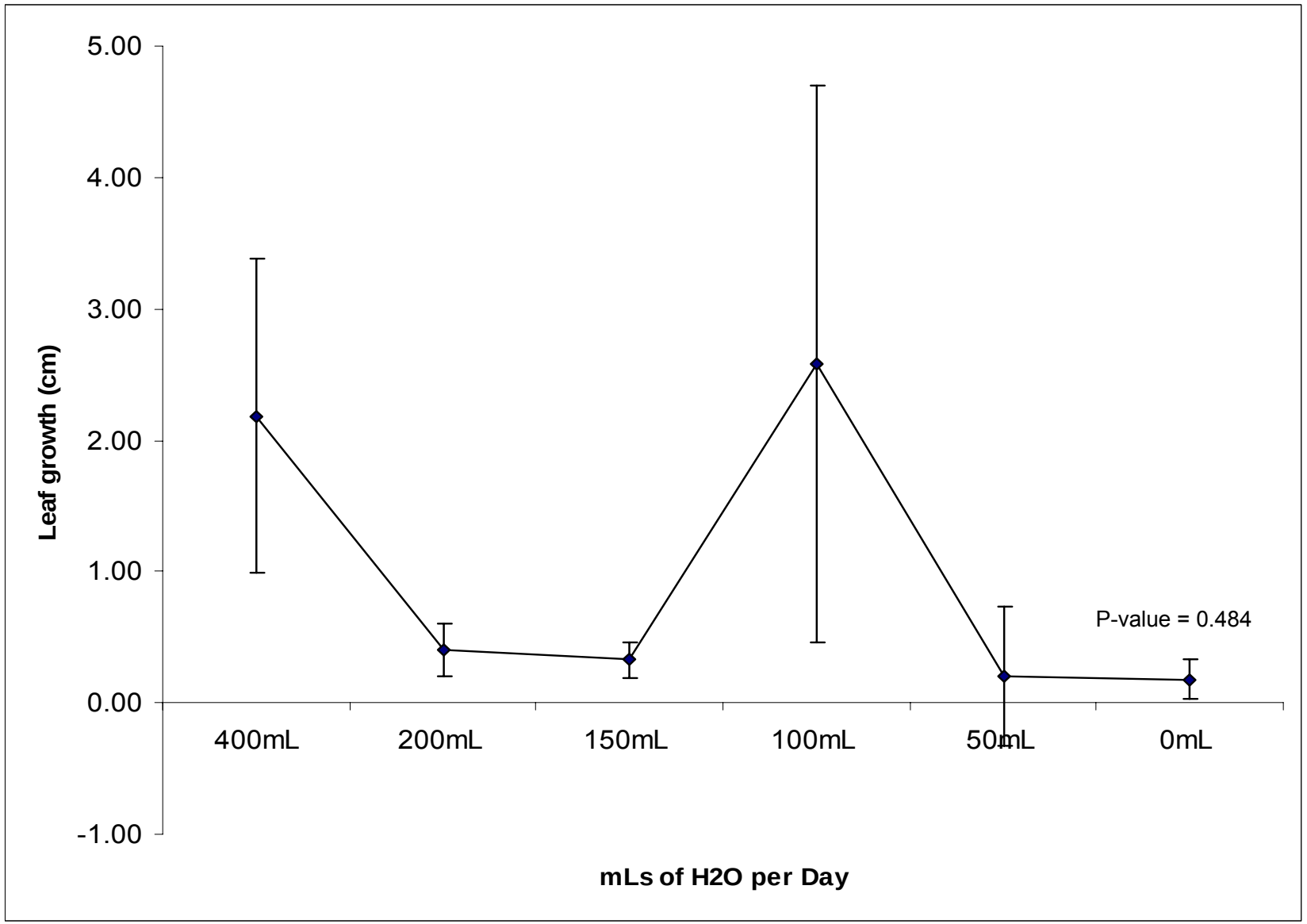
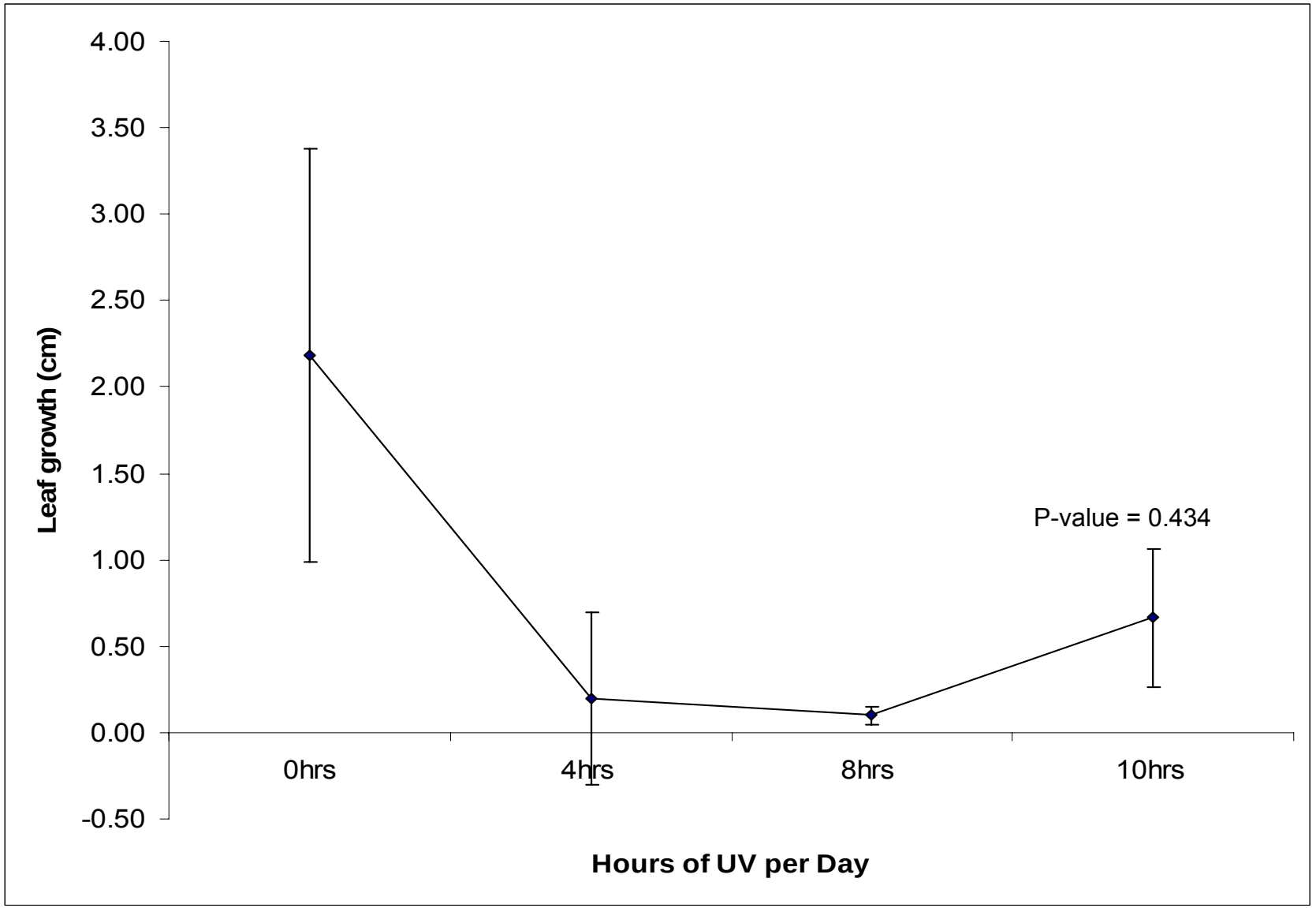

5.00
4.00
3.00
2.00
1.00
0.00
-1.00
Leaf growth (cm)
P-value = 0.484
400mL
200mL
150mL
100mL
50mL
0mL
mLs of H2O per Day

Figure 2F


4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
Leaf growth (cm)
P-value = 0.434
0hrs
4hrs
8hrs
10hrs
Hours of UV per Day

***Figures 2A-2F.*** Results of single-stress pilot experiment where varying levels of drought stress and UV stress were applied to maize seedlings and the effects on four traits were recorded. Figure 1A shows the mean±s.e. root and shoot dry weights recorded in each drought treatment group. Drought stress is increasing from left to right. Figure 1B shows the mean±s.e. root and shoot dry weights recorded in each UV treatment group with UV stress increasing from left to right. Figure 1C shows the mean±s.e. change in plant height recorded in each drought treatment group while Figure 1D shows the mean±s.e. change in plant height recorded in each UV treatment group. Figures 1E and 1F show the mean±s.e. change in leaf length recorded in each drought and UV treatment group, respectively. n = 3 and p values indicate whether the treatments had significant effect on the measured trait ($p < 0.05$ = significant).

Figure 3A

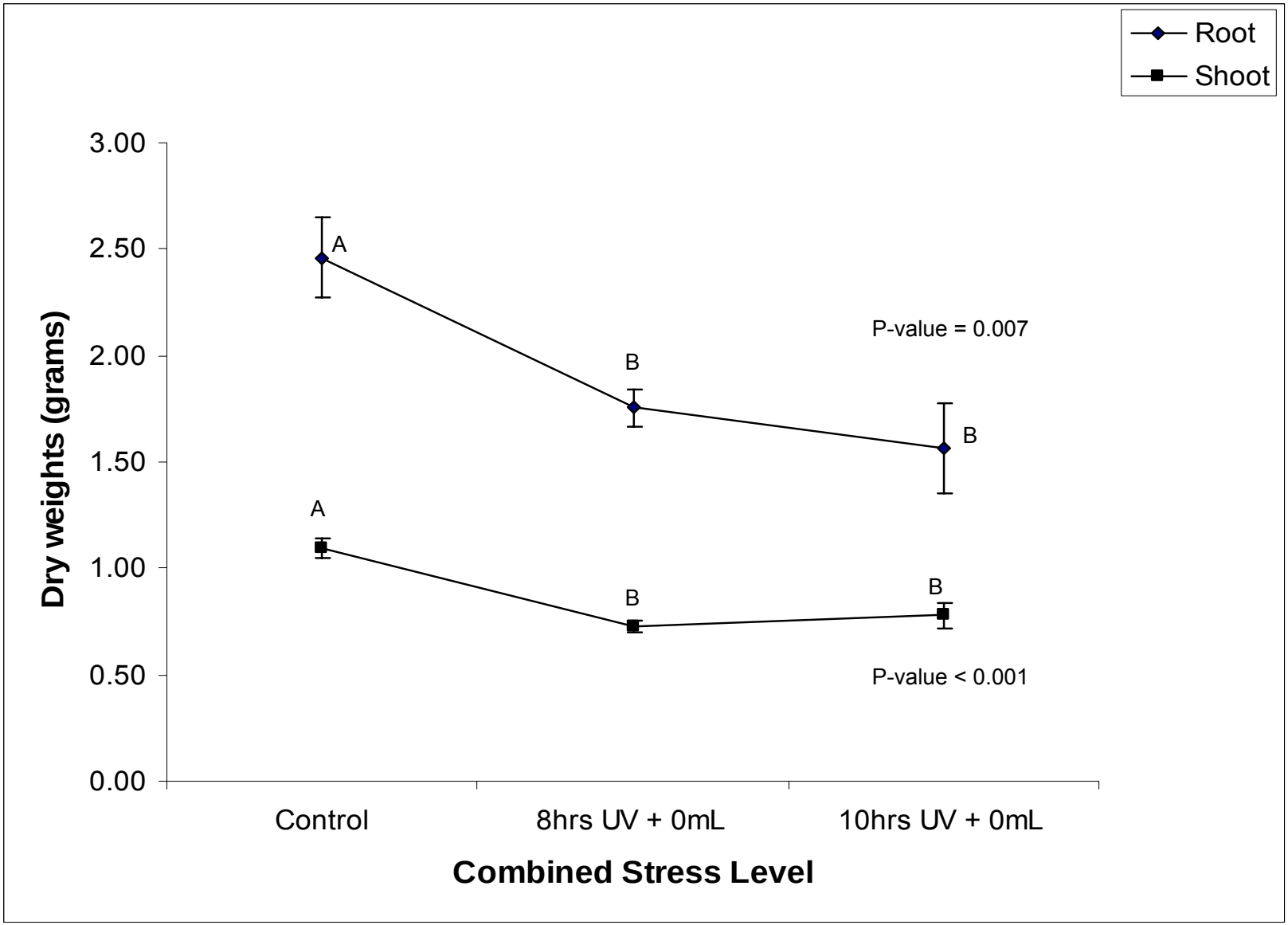

Root
Shoot
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Dry weights (grams)
A
B
B
A
B
B
P-value = 0.007
P-value < 0.001
Control
8hrs UV + 0mL
10hrs UV + 0mL
Combined Stress Level

Figure 3B

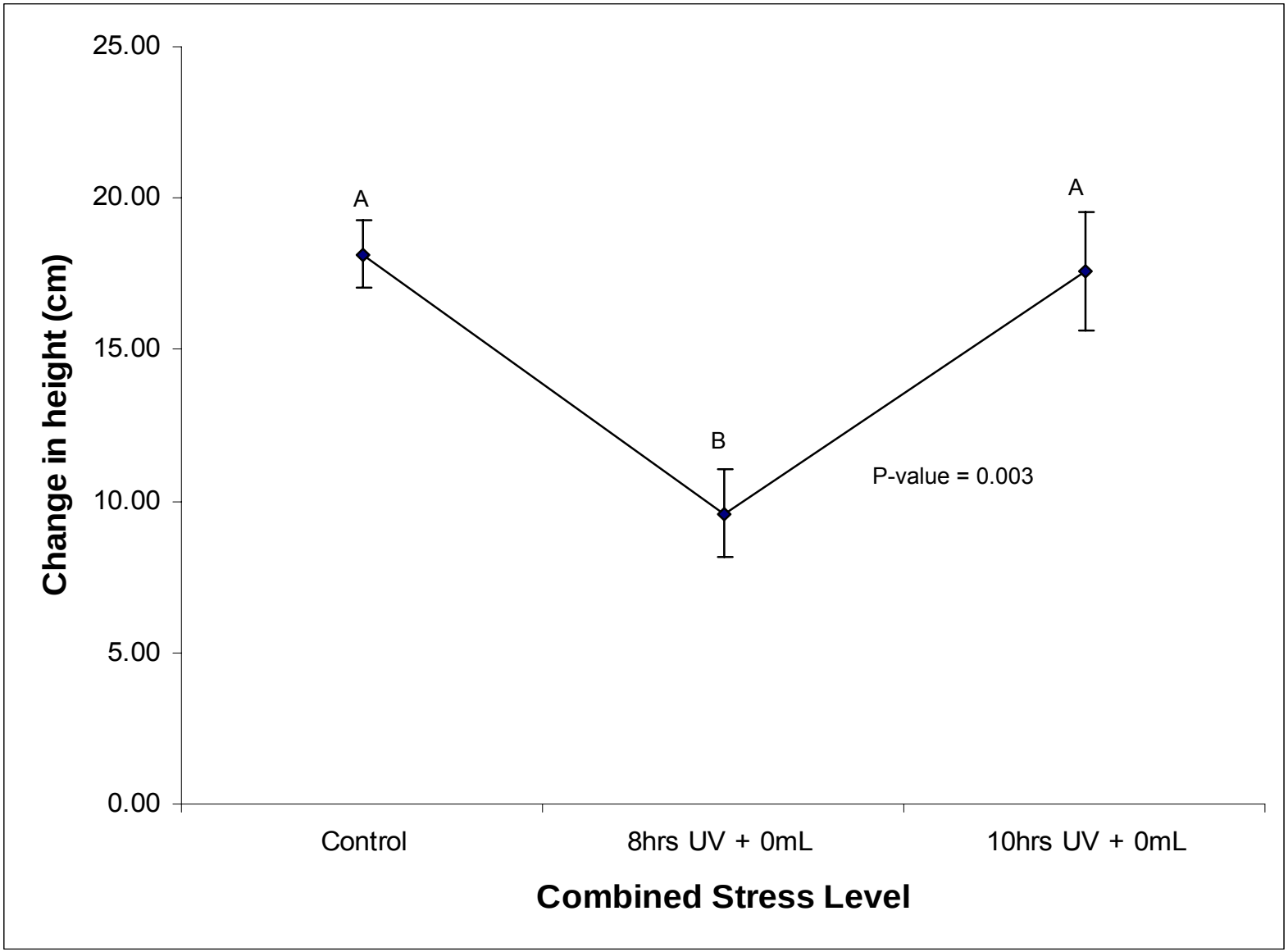
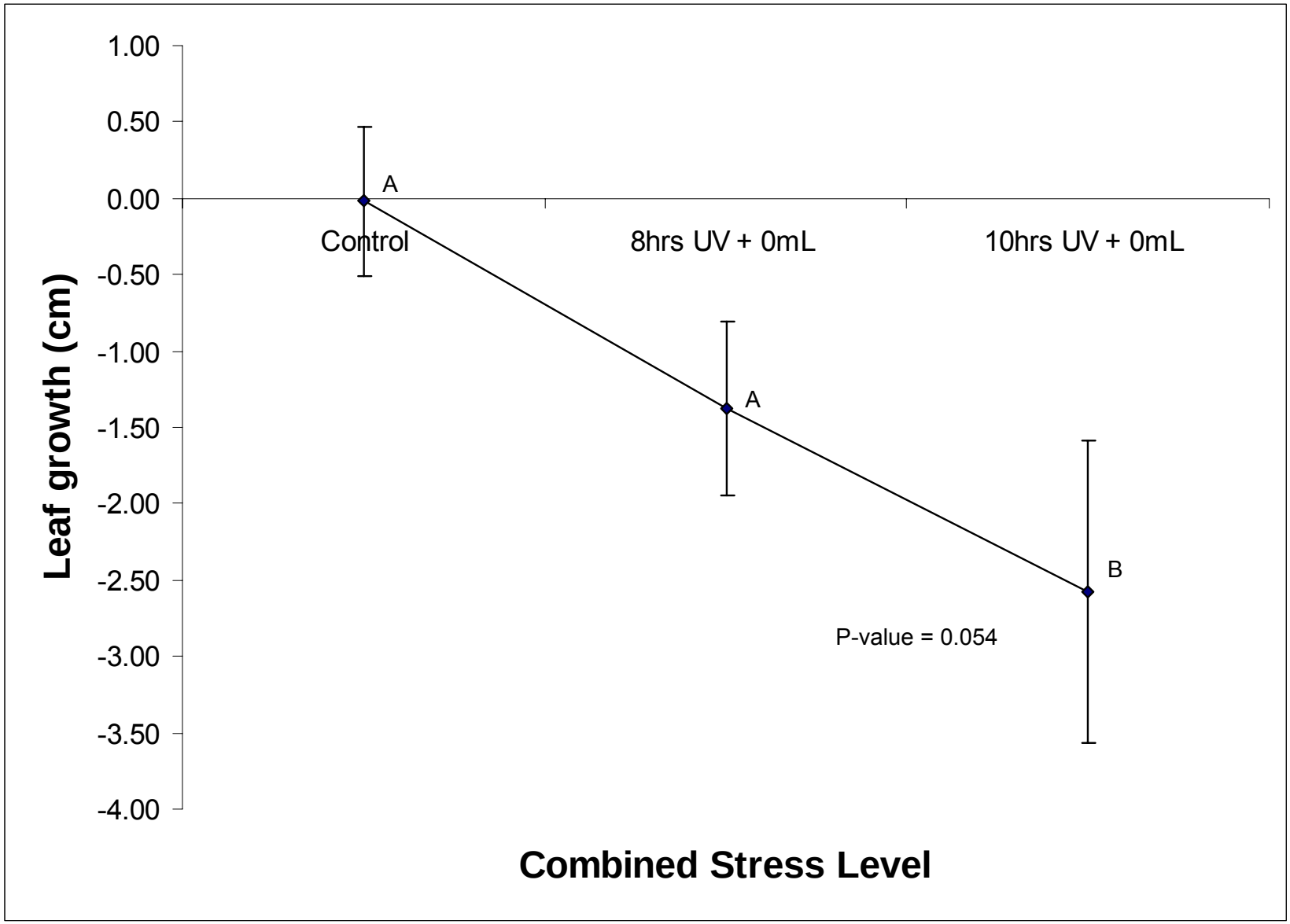

25.00
20.00
15.00
10.00
5.00
0.00
Change in height (cm)
A
B
A
P-value = 0.003
Control
8hrs UV + 0mL
10hrs UV + 0mL
Combined Stress Level

Figure 3C


1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
Leaf growth (cm)
A
Control
8hrs UV + 0mL
10hrs UV + 0mL
A
B
P-value = 0.054
Combined Stress Level

***Figures 3A-3C.*** Results of combination-stress pilot experiment where varying levels of drought stress and UV stress were applied, in combination, to maize seedlings and the effects on four traits were recorded. Figure 2A shows the mean±s.e. root and shoot dry weights recorded in each drought + UV treatment group with stress increasing from left to right. Figure 2B shows the mean±s.e. change in height recorded in each group while Figure 2C shows the mean change in leaf length recorded in each group. n = 4 and p values indicate whether the treatments had significant effect on the measured trait ($p < 0.05$ = significant). Designations of A and B indicate which treatment groups were significantly different from each other.

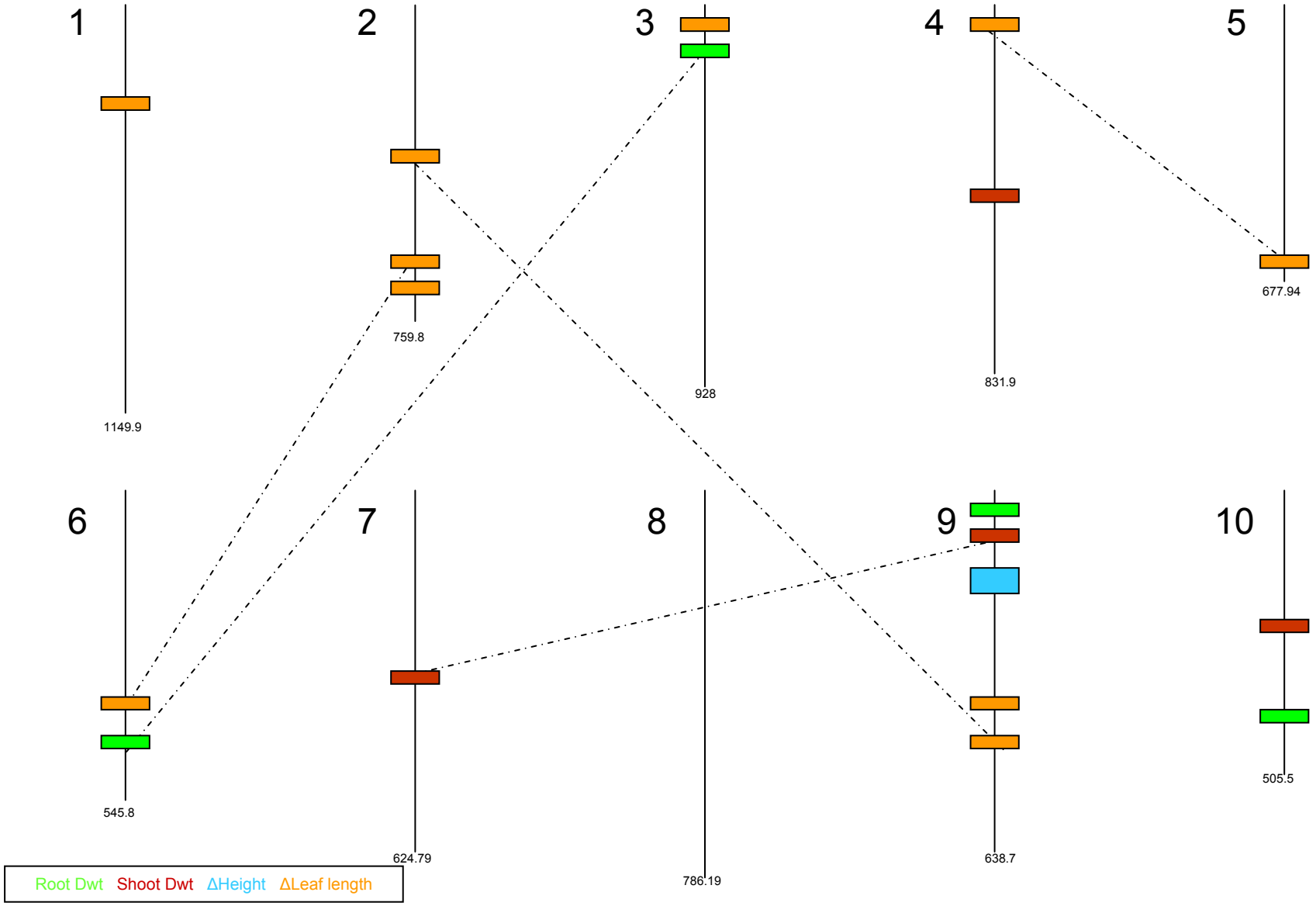



***Figure 4.*** Complete map of all QTLs detected in the non-adjusted data using the QTL Network software. Different colors represent QTLs associated with a particular trait. QTL region sizes are represented by the relative sizes of the colored blocks. Dashed lines between QTLs on different chromosomes indicate results from 2-D analyses and epistasis.

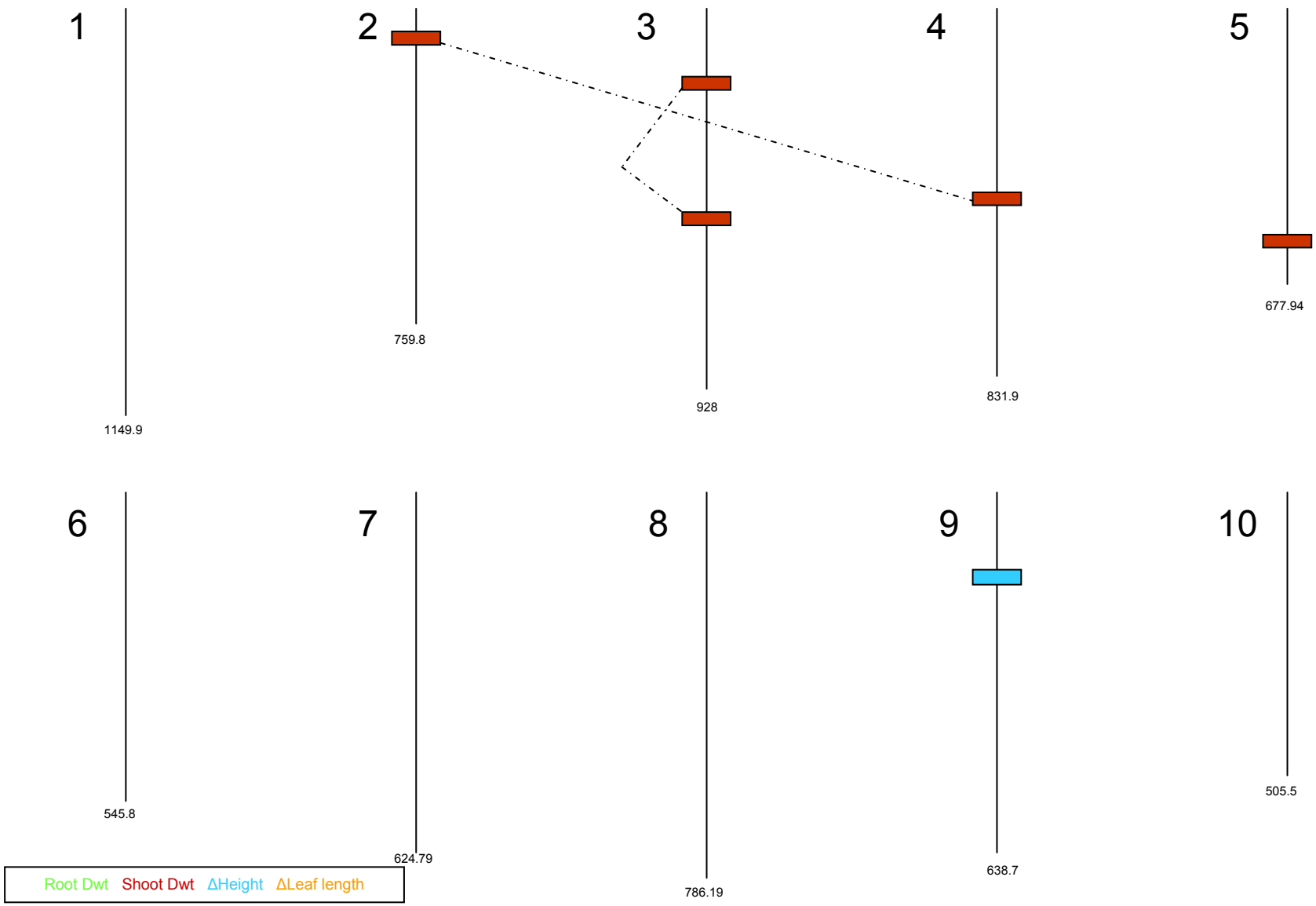
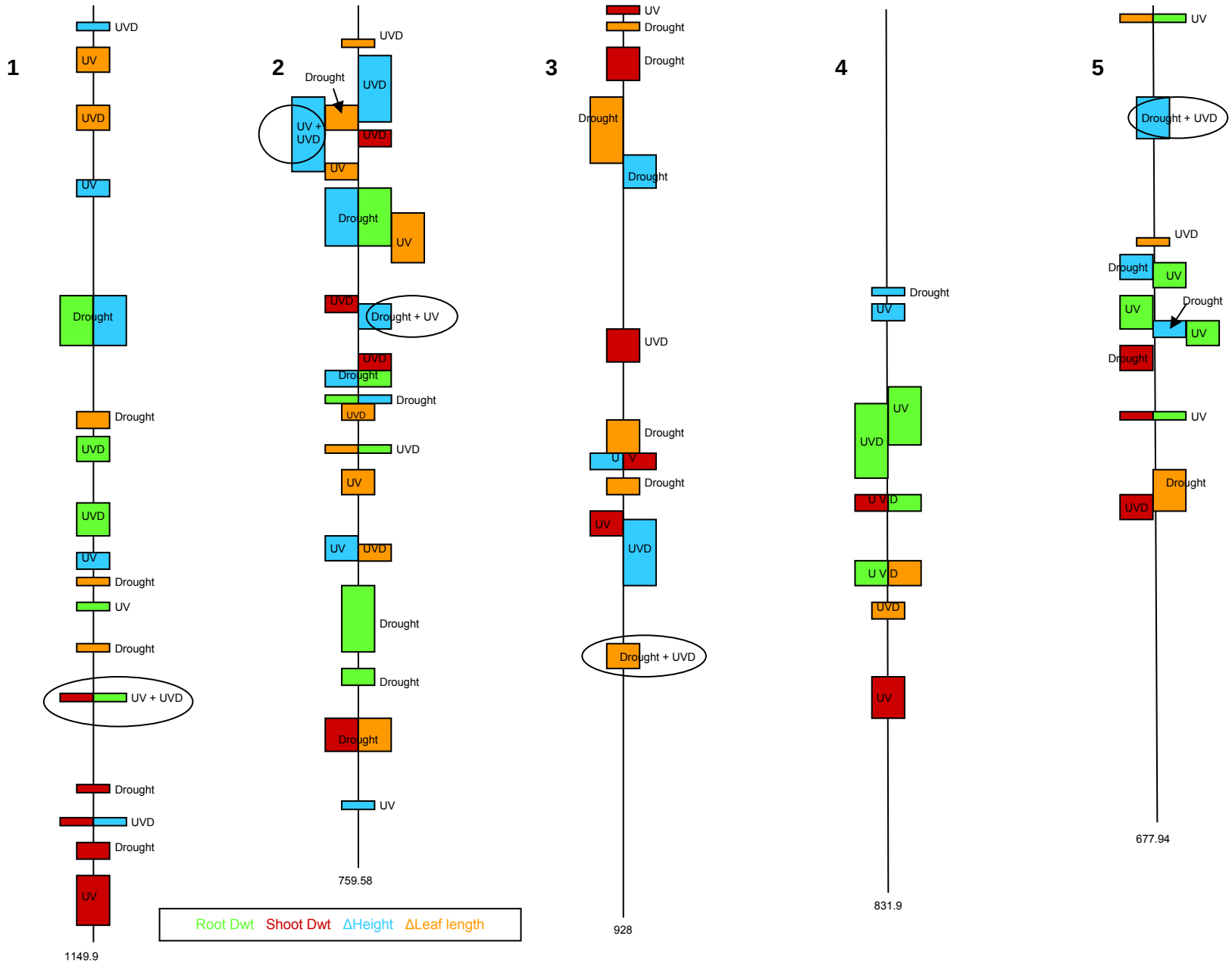



***Figure 5.*** Complete map of all QTLs detected in the temporally adjusted data using the QTL Network software. Different colors represent QTLs associated with a particular trait. QTL region sizes are represented by the relative sizes of the colored blocks. Dashed lines between QTLs on different chromosomes indicate results from 2-D analyses and epistasis.


1
UVD
UV
UVD
UV
Drought
Drought
UVD
UV
Drought
UV
Drought
UV + UVD
Drought
UVD
Drought
UV
1149.9
2
Drought
UVD
UV + UVD
UVD
UV
Drought
UV
UVD
Drought + UV
UVD
Drought
Drought
UVD
UVD
UV
UV
UVD
Drought
Drought
Drought
UV
759.58
3
UV
Drought
Drought
Drought
Drought
UVD
Drought
U V
Drought
UV
UVD
Drought + UVD
928
4
Drought
UV
UV
UVD
U VD
U VD
UVD
UV
831.9
5
UV
Drought + UVD
UVD
Drought
UV
UV
Drought
UV
Drought
UV
Drought
UVD
677.94
Root Dwt
Shoot Dwt
ΔHeight
ΔLeaf length

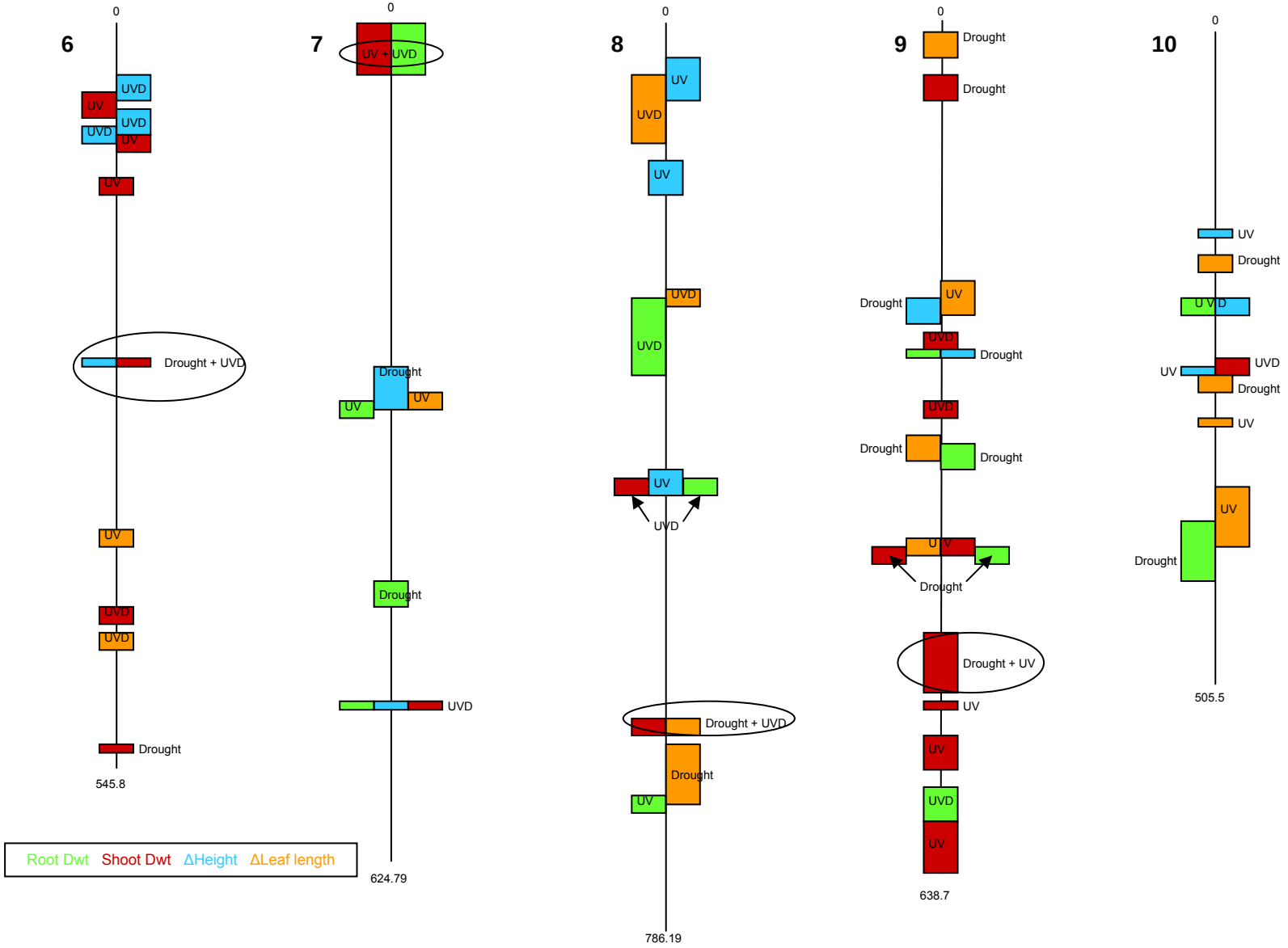



***Figure 6.*** Complete map of all QTLs detected using Chromoscan analysis. Different colors represent QTLs associated with a particular trait and the treatment(s) with which they are associated are labeled on each QTL. QTL region sizes are represented by the relative sizes of the colored blocks and overlap is represented by blocks that occupy the same vertical space on each chromosome. Regions that perfectly overlap were detected in each of the indicated traits and circles represent QTLs that were detected under multiple treatment conditions.