

PATTERN OF GENOMIC LOCI CONTROLLING MORPHOLOGICAL RESPONSES TO  
UV-B RADIATION IN MAIZE (*ZEA MAYS L.*)

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## ABSTRACT

The sessile nature of plants determines that they tolerate rather than escape from environmental changes. Therefore, studying plant responses to ultraviolet radiation (UV) is important for understanding how plants respond to environmental challenges. Although numerous UV responses have been reported, little is known about the genetics controlling quantitative natural variation in those UV responses. To address this question, I examined morphological UV responses in maize (*Zea mays*). First, dose-response and reciprocity experiments were conducted to find a standard experimental UV dose of six hours per day for four days. Second, a 84 subset of 94 mapping lines from the recombinant inbred of maize (IBM) population was planted in a greenhouse in a completely randomized design. Maize UV responses including ratio of leaf rolling, plant height, dry weight of second and third leaf, and dry weight of root, were compared for “control” and “UV” environments. A composite interval mapping (CIM) analysis detected 12 significant quantitative trait loci (QTL) affecting at least one of five traits. A total of 8 significant QTL were identified by multitrait composite interval mapping (MCIM). Only two QTL were detected by both CIM and MCIM. The allelic sensitivity model was supported most often. Genome-wide QTL mapping is an efficient way to generate a more complete understanding of the genetic basis of plant responses to UV irradiation.

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Many, many thanks also to my family and friends, especially Carletha Blanding, Tiandao Li, Bing Zhang, Yusheng Zai and Yuan Liu who all helped me get through the tough times. I couldn't have made it without them and a little help from god.

## DEDICATION

I would like to dedicate this thesis to my father, Zhaoling Fu and my mother, Yuzhong Shu, whose lifting words rattled my ears from the time I was a little kid. Thanks for always being my guiding light and for teaching me that high expectations and goals are attainable through hard work and dedication. Your loving support means more to me than you will ever know.

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## INTRODUCTION

Ultraviolet radiation (UV) comprises a small portion of the whole sunlight spectrum, and can be separated into three groups on the basis of wavelength: UV-A (400-320 nm), UV-B (320-280 nm) and UV-C (280-250 nm). UV-A wavelengths may reach the earth's surface; in contrast, the atmosphere will effectively absorb UV-C wavelengths before it reaches the earth's surface; interestingly, UV-B wavelengths will be absorbed partially by the ozone layer (Lumsden, 1997). UV has been shown to cause damage. Stapleton (1992) classified such damage into two categories: damage to DNA and changes in physiological processes. In the past decades, a number of researchers have examined UV effects on a wide variety of plants (Tosserams and Rozema, 1995; Rousseaux et al., 1999; Liu et al., 2000; Ries et al., 2000). Some of the most important of these responses are changes in morphology and growth characteristics. However, little is known about the genetic mechanisms plants use for adaptation to ultraviolet radiation stress. Furthermore, as ozone levels decrease, it is becoming more and more urgent to understand these mechanisms.

Many traits show complex patterns of natural variation. This variability, in general, is attributable to genetic differences, environmental differences and the interaction between them. The ability of a genotype to produce multiple phenotypes in response to different environments is referred to as phenotypic plasticity (Kliebenstein et al., 2002). The marginal difference in measurements between two different environments is referred to as environmental sensitivity (Ungerer et al., 2003). There are a number of theoretical models that have been developed to explain phenotypic plasticity. Two of the most widely tested models are (1) the gene regulation model that considers that certain gene regulatory sequences determine phenotypic plasticity, and in contrast (2) the allelic sensitivity model, which argues that different genes expressed in

different environments cause phenotypic plasticity (as cited in Ungerer et al., 2003). Few studies that have been conducted to examine these hypotheses (Kliebenstein et al., 2002; Ungerer et al., 2003). Therefore, it will be helpful to our understanding of the mechanisms that plants use for adaptation to environmental changes to examine phenotype plasticity in ultraviolet radiation responses.

The development of genetic maps and powerful statistical tools, including the use of quantitative trait locus (QTL) mapping techniques, provide an opportunity to investigate the underlying genetic mechanisms. QTL mapping is the process of finding and estimating associations between a continuous quantitative trait and a set of DNA markers that have been previously placed in a genetic map, with the ultimate goal of determining the genetic architecture of a trait, or finding markers that can be used to select for preferred values of the trait (Ball, 2001). Basically, there are two strategies to map QTL (Grisel, 2000): (1) analyzing an F2 population or (2) analyzing a population of recombinant inbred (RI) lines. In this study, I used the latter method, since RI families offer permanent research materials in which homozygosity is nearly complete; and a genotype in RI families was represented by an inbred line, rather than by an individual, so RI families can be utilized in different environments (Burr et al., 1988). The use of RI families is preferred for evaluating QTL x Environment interactions (Vieira et al., 2000).

Numerous QTL mapping experiments have been conducted to examine the genetic architecture of different environmental stresses (Menendez et al., 2002; Loudet et al., 2003). Several model organisms, such as *Drosophila melanogaster* and *Arabidopsis thaliana* have RI line resources available (Juenger et al., 2000; Zimmerman et al., 2000; Workman et al., 2002). To my knowledge, my study is the first example of genetic analysis of ultraviolet radiation

responses in maize, and my results may open the way to a better understanding of the architecture of regulatory processes.

## MATERIALS AND METHODS

### Plant Materials and Growth

#### Plant Materials

Inbred line B73 seed was supplied by M. Lee, Iowa State University, and increased in the field nursery at Clayton, NC. B73 is one parent of the IBM94 mapping line set. We obtained the IBM 94 recombinant inbred lines from the Maize Genetics Coop at URL:

[www.agron.missouri.edu](http://www.agron.missouri.edu) (Lee et al.). The seed was increased at the field nursery in Clayton, NC. For ultraviolet radiation experiments, seeds were grown in vermiculite-filled 36-cell flats in the greenhouse at the University of North Carolina at Wilmington. For dose-response experiments, seeds were planted at a density of one per pot. For the QTL mapping experiment, seeds were planted at a density of three to four per pot and thinned to one per pot after germination.

#### Ultraviolet Irradiation Conditions

After germination the seedlings were assigned randomly to control and treatment groups. The treatment group was placed on a shelf 0.8 meter under a pair of UV-313 lamps (Q-Panel Lab Products). The control group used the same lamps but Mylar (United Plastics) covered the bulbs to block UV-B radiation. This experimental system is widely used and has been used for previous work in our lab (Cartwright et al., 2001). When the majority of seedlings had a visible third leaf (after 7-10 days of growth) the UV lamps were turned on. After irradiation, the seedlings were allowed to recover for 24 h before measuring.

A dose-response and reciprocity experiment was conducted to determine the best dose of ultraviolet radiation for the QTL mapping experiment. The maize seedlings were irradiated for 4, 6, 8 and 10 h per day, for 4 days during this experiment.

For the QTL mapping experiment, the maize (RIL) seedlings were irradiated for 6 h per day, from 3PM to 9PM, for 4 days.

#### Experimental Design

For dose-response and reciprocity experiments, plants were divided evenly into treatment and control groups (n=6-8), using a paired design so that each treatment group had a corresponding control group.

For the QTL mapping experiment, there were 4 groups for control and for treatment; 5 trays constituted each group. Two of eight maize lines were randomly assigned in one group. There were 8 replicates for each of 86 lines (84 mapping lines plus each parent line) in control and treatment group separately. Due to germination problems, one of the 84 RI lines was removed from the analysis due to a lack of data. A total of 1229 plants were analyzed for this experiment for an average of 7 plants per line per treatment.

#### Trait Measurements

**Height:** Plant height was measured as the perpendicular length between the tip and the base node of each plant, using a centimeter ruler. For some tilted plants, I let the plant stand still and measured from the soil surface to the node. Height measurements were conducted before UV irradiation and after UV irradiation. The difference between final height and initial height was used for all analyses.

**Leaf rolling:** Rolling was measured essentially as previously described (Cartwright et al., 2001). The second leaf was cut at approximately 90 degrees to the midrib at the half position of the leaf. The cut edge of the leaf was dipped in black lithographic ink (Hunt Manufacturing, Statesville, NC, USA) and then pressed onto a white paper to produce a rolled leaf width; the cut edge was flattened and pressed on the paper to produce the flat width. Both the curled and flat

margin-to-margin length were measured and recorded from the prints using digital calipers (Starrett, Althol, MA, USA). The ratio of treatment/control was used for all analyses.

**Dry weight:** The second and third leaves were cut at the sheath junction; the root was cut from the base node and cleaned thoroughly. The three parts of a plant were put in an envelope. All plants were dried in an oven at 75°C for one day. An analytical balance (Ohaus Corporation, d = 0.1 mg) was used to determine the dry weight of each part in grams.

#### Statistical Analysis

Excel and SAS/STAT version 8e were utilized for all statistics (SAS Institute, Cary, NC). For the dose-response experiments, an ANOVA and multiple range tests were used to analyze differences between control and treatment.

For the QTL mapping experiment, two-way ANOVA using the SAS GLM module was used to compare the lines under two treatments. The following model was used:

$$Y_{ij} = U + G_i + E_j + I_{ij} + \mathcal{E}$$

Where  $y_{ij}$  is the observed phenotype,  $U$  is the mean phenotype in the population,  $G_i$  and  $E_j$  are the effects due to an individual having genotype  $i$  and environment  $j$ ,  $I_{ij}$  is the interaction effect between  $i$  and  $j$ , and  $\mathcal{E}$  is a random contribution to the phenotype.

All data were tested for normality by Q-Q plot. If normality failed, transformations were attempted.

#### QTL Mapping

Composite interval mapping and multiple interval mapping were utilized in the Windows version 2.0 of QTL Cartographer (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>). Genome-wide 0.05 significance thresholds for each trait were used, with 500 permutations conducted to

determine appropriate threshold likelihood ratio scores. Each measured trait was mapped as three separate traits: control condition, treatment condition and combined condition. The RI line x Treatment medians were used for QTL mapping.



## RESULTS

### Dose-response and Reciprocity Experiment

I measured four traits under control and treatment conditions, they are dry weight of second leaf, dry weight of third leaf, dry weight of root, and plant height (sensitivity) in this experiment. From two-way ANOVA (Table 1), there is a significant difference for the interaction of UV dose and treatment for dry weight of second and third leaf. Furthermore, a Ryan-Einot-Gabriel-Welsch Multiple Range Test for above two traits, presented in Table 2, showed there is no significant difference for the combinations among 6, 8 and 10 h/day UV irradiation under UV condition, but the three combinations are significantly different from other combinations.

### Normal Distribution Test and Transformation

Q-Q plots showing distributions of residuals of five measured traits suggest a normal distribution for all traits except leaf rolling. Following transformation, leaf-rolling data were approximately normal (Fig. 1f). Analyses of variance (ANOVA), presented in Table 3, demonstrated the presence of significant variability for all the five traits in the IBM94 population, thus permitting further QTL analyses. Moreover, in respect to ANOVA, dry weight of root and leaf-rolling were affected significantly by environment.

The distribution of IBM94 lines for median of all five traits clearly showed the effect of UV for some lines (Figure2-Figure11).

Table 1. Analysis of variances for four traits in Dose-response and Reciprocity Experiment

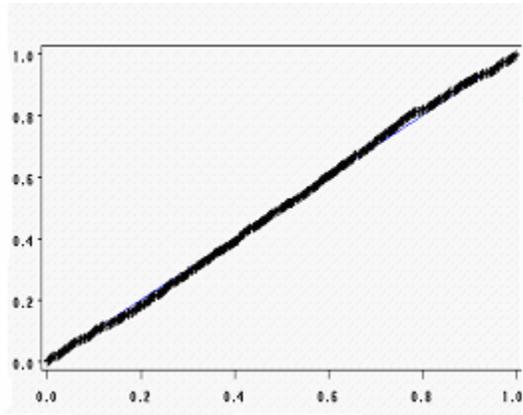
Trait	Treatment		UV dose		Treatment*UV dose	
	F	P	F	P	F	P
Dry weight of second leaf	0.03	0.8723	7.31	0.0094**	5.11	0.0281**
Dry weight of third leaf	0.00	0.9736	5.39	0.0244**	4.57	0.0375**
Dry weight of root	0.00	0.9450	1.65	0.2051	1.59	0.2139
Plant height (sensitivity)	0.93	0.3402	0.44	0.5116	0.99	0.3234

\*\*Significant at  $P < 0.01$ . F is the F value from the type III sum of squares ANOVA for each factor and P is the estimated probability of obtaining this F value under the null hypothesis.

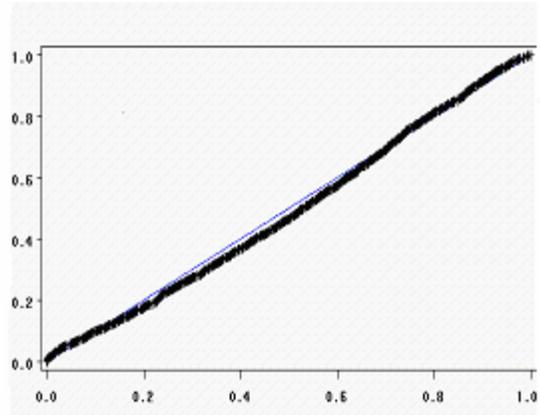
Table 2. Ryan-Einot-Gabriel-Welsch Multiple Range Test

Dry weight of second leaf			Dry weight of third leaf		
REGWQ grouping	Mean	Treatment*UV dose	REGWQ grouping	Mean	Treatment*UV dose
A	0.020843	Control*4 h UV	A	0.039957	Control*4 h UV
A	0.020586	Treatment*4 h UV	A	0.039571	Treatment*4 h UV
A	0.020186	Control*10 h UV	A	0.039371	Control*10 h UV
A	0.019250	Control*8 h UV	A	0.039250	Control*8 h UV
A	0.019071	Control*6 h UV	A	0.038657	Control*6 h UV
B	0.014283	Treatment*8 h UV	B	0.030750	Treatment*8 h UV
B	0.014171	Treatment*6 h UV	B	0.030329	Treatment*6 h UV
B	0.014086	Treatment*10 h UV	B	0.030043	Treatment*10 h UV

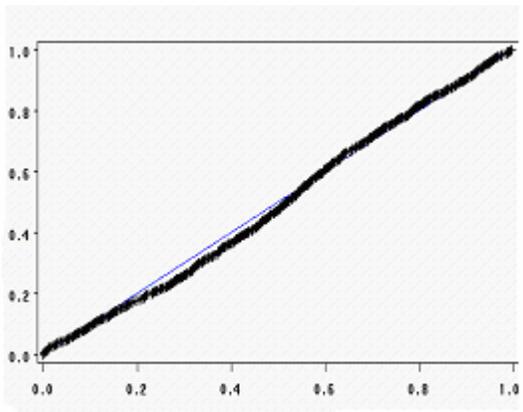
Means with the same letter are not significantly different



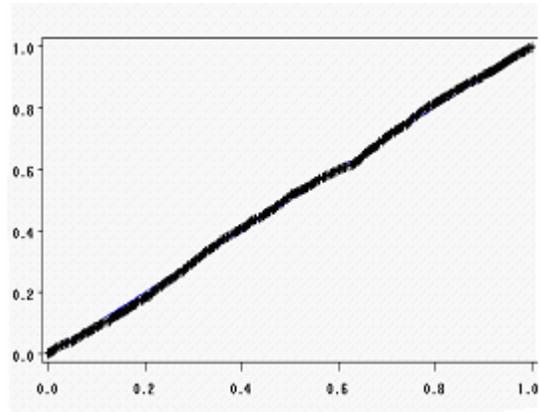
a. Dry weight of second leaf



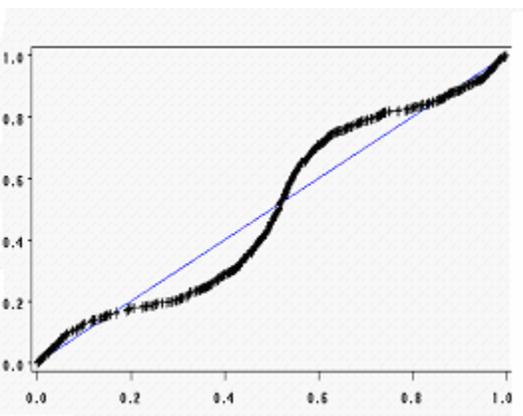
b. Dry weight of third leaf



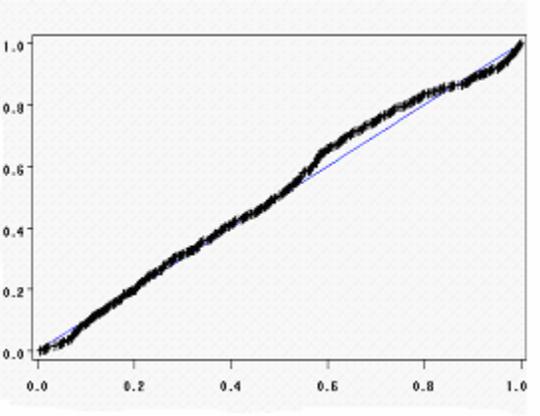
c. Dry weight of root



d. Plant height



e. Leaf rolling



f. Leaf rolling (Transformed)

Figure 1. Q-Q plot of residuals of five metric traits and transformation data

Table 3. Analysis of variance results for five traits in IBM94 population

Trait	Line		Treatment		Line x Treatment	
	F	P	F	P	F	P
Dry weight of root	10.12	0.0001**	7.23	0.0073**	0.81	0.8812
Dry weight of second leaf	16.86	0.0001**	0.02	0.8801	1.10	0.2586
Dry weight of third leaf	17.67	0.0001**	0.13	0.7139	0.83	0.8627
Plant height (sensitivity)	8.5	0.0001**	0.01	0.9244	0.9	0.7240
Leaf rolling (transformed)	1.49	0.0042**	846.71	0.0001**	1.02	0.4258

\*\*Significant at  $P < 0.01$ . F is the F value from the type III sum of squares ANOVA for each factor and P is the estimated probability of obtaining this F value under the null hypothesis.

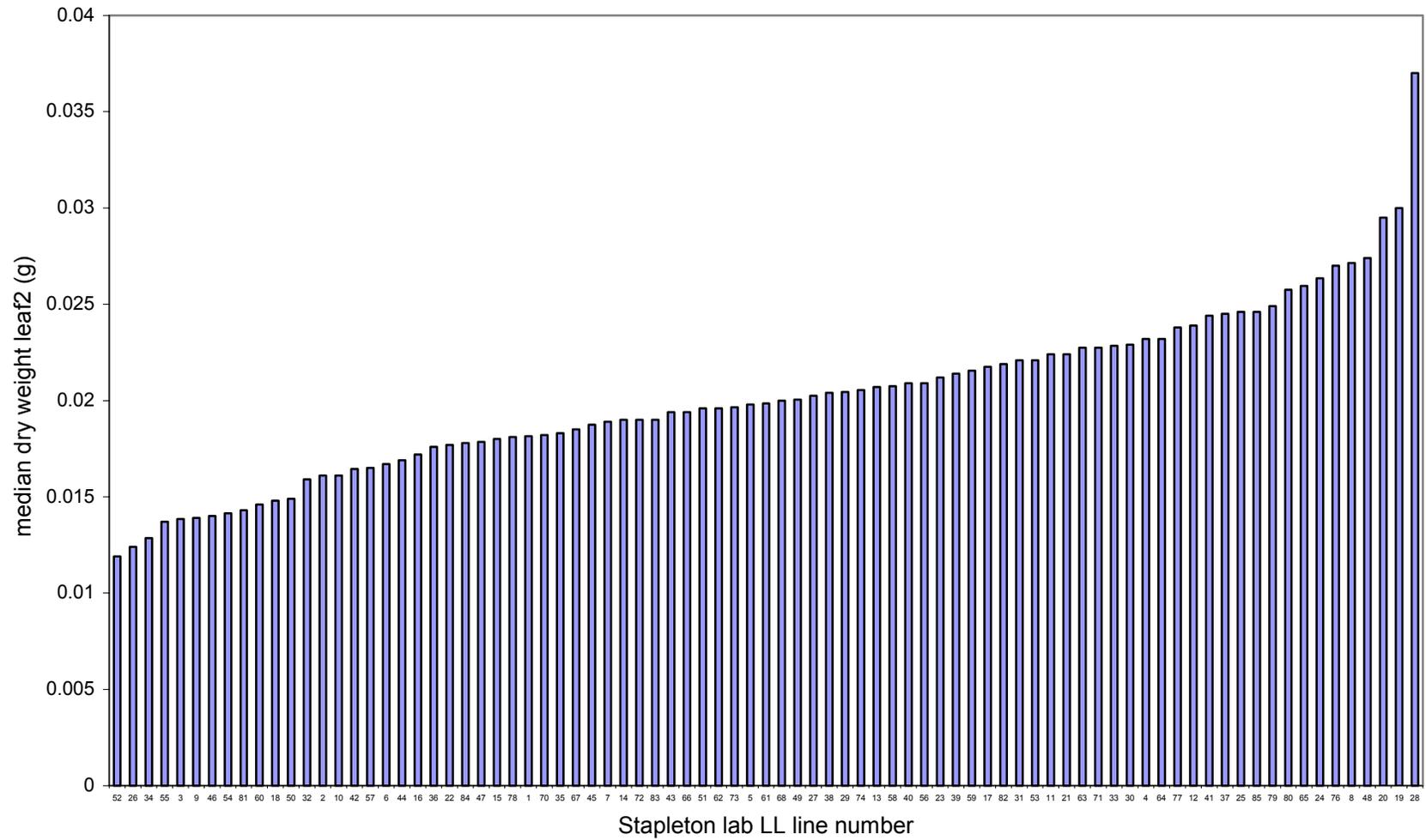


Figure 2. Distribution of Stapleton lab LL for median of dry weight of second leaf.

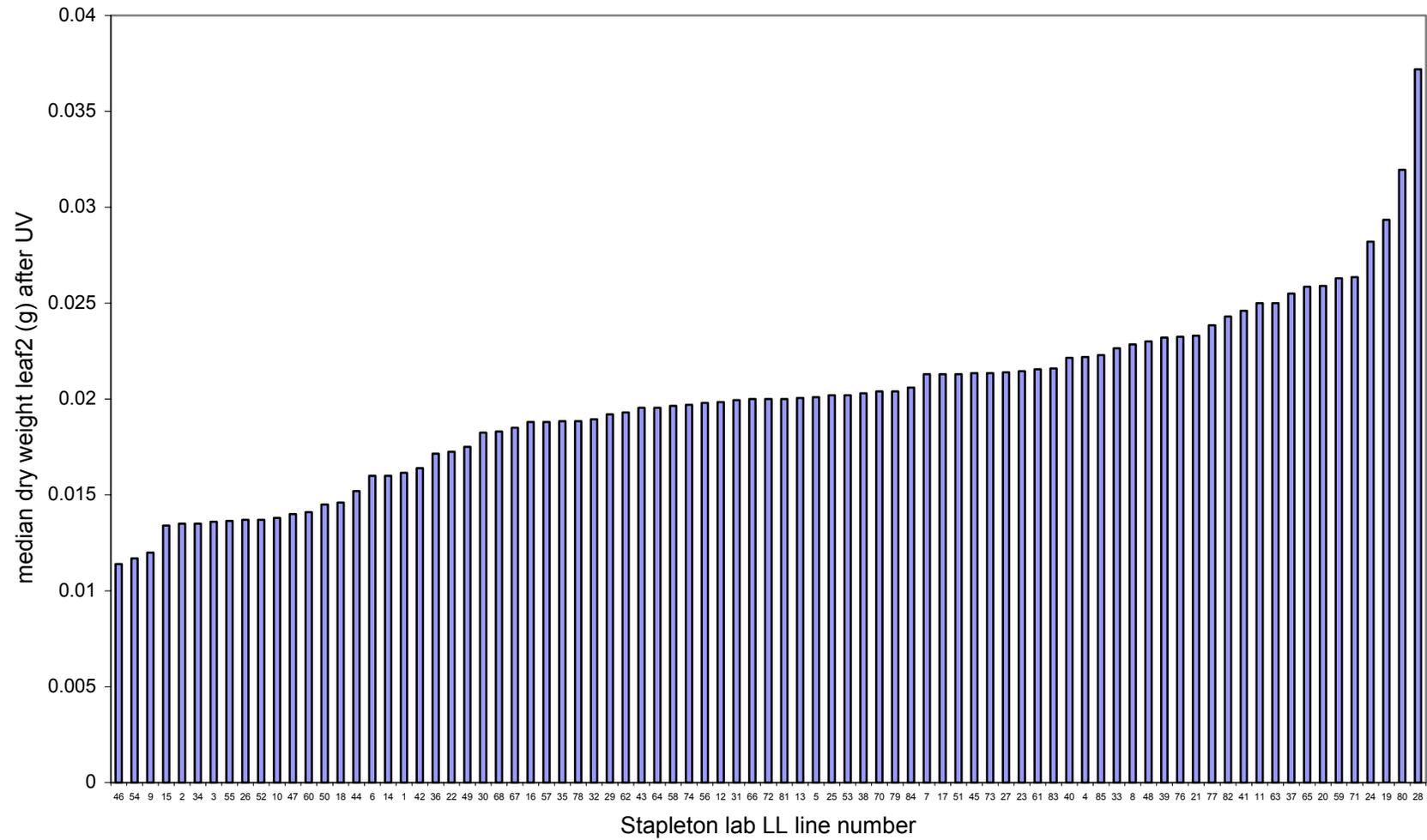


Figure 3. Distribution of Stapleton lab LL for median of dry weight of second leaf after UV.

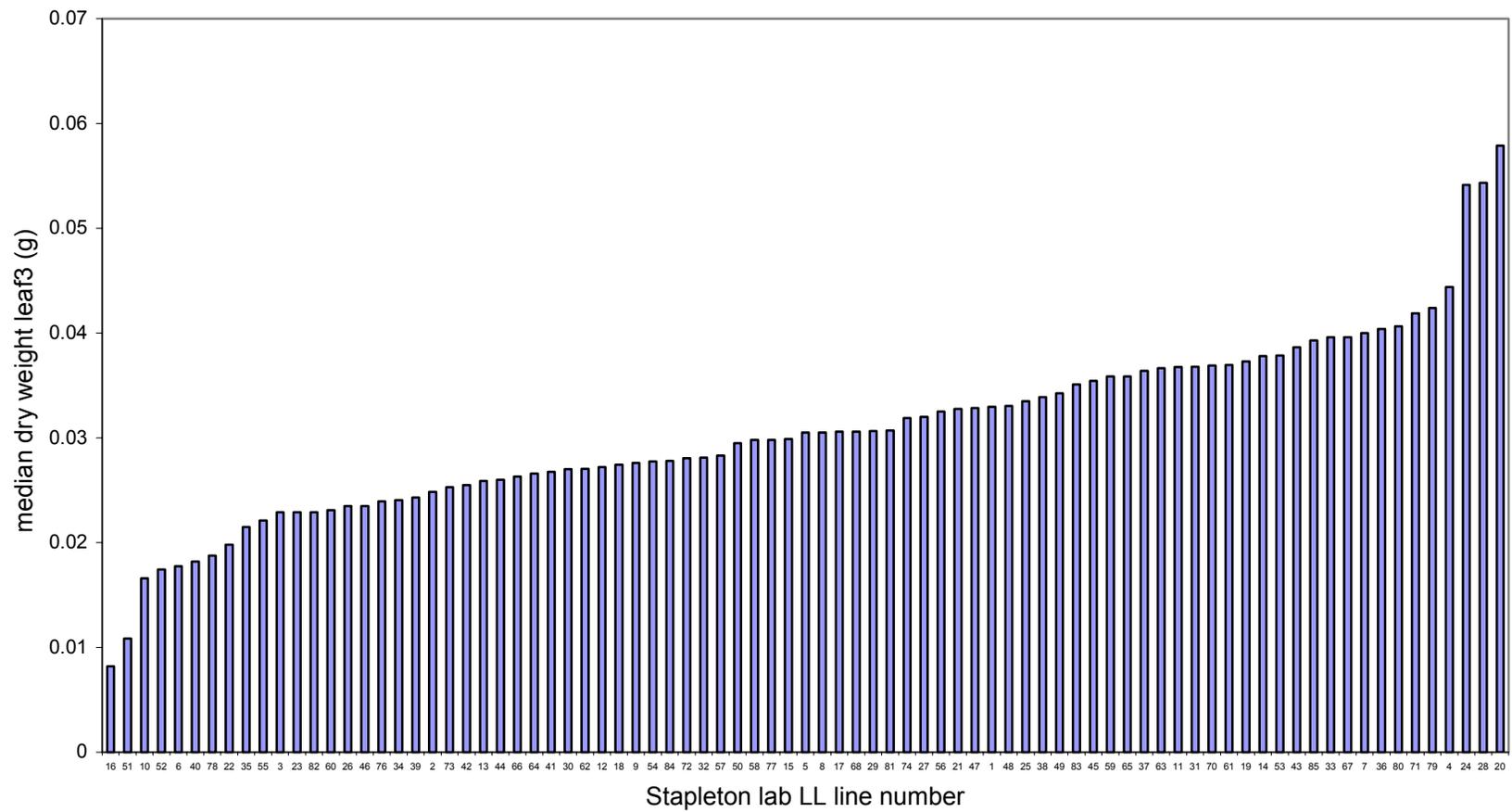


Figure 4. Distribution of Stapleton lab LL for median of dry weight of third leaf.

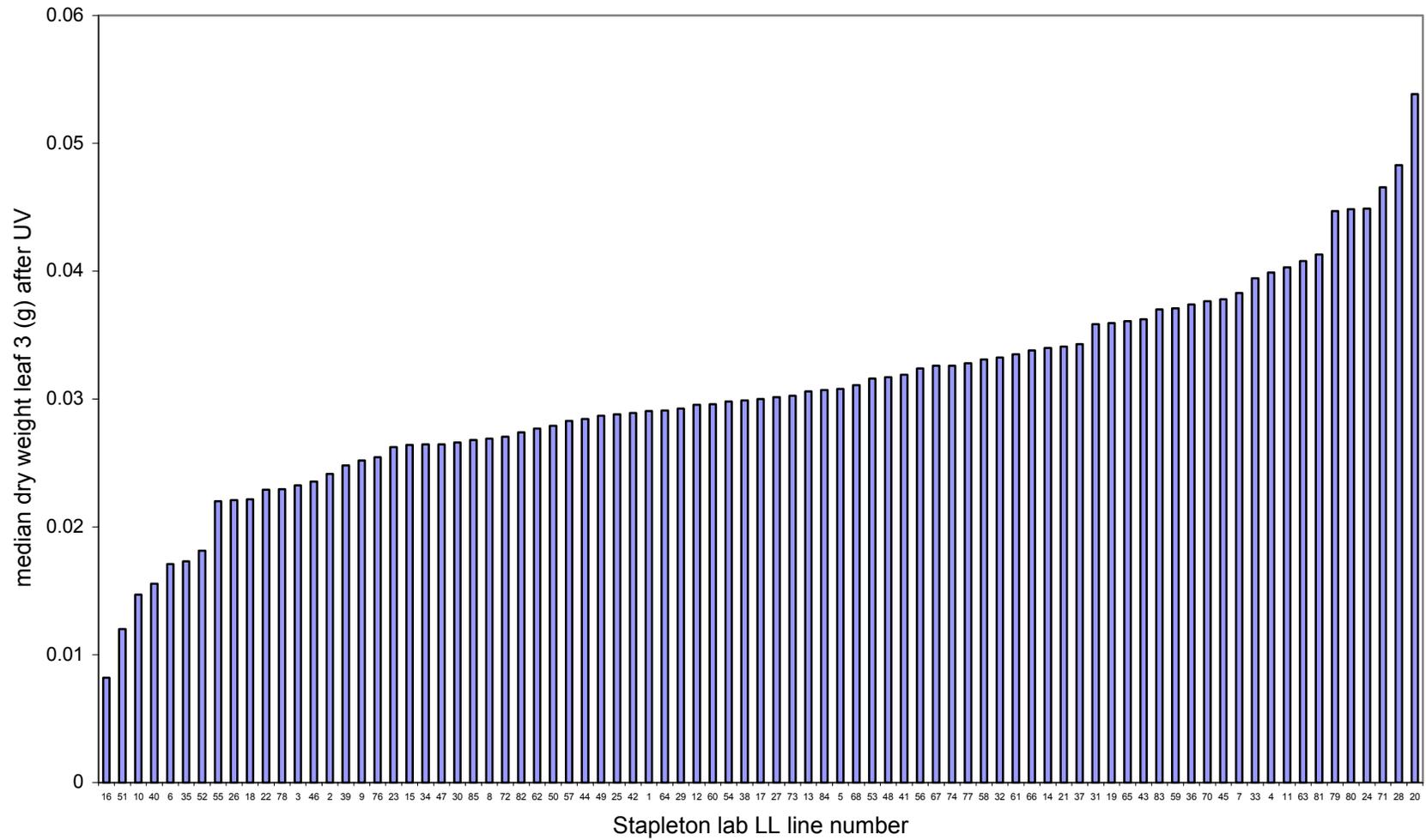


Figure 5. Distribution of Stapleton lab LL for median of dry weight of third leaf after UV.

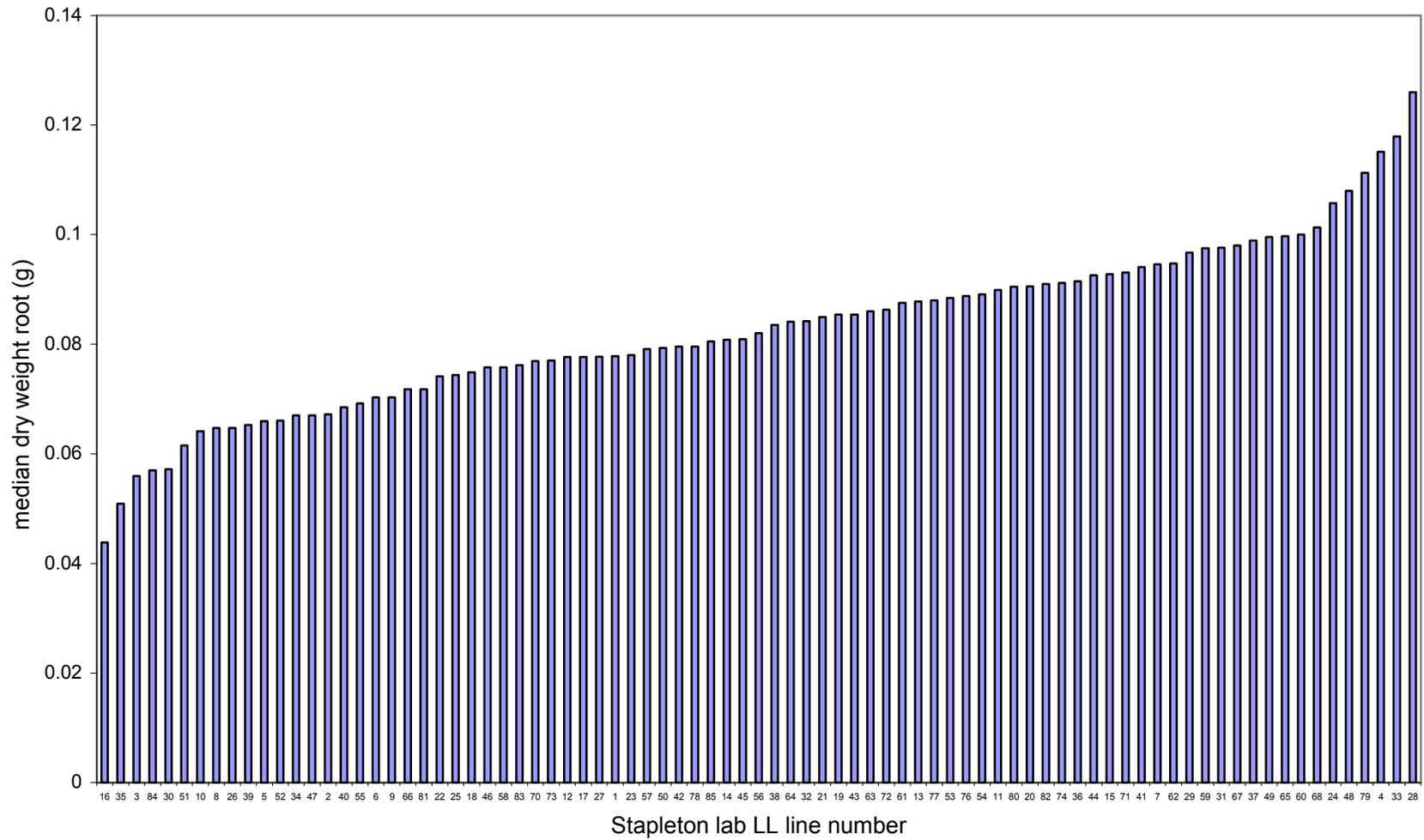


Figure 6. Distribution of Stapleton lab LL for median of dry weight of root.

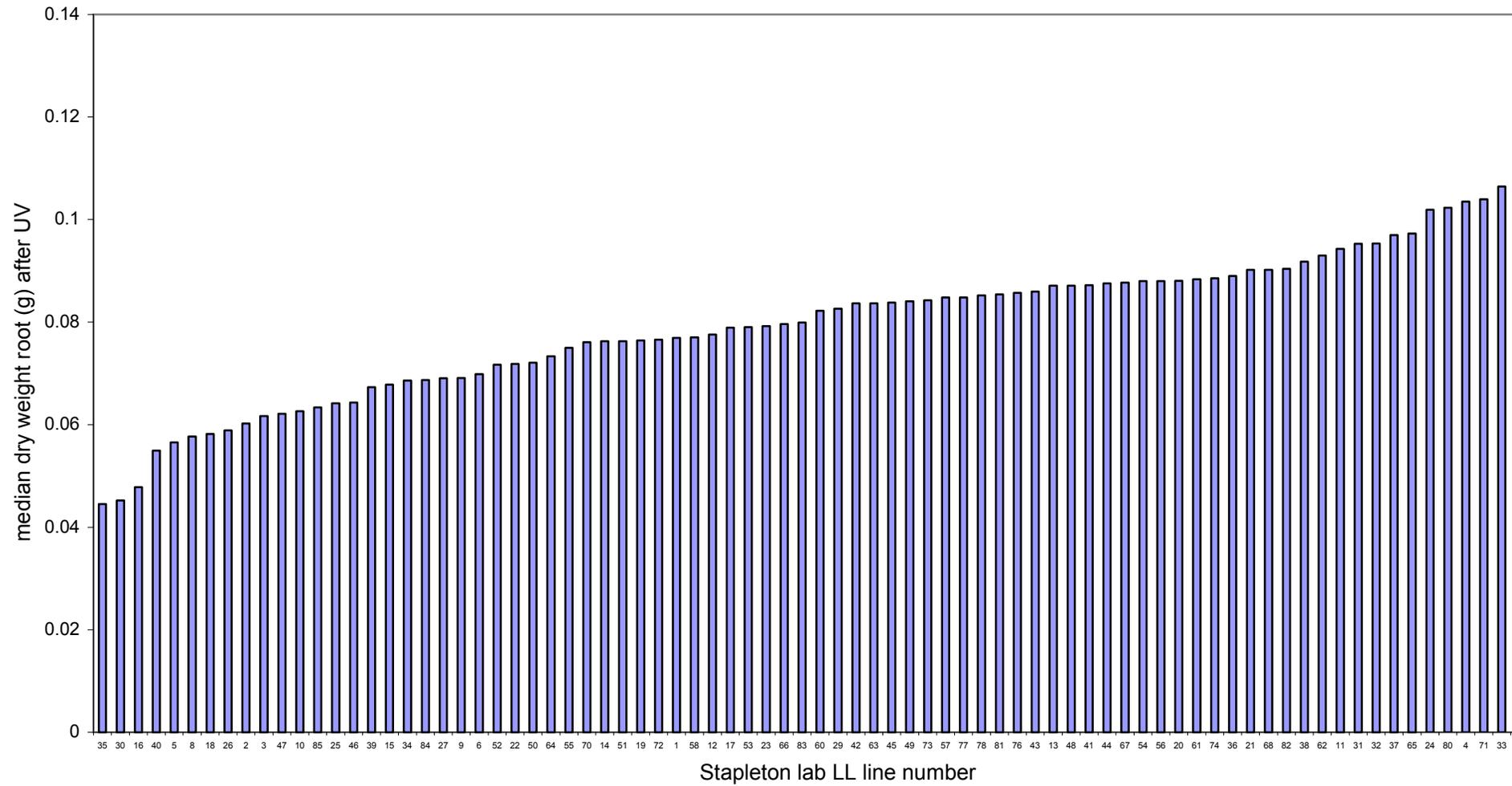


Figure 7. Distribution of Stapleton lab LL for median of dry weight of root after UV.

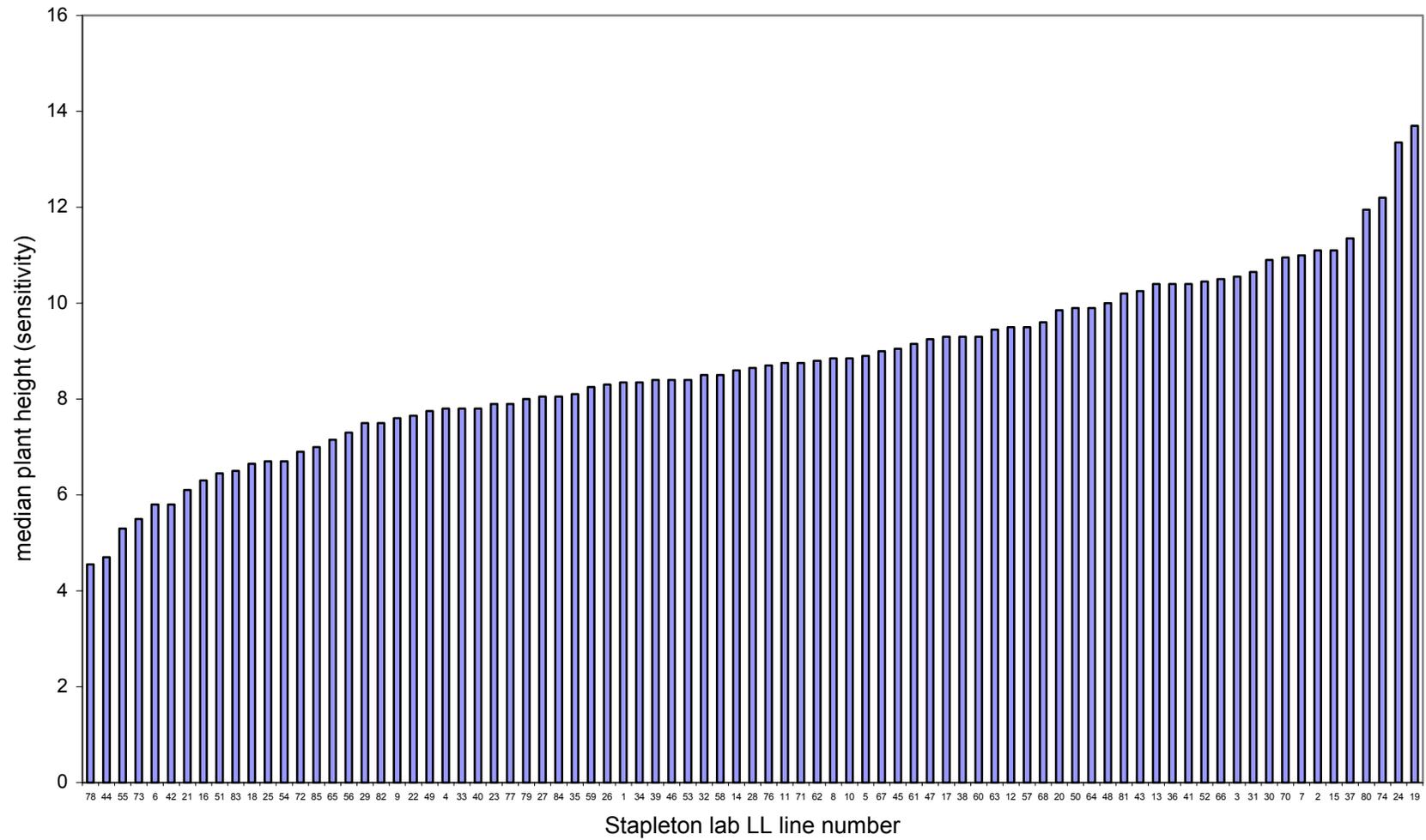


Figure 8. Distribution of Stapleton lab LL for median of plant height (sensitivity).

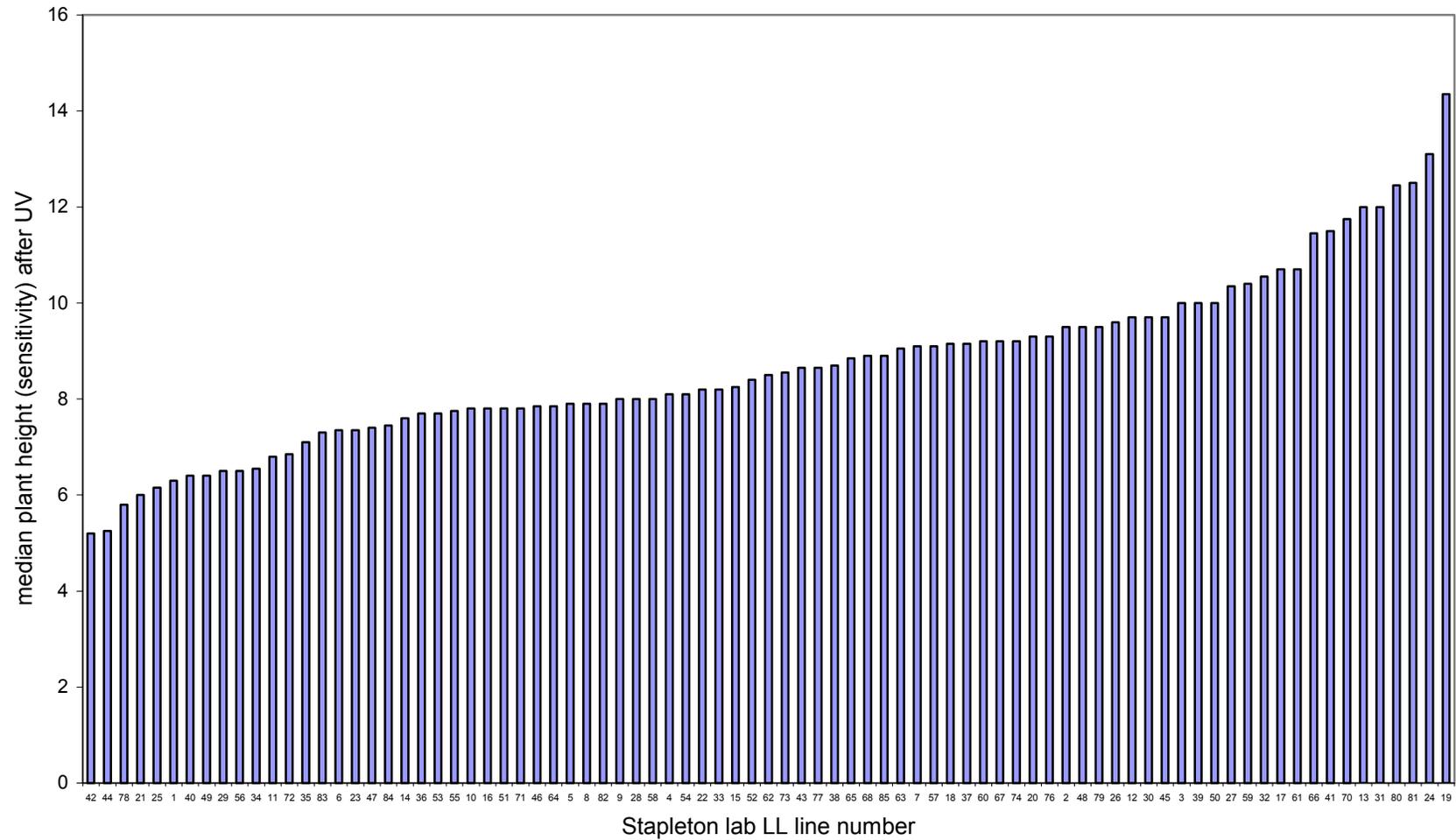


Figure 9. Distribution of Stapleton lab LL for median of plant height after UV (sensitivity).

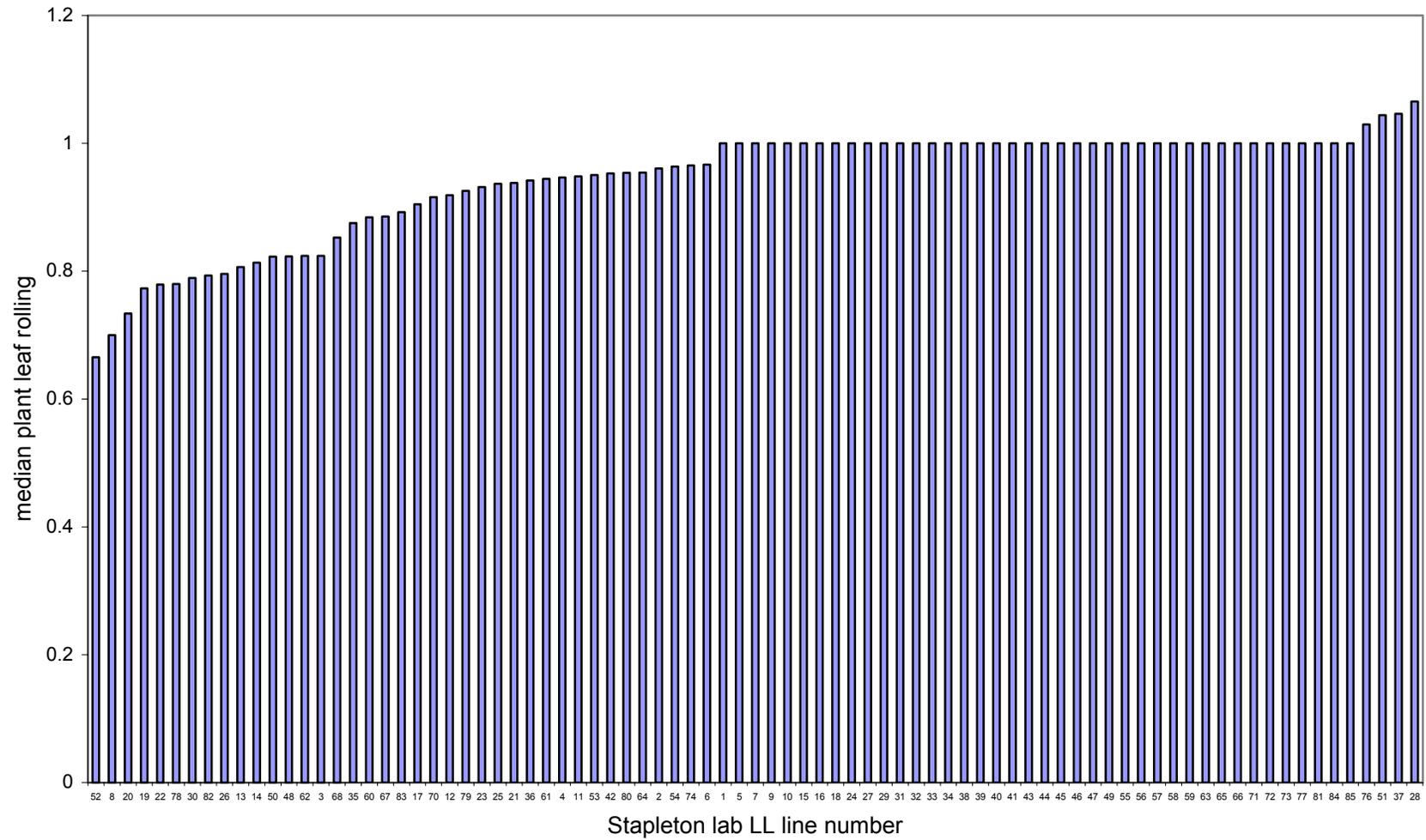


Figure 10. Distribution of Stapleton lab LL for median of plant leaf rolling.

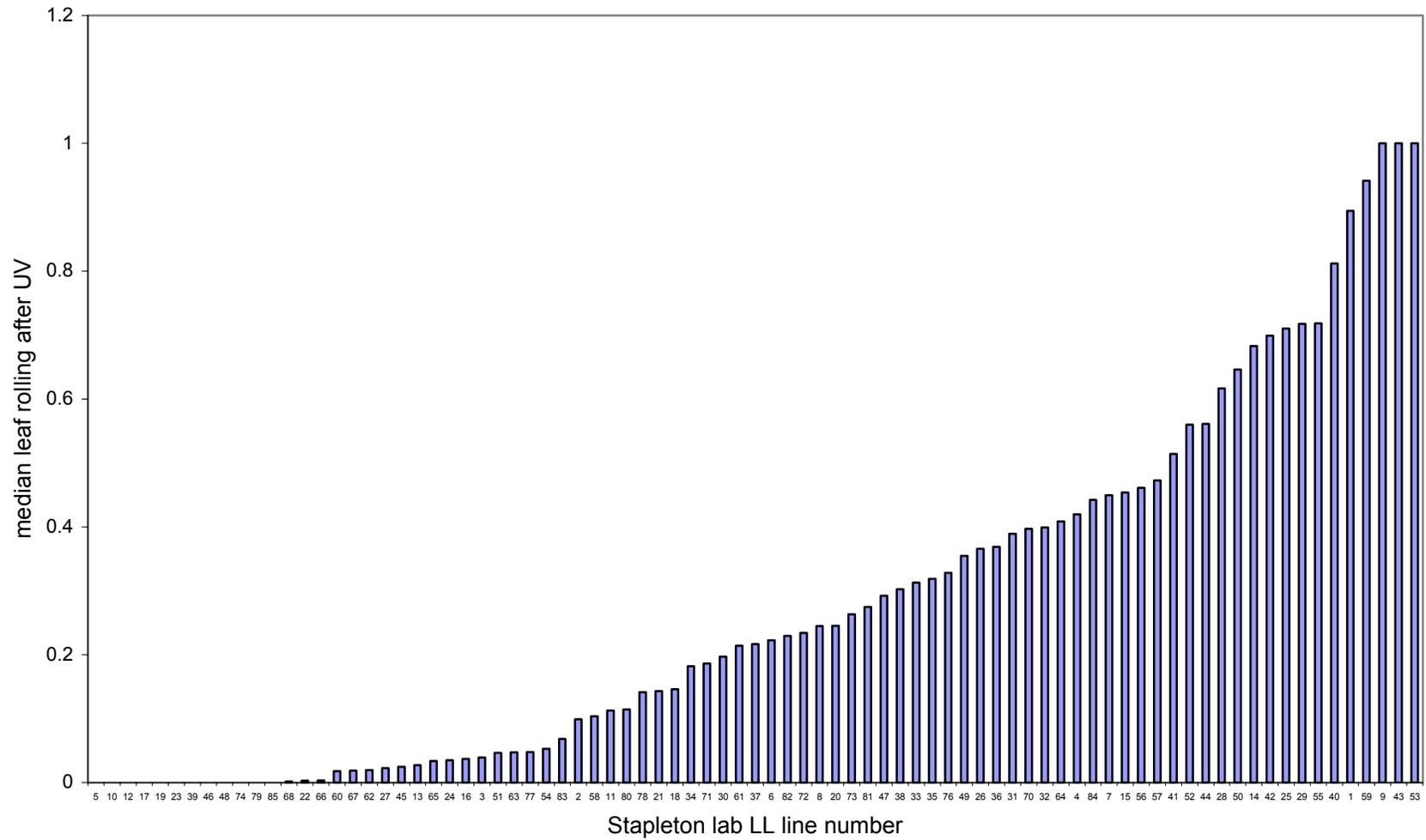


Figure 11. Distribution of Stapleton lab LL for median of plant leaf rolling after UV (sensitivity).

## Composite Interval Mapping (CIM) of QTL

Results of composite interval mapping (CIM) are listed in Table 4 and likelihood-ratio statistic profile plots for each trait are presented from Figure 3 to Figure 12. Overall, 12 QTL with LOD scores (logarithm of odds ratio) ranging from 3.3 to 4.4, and spread over five chromosomes were identified. A QTL between Marker 5 and Marker 6 on chromosome 7 was detected in three traits, which are dry weight of second leaf, dry weight of third leaf and dry weight of root. In addition to this QTL, another QTL (near Marker 18 on chromosome 2) was detected for both height and leaf rolling traits.

## Multitrait Composite Interval Mapping (MCIM)

MCIM was conducted for the same trait in different environments (control and treatment) for five traits separately. Results of multitrait composite interval mapping (MCIM) are listed in Table 5 and likelihood-ratio statistic profile plots for each trait are presented from Figure 13 to Figure 17. A total of 8 QTL were identified. Of these 8 QTL, two were detected by control MCIM, treatment MCIM and joint MCIM; three were detected by both control MCIM and joint MCIM; one was detected by treatment MCIM and joint MCIM; only one was just found by joint MCIM. No QTL was detected by multitrait composite interval mapping about traits of dry weight of third leaf and leaf rolling. Only two QTL, which are near Marker 8 and between Marker 5 and Marker 6 on chromosome 7, were detected by both CIM and MCIM.

Table 4. Composite interval mapping

Trait	Control			Treatment		Treatment - Control	
	Chromosome	Marker	LOD score	Marker	LOD score	Marker	LOD score
Dry weight of leaf 2	7	5-6& 8 ( <i>php20581a-umc1600</i> & <i>umc1983</i> )	3.5	-	-	2 ( <i>umc1378</i> )	3.7
Dry weight of leaf 3	5	-	-	16 ( <i>nbp35</i> )	3.5	-	-
	6	-	-	-	-	14 ( <i>umc38a</i> )	3.3
	7	5-6 ( <i>php20581a-umc1600</i> )	3.6	5-6 ( <i>php20581a-umc1600</i> )	3.5	-	-
Dry weight of root	7	-	-	5-6 ( <i>php20581a-umc1600</i> )	3.6	-	-
Plant height	2	-	-	-	-	18 ( <i>umc1604</i> )	4.1
	4	-	-	22 ( <i>umc1842</i> )	3.5	-	-
Leaf rolling	2	-	-	18 ( <i>umc1604</i> )	4.4	18 ( <i>umc1604</i> )	3.9

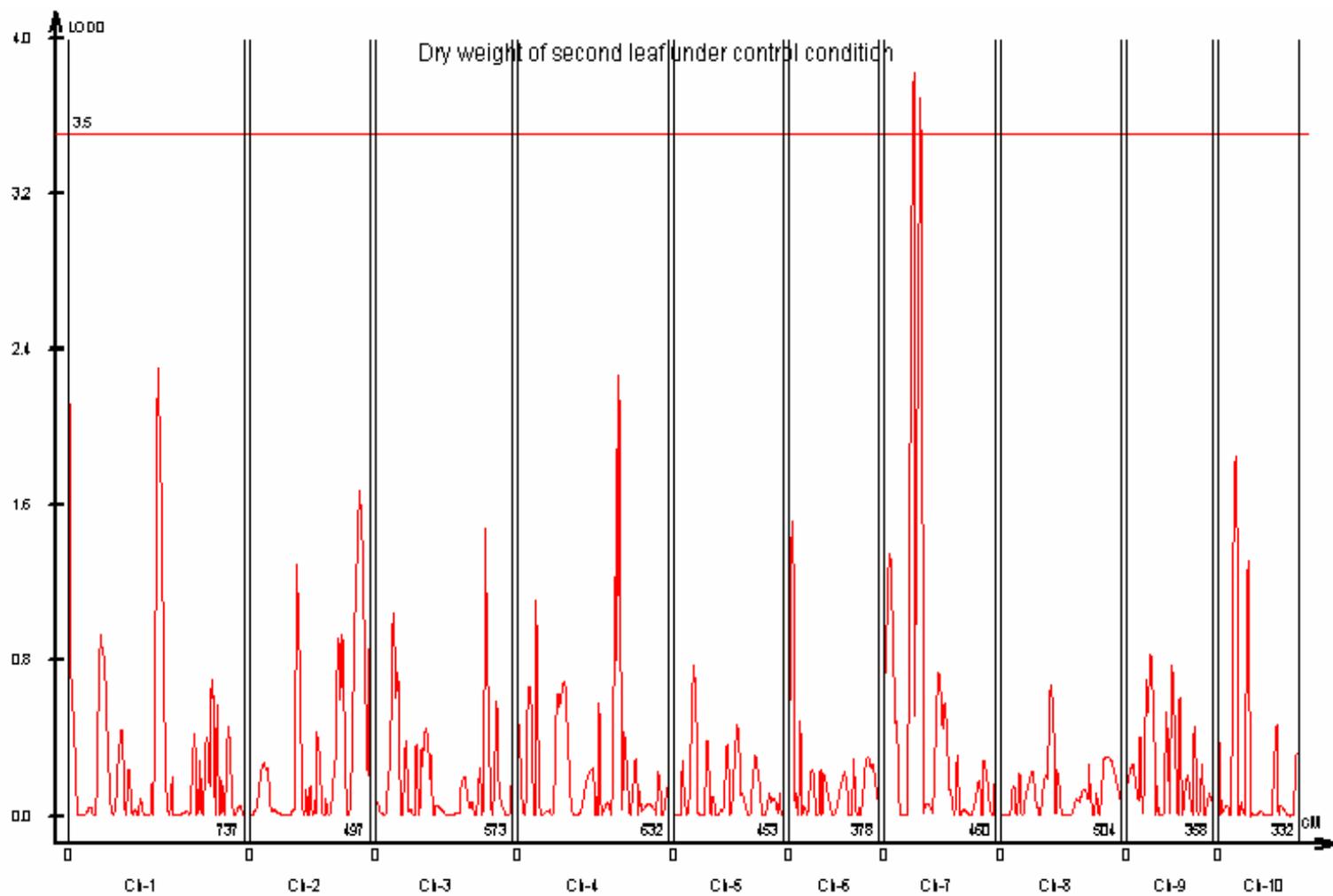


Figure 12. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of second leaf under control condition.

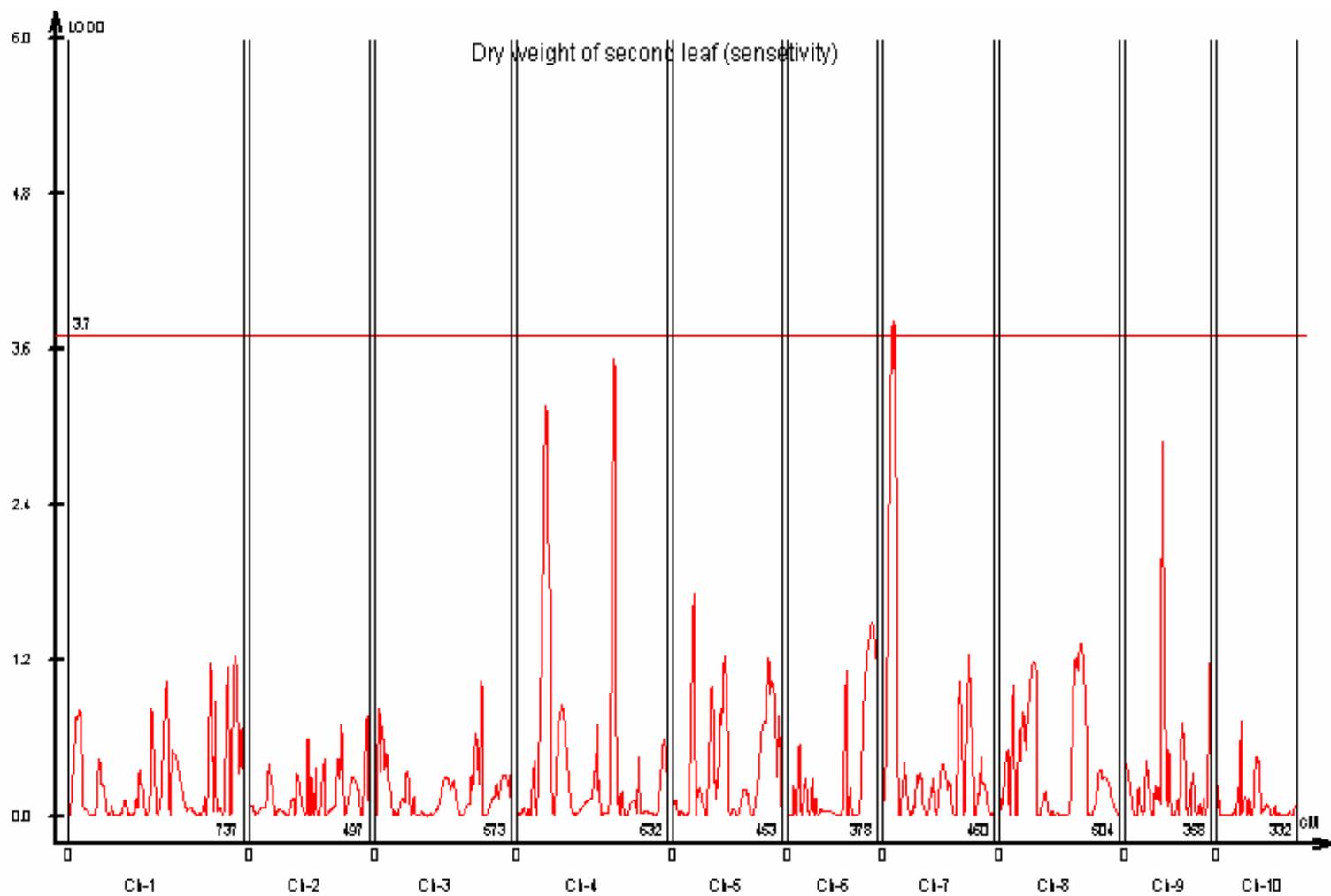


Figure 13. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of second leaf (Sensitivity).

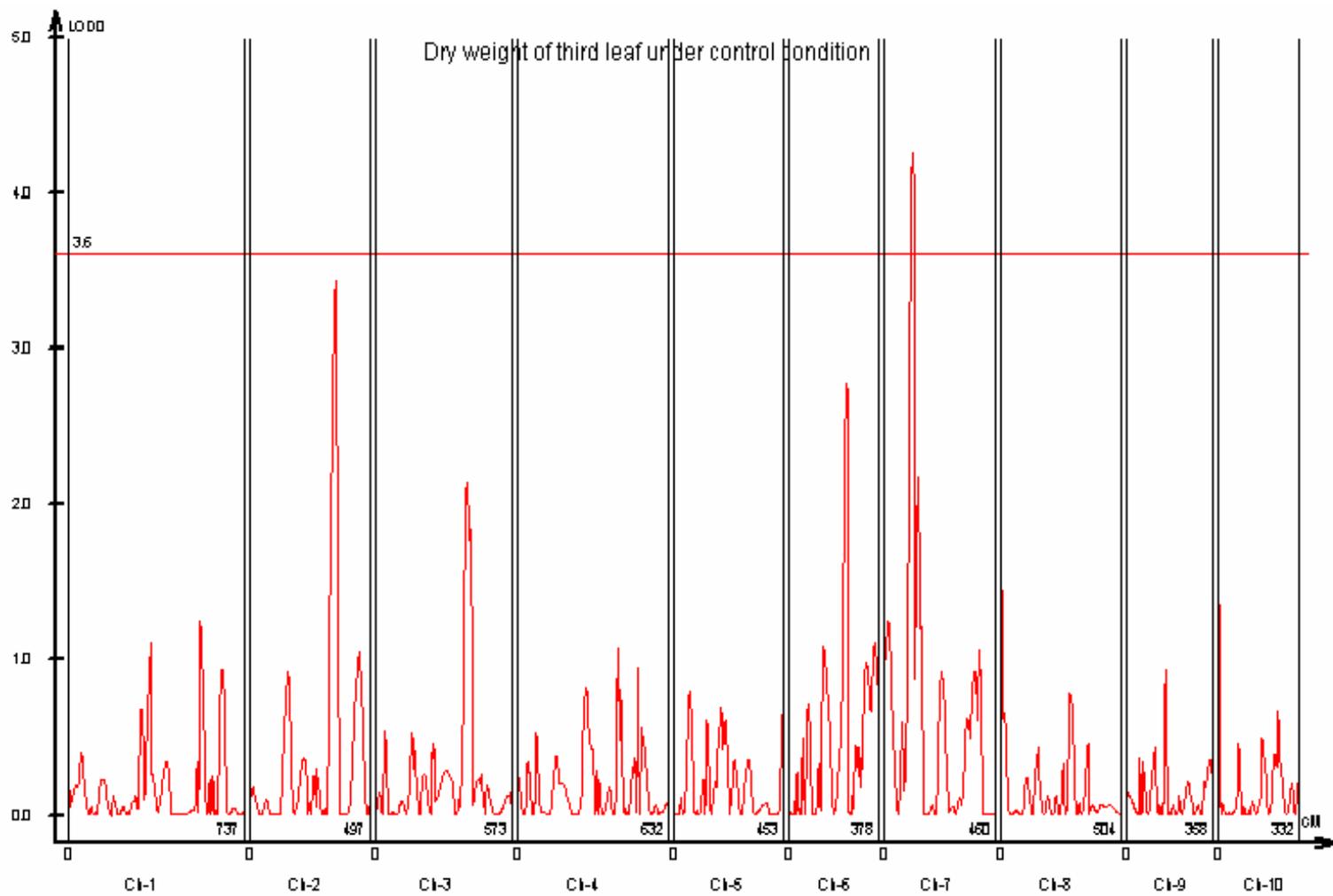


Figure 14. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of third leaf under control condition.

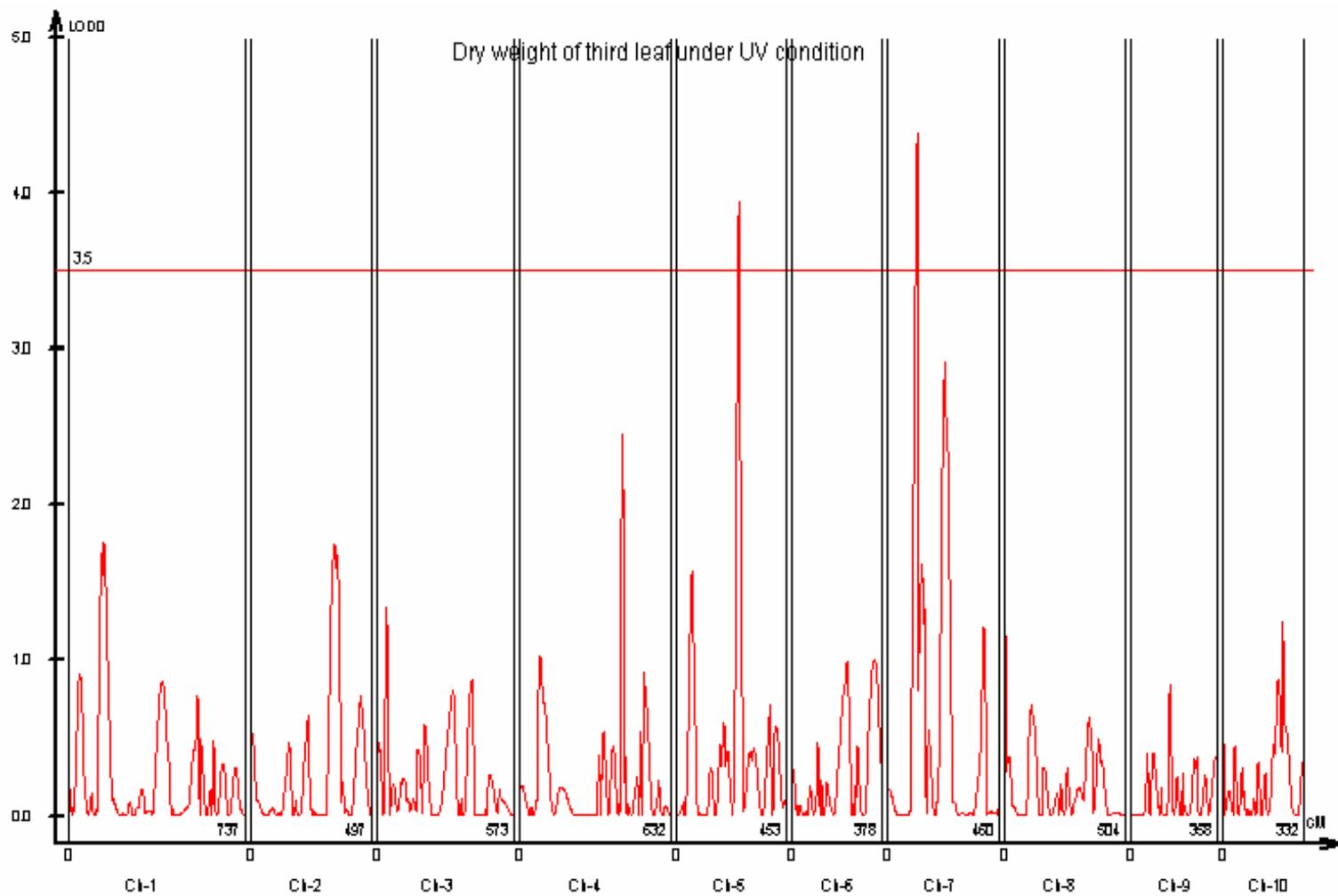


Figure 15. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of third leaf under UV condition.

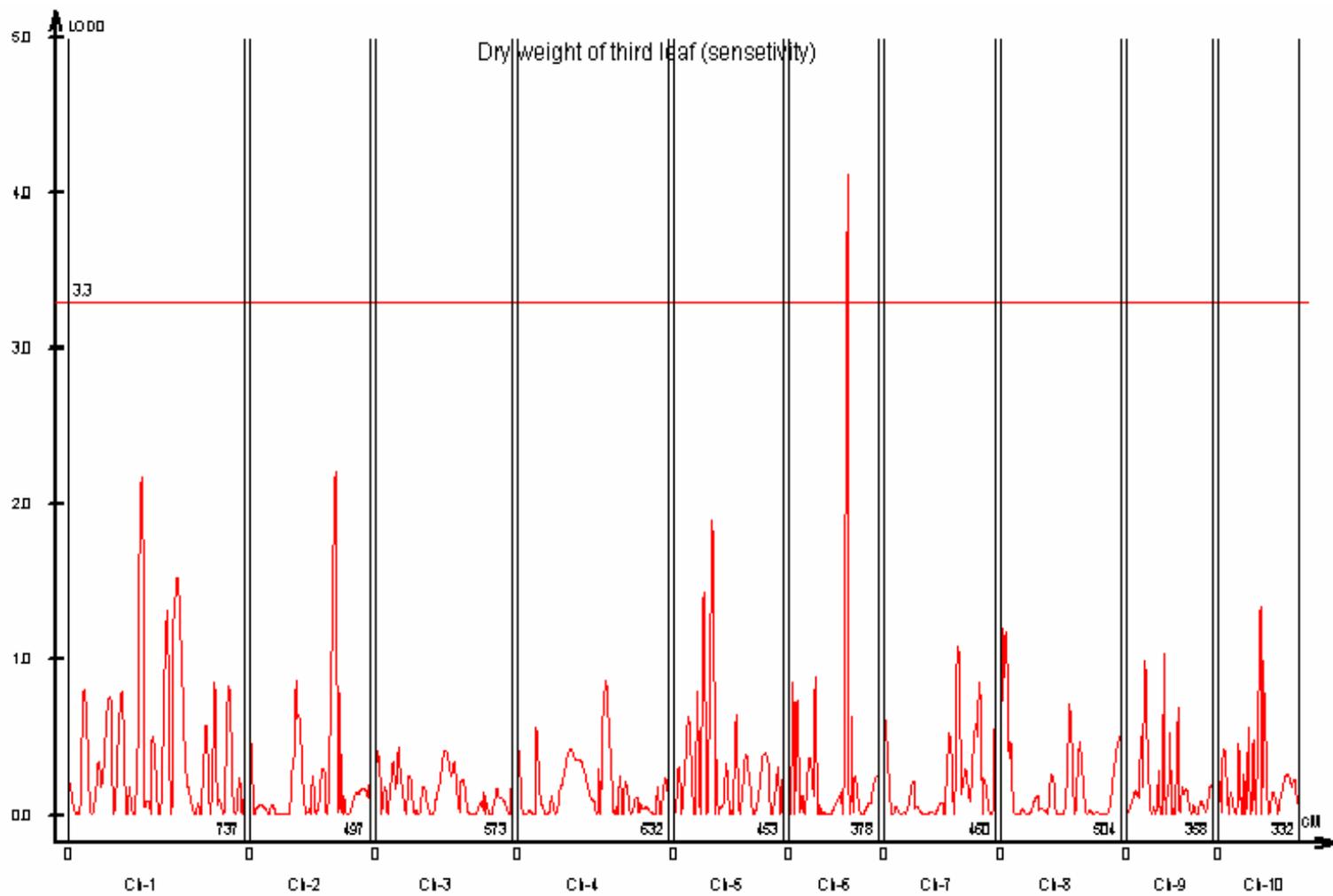


Figure 16. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of third leaf (sensitivity).

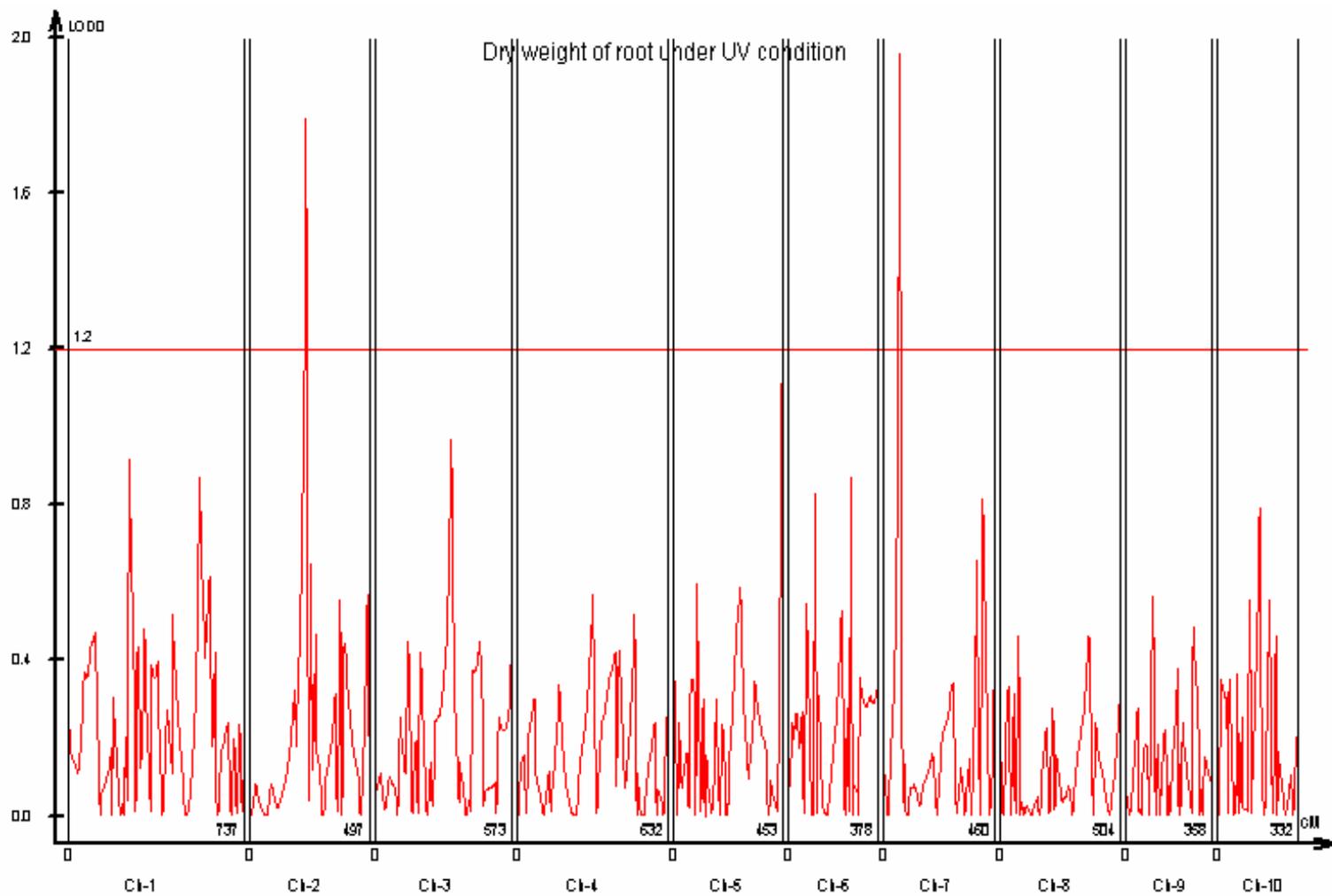


Figure 17. LOD statistic profile plots generated from composite interval mapping analyses for dry weight of root under UV condition.

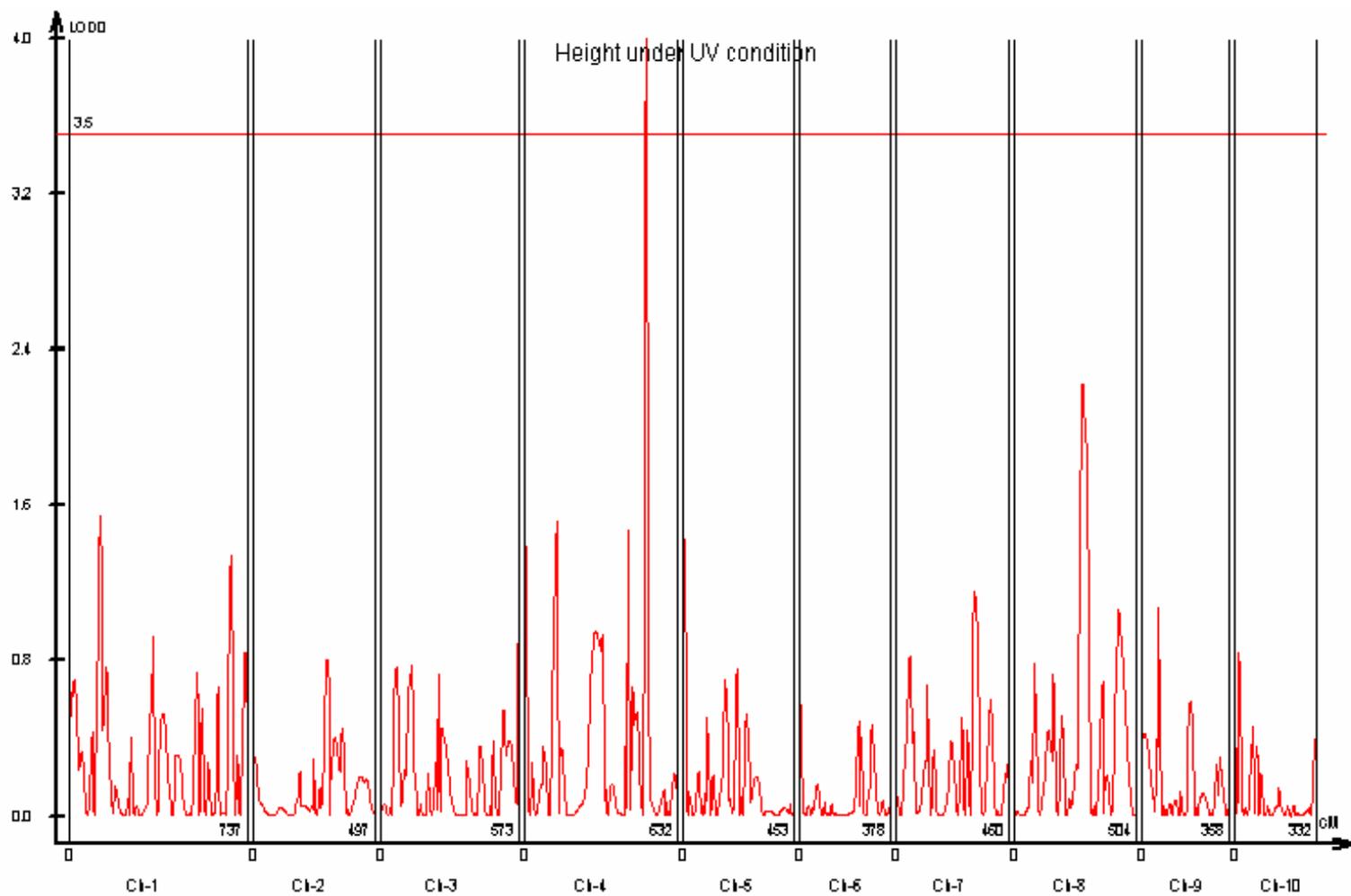


Figure 18. LOD statistic profile plots generated from composite interval mapping analyses for plant height under UV condition.

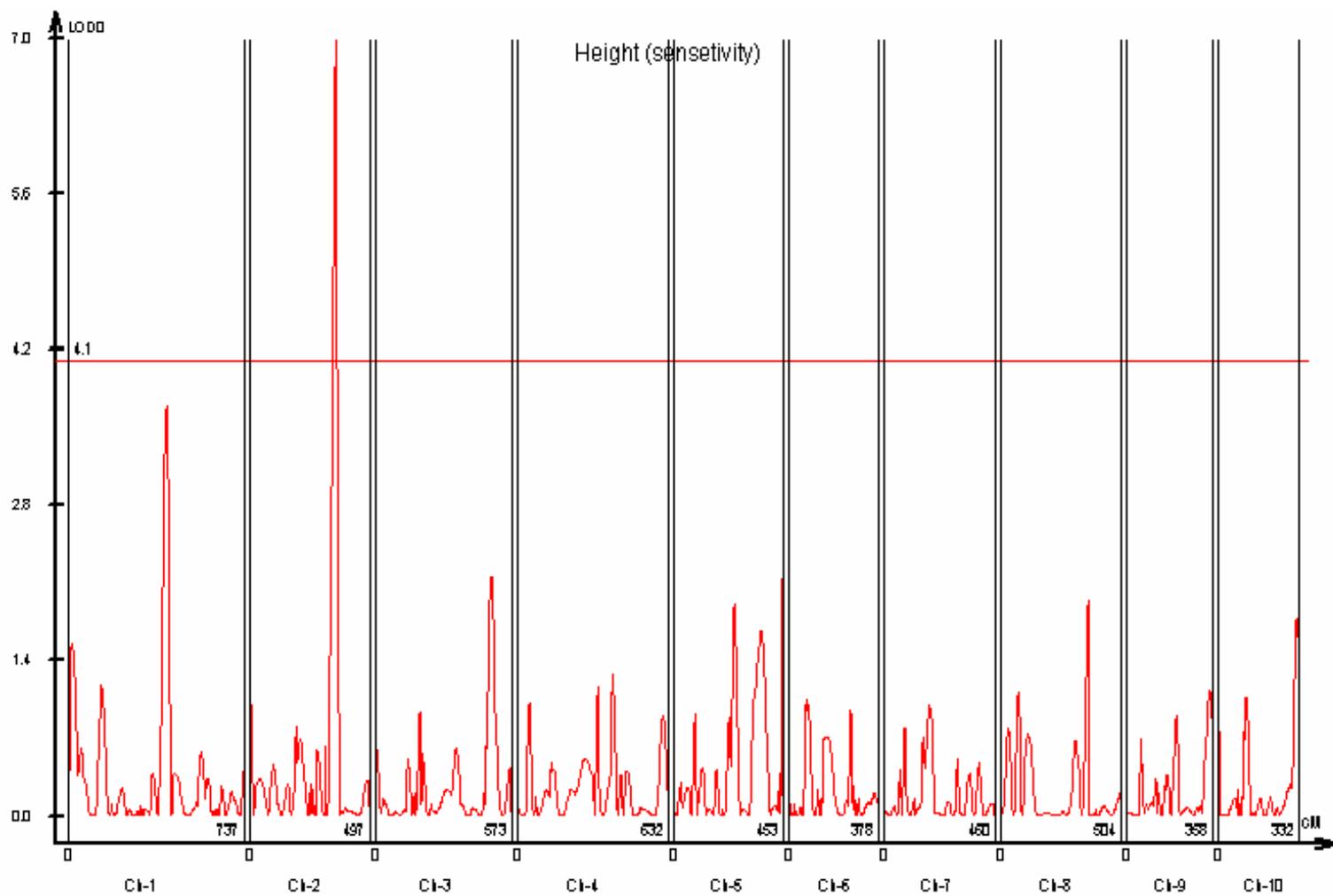


Figure 19. LOD statistic profile plots generated from composite interval mapping analyses for height (sensitivity).

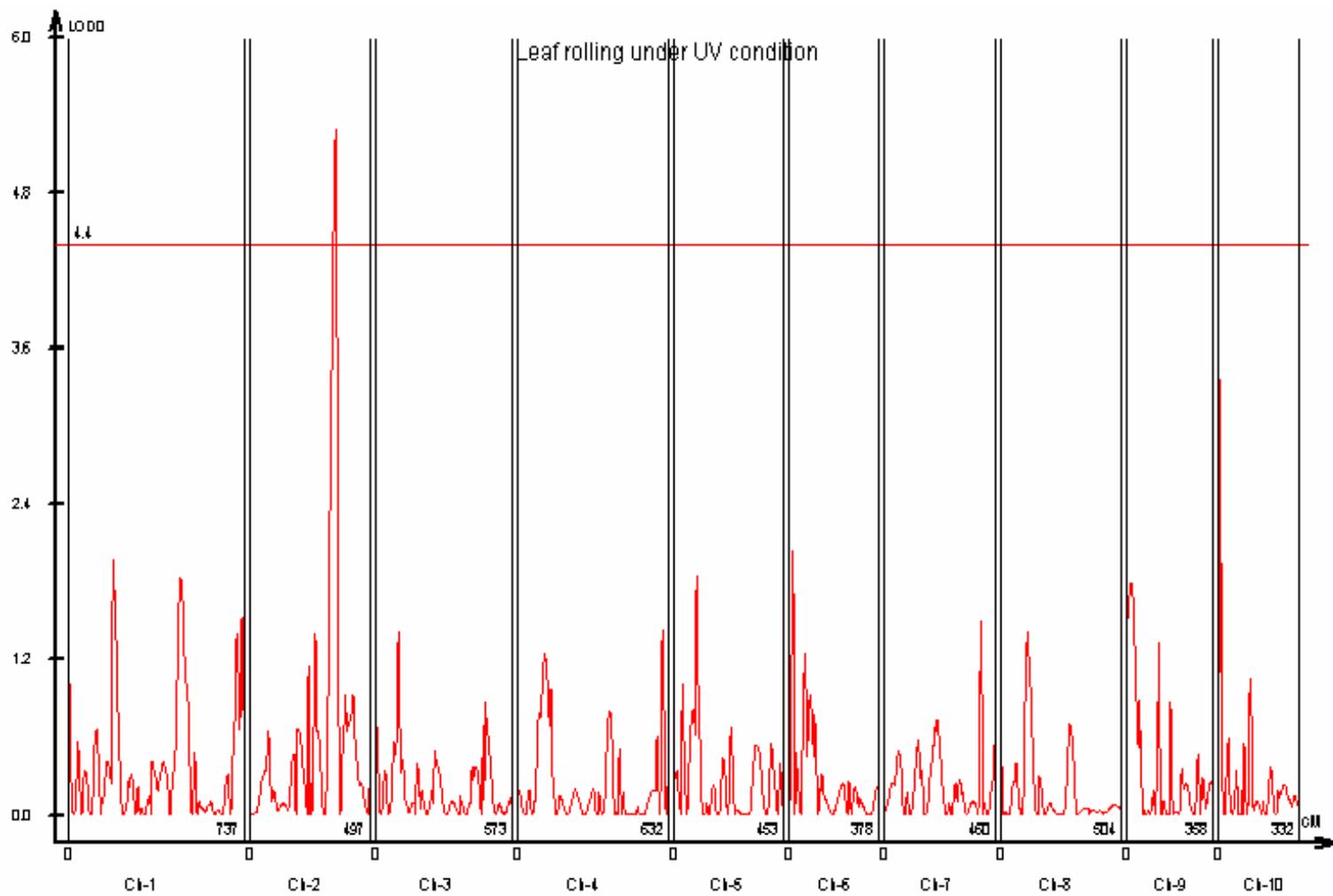


Figure 20. LOD statistic profile plots generated from composite interval mapping analyses for Leaf rolling under UV condition.

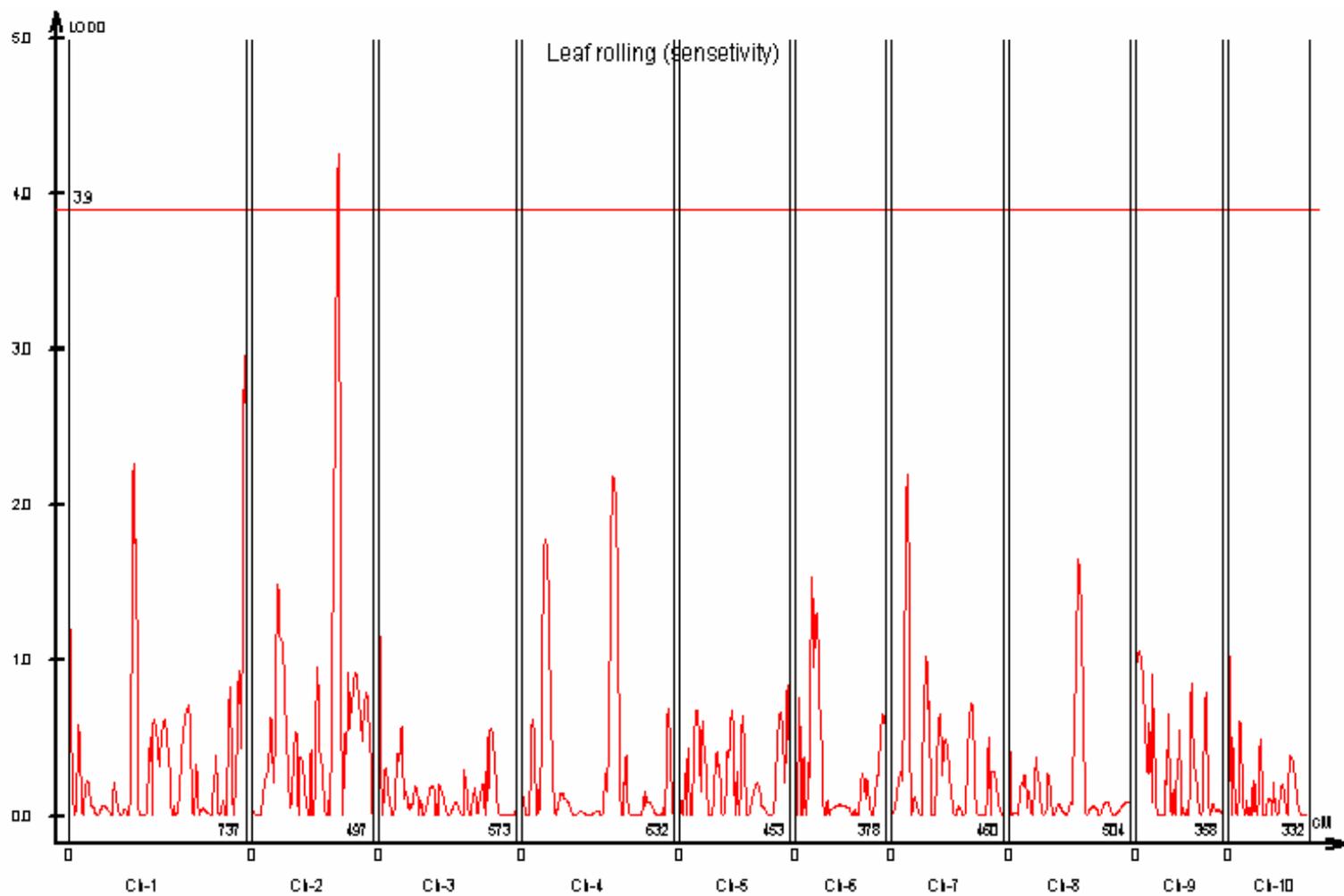


Figure 21. LOD statistic profile plots generated from composite interval mapping analyses for leaf rolling (sensitivity).

Table 5. Mutitrait composite interval mapping

Trait	Control			Treatment		Joint traits	
	Chromosome	Marker	LOD score	Marker	LOD score	Marker	LOD score
DW2	1	2 ( <i>tub1</i> )	3.6	2 ( <i>tub1</i> )	3.6	2 ( <i>tub1</i> )	3.6
	7	5-6& 8 ( <i>php20581a-umc1600</i> & <i>umc1983</i> )	3.6	5-6 ( <i>php20581a-umc1600</i> )	3.6	5-6& 8 ( <i>php20581a-umc1600</i> & <i>umc1983</i> )	3.6
	10	-	-	5-6 ( <i>nip285a-umc2069</i> )	3.6	5-6 ( <i>nip285a-umc2069</i> )	3.6
DW3	-	-	-	-	-	-	-
DWR	7	-	-	-	-	12-13 ( <i>bnlg1070-umc56</i> )	3.3
	10	14 ( <i>bnl10.13a</i> )	3.3	-	-	14 ( <i>bnl10.13a</i> )	3.3
Plant height	3	28 ( <i>umc1641</i> )	3.5	-	-	28 ( <i>umc1641</i> )	3.5
	4	11 ( <i>bnlg490</i> )	3.5	-	-	11 ( <i>bnlg490</i> )	3.5
Leaf rolling	-	-	-	-	-	-	-

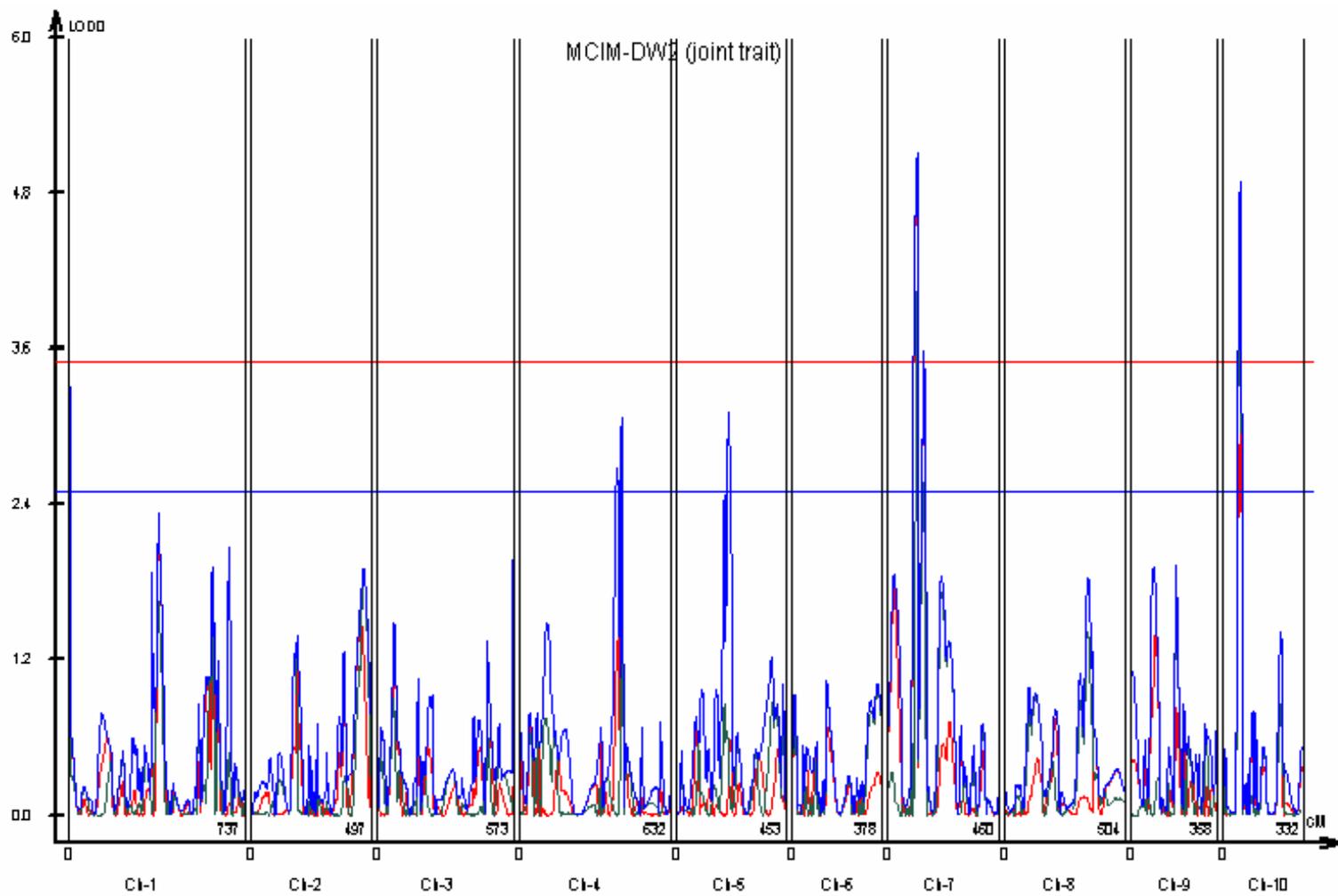


Figure 22. LOD statistic profile plots generated from multitrait composite interval mapping analyses for dry weight of second leaf.

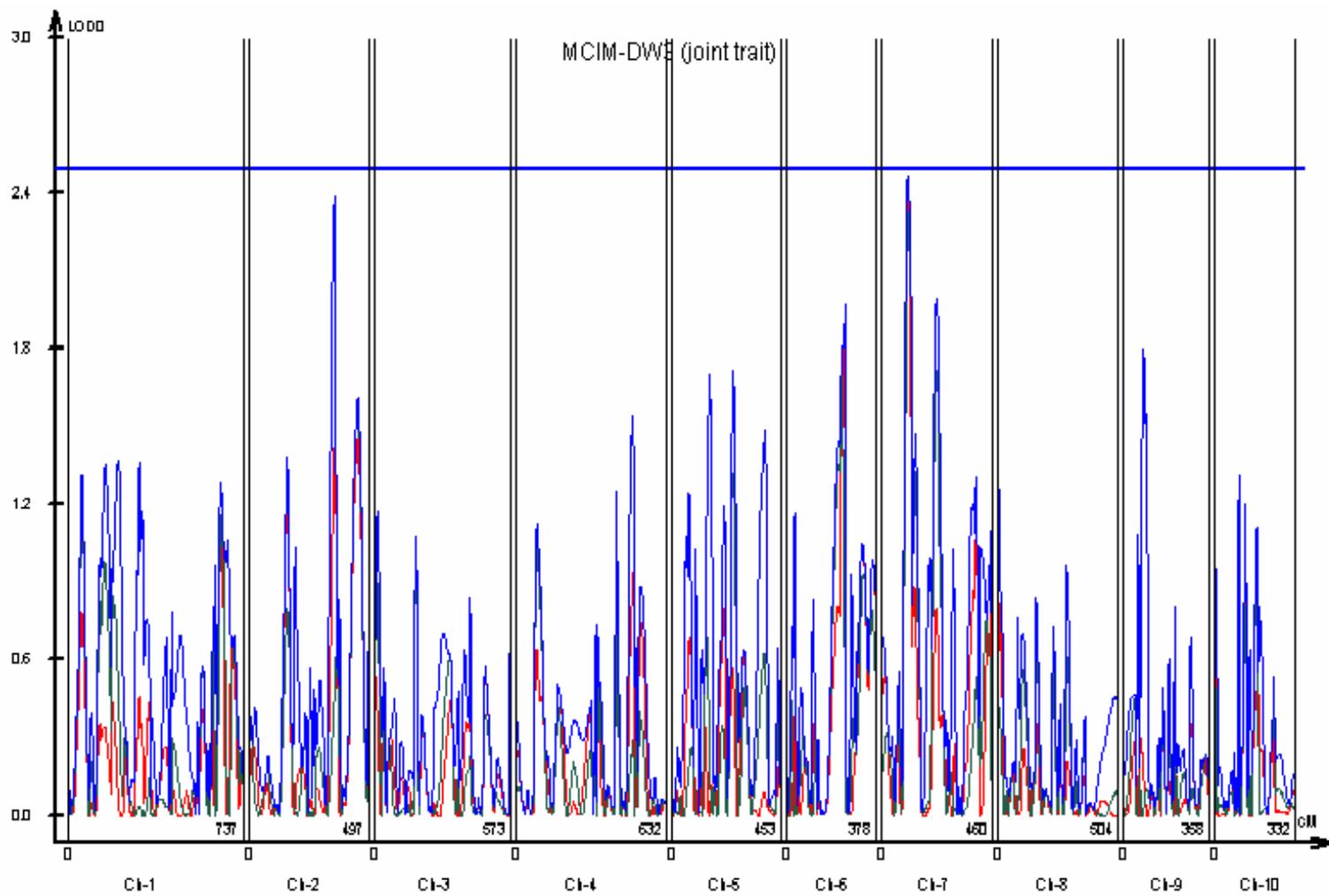


Figure 23. LOD statistic profile plots generated from multitrait composite interval mapping analyses for dry weight of third leaf.

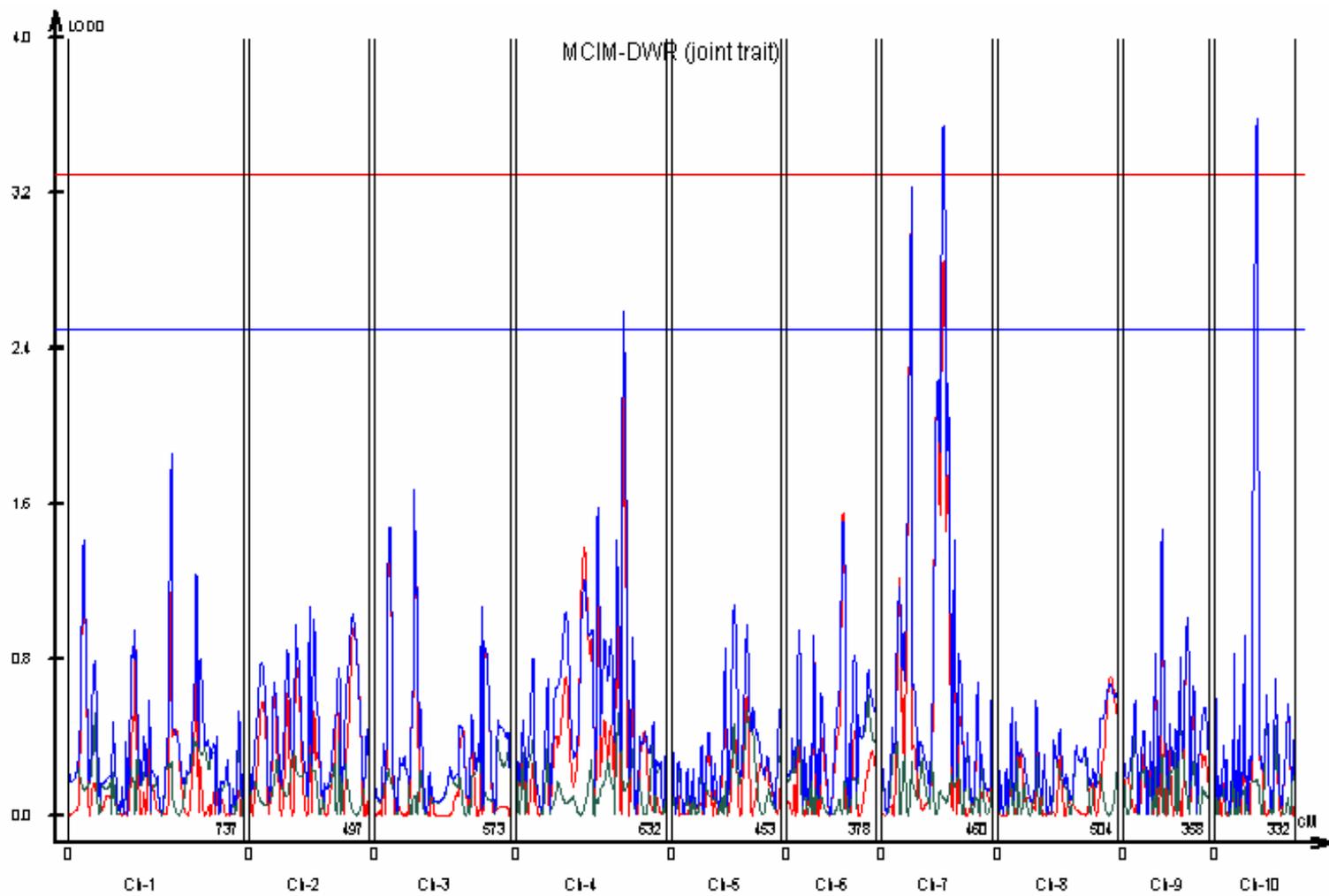


Figure 24. LOD statistic profile plots generated from multitrait composite interval mapping analyses for dry weight of root.

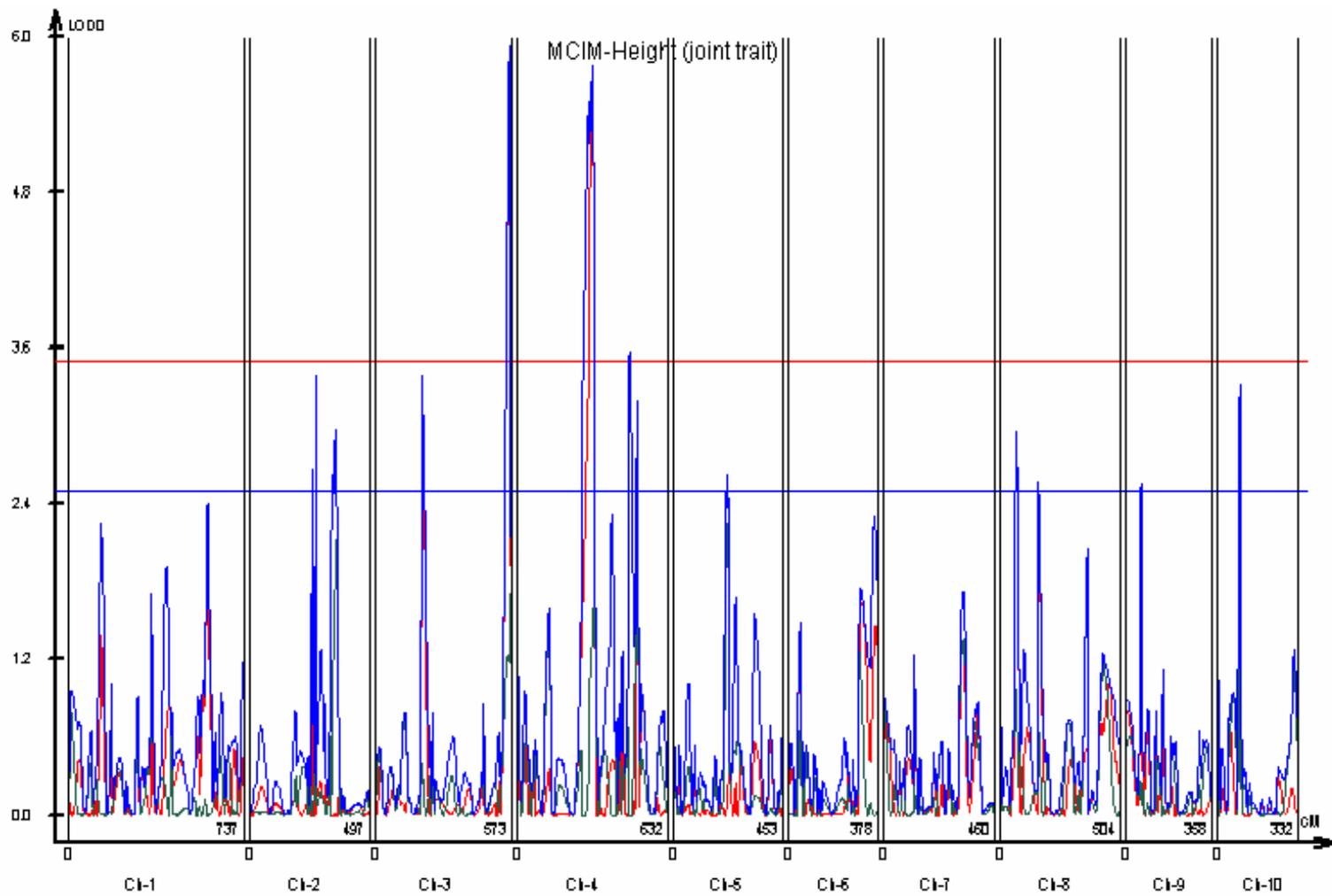


Figure 25. LOD statistic profile plots generated from multitrait composite interval mapping analyses for dry weight of plant height.

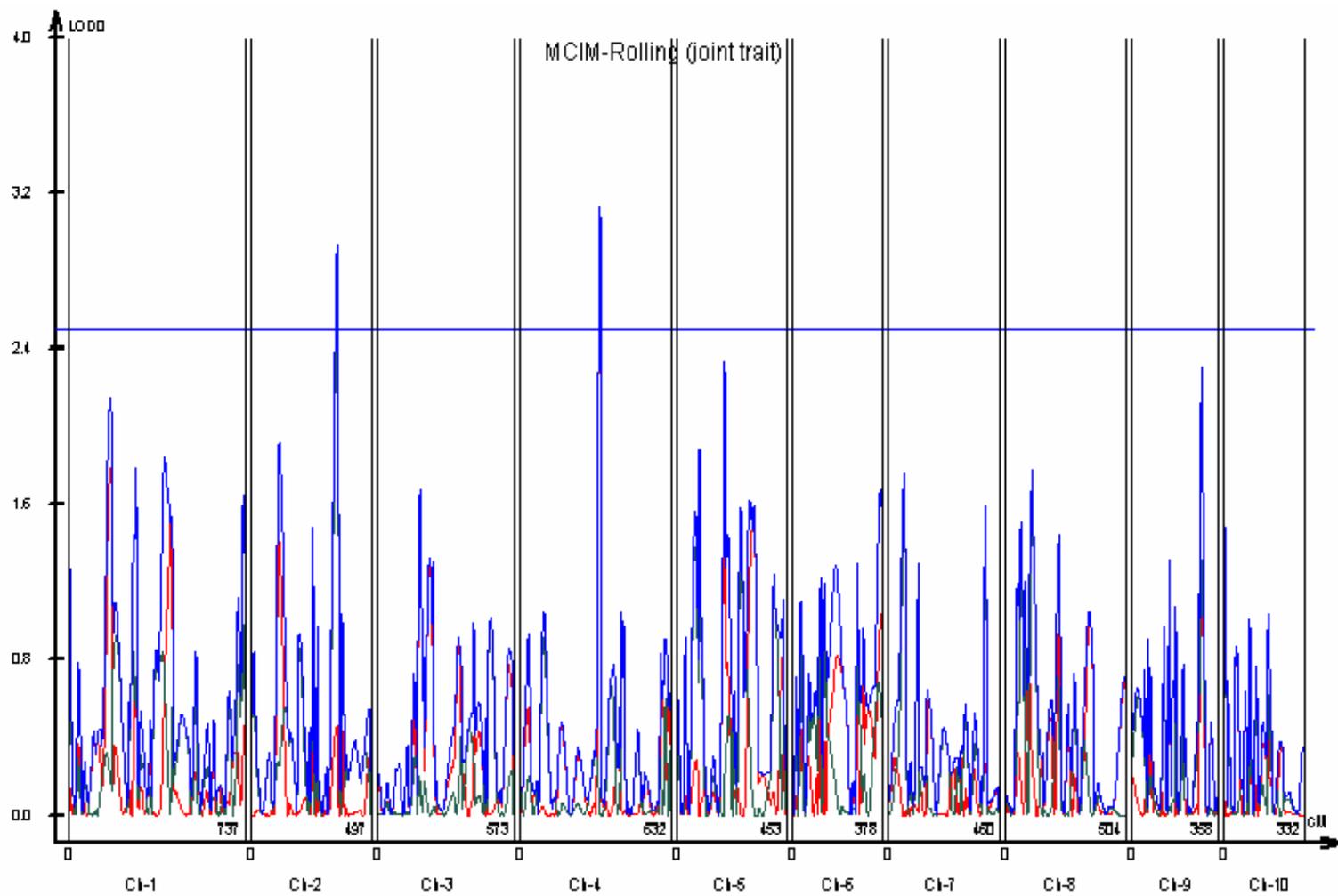


Figure 26. LOD statistic profile plots generated from multivariate composite interval mapping analyses for leaf rolling.

## DISCUSSION

If there is a linear relationship between the measured response and the absolute dose of UV, then increased dose will cause the same response, whether that dose is applied over a long or short time period. This kind of linear response is termed reciprocity (Coohill, 1992). I found that the timing of applied dose does affect the response, so reciprocity does not hold for the maize UV responses I measured.

As reciprocity does not hold, I increased the time of exposure per day to choose an appropriate dose for mapping of UV responses. The results of this time course (six, eight and ten hours per day for four days) were used to determine a UV dose that gave a significant response for 3 of 5 of the measured traits.

In the present study, the experimental results show that there is a set of genes for each different kind of UV response. Using CIM with five traits under three conditions, I identified as many as 12 putative QTL that were found to be present on five of the ten chromosomes. Of these 12 QTL, four QTL (between marker 5 and marker 6) located on chromosome 7 and three QTL (near marker 18) located on chromosome 2 may represent cases where the same QTL is affecting more than one trait. It is reasonable that a QTL is shared by DW2, DW3 and DWR, but it is interesting that a QTL is regulating both seedling height and leaf rolling. At present, most researchers agree that MCIM is an extension of CIM for improving the power and precision of QTL mapping (Jiang and Zeng, 1995). In this study, 6 of the 8 QTL detected by joint MCIM were new, another two loci for dry weight of second leaf at chromosome 7 were also detected by CIM. That means these two QTL may be considered the real detected QTL, and other QTL should therefore be considered candidate QTL until further evaluation.

Four QTL for sensitivity scores (treatment – control) for all traits were detected. Only one trait (leaf rolling) has a QTL at marker 18 that is significant for both treatment and for environment sensitivity, none of the other QTL for sensitivity scores was detected at the same region as those QTL for both control and treatment conditions. In my experiments the allelic sensitivity model was supported most often, in agreement with previous research (Leips and Mackay, 2000; Kliebenstein et al., 2002; Ungerer et al., 2003).

There are several caveats with respect to this study. First, I used the IBM 94 lines for this research; the precision of QTL localization will be affected by use of this small line set, because the marker space is too large in some intervals in the genetic map of the IBM 94 lines. For example, the marker distance between marker 5 and marker 6 at chromosome 7 is 33 cM that makes it difficult to precisely localize the QTL between two markers. In future experiments, fine-scale mapping may improve estimates of QTL positions. Further fine-scale mapping is possible, as more IBM lines and > 2000 markers are now available. Secondly, CIM and MCIM are not the final answer for QTL mapping; this method suffers from the choice of models, although there are some advantages, such as allowance of some marker genotype missing, multiple QTL detecting and great power to detect QTL (Broman, 2001). Therefore, new statistical tools must be developed to localize QTL accurately. Several new approaches have been theoretically developed recently, including Bayesian methods and the use of a genetic algorithm (Carlborg et al., 2000; Yi and Xu, 2000; Nakamichi et al., 2001).

In conclusion, my research is the first to detect QTL for maize UV responses. I have found a number of QTL with effects on maize morphological change under UV irradiation. I have set up a primary QTL pattern of UV responses that is a foundation to understanding the mechanisms plants use for adaptation to UV stress. Future study will include QTL mapping of

UV responses at multiple levels, including gene expression and improve the resolution of the QTL detection.

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