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Assessing the use of reflectance spectroscopy in determining CsCl stress in the model species *Arabidopsis thaliana*

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Reflectance spectroscopy is a rapid and non-destructive analytical technique that may be used for assessing plant stress and has potential applications for use in remediation. Changes in reflectance such as that due to metal stress may occur before damage is visible, and existing studies have shown that metal stress does cause changes in plant reflectance. To further investigate the potential use of reflectance spectroscopy as a method for assessing metal stress in plants, an exploratory study was conducted in which *Arabidopsis thaliana* plants were treated twice weekly in a laboratory setting with varying levels (0 mM, 0.5 mM, or 5 mM) of caesium chloride (CsCl) solution, and reflectance spectra were collected every week for three weeks using an ASD FieldSpec Pro spectroradiometer with both a contact probe (CP) and a field of view (FOV) probe at 36.8 and 66.7 cm above the plant. Plants were harvested each week after spectra collection for determination of relative water content and chlorophyll content. A visual assessment of the plants was also conducted using point observations on a uniform grid of 81 points. A mixed effects model analysis was conducted for each vegetation index to determine the effects of length of treatment, treatment level, view with which spectra was acquired, and the interactions of these terms. Two-way ANOVAs were performed on the aforementioned endpoints (e.g. chlorophyll content) to determine the significance of the effects of treatment level and length of treatment. Multiple linear regression was used to develop a predictive model for each endpoint, considering VI acquired at each view (CP, high FOV, and low FOV). Of the 14 VI considered, 8 were included in the MLR models, with YI (at high FOV), R_{1390}/R_{1454} (at CP), and R_{1676}/R_{1933} (at CP) being the most common. Contact probe readings and field of view readings differed significantly. Field of view measurements were generally consistent at each height.

Keywords: reflectance spectroscopy; remote sensing; caesium; *Arabidopsis thaliana*

1. Introduction

1.1. Background

When light interacts with a material, some may be reflected back depending on the wavelength of light and the properties of the material. Reflectance spectroscopy, the collection and analysis of reflectance spectra, provides a quick, non-destructive analytical technique that has found use in numerous fields. (Burns, ed. 2001; Pasquini 2003) Fresh plant reflectance in the visible region (400 to 700 nm) of the electromagnetic spectrum is associated with composition, amount, and distribution of pigments. Plant reflectance in the near-infrared region (700 to 1300 nm) is associated with leaf structure, and within the mid-infrared region (1300 to 2500 nm) reflectance is associated with water content (Gates et al 1965; Knipling 1970; Van der Meer and de Jong, eds. 2006). Shifts in infrared reflectance of leaves in response to disease, senescence, or stress can vary; at times reflectance will decrease and other times increase, depending on the situation (Van der Meer and de Jong, eds. 2006). Numerous studies have been conducted to qualitatively and/or quantitatively relate reflectance intensity at the leaf, whole plant, or canopy scale to various plant characteristics and conditions (Card, Peterson, and Matson 1988; Carter 1993; Carter and Knapp 2001; Curran et al 1992; Horler, Dockray, and Barber 1983; Knapp and Carter 1998; Knipling 1970; Gamon, Peñuelas, and Field 1992; Gates et al 1965; Gausman et al 1970; Gitelson, Chivkunova, and Merzlyak 2009; Grzesiak et al 2010; Ourcival, Joffre, and Rambal 1999; Peng and Gitelson 2012; Pinder and McLeod 1999; Serbin et al 2012; Serrano 2008; Shull 1929; Sims and Gamon 2002; Slaton, Hunt, and Smith 2001; Viña et al 2011; Wang and Pingheng 2012; Woodhouse et al 1994; Yoder and Pettigrew-Crosby 1995 and others), and among the applications of remote sensing is the early detection of plant stress

79 (Peñuelas and Filella 1998). Changes in reflectance due to stress have already been
80 demonstrated to occur before damage is visible (e.g. Chaerle and Van Der Stragen 2000;
81 Milton et al 1989). Although imaging is a powerful technique for visualizing, diagnosing,
82 and quantifying plant stresses, many different stressors have similar intermediate
83 responses that may be indistinguishable (Jones and Schofield 2008).

84 The purpose of this exploratory study was to determine whether a quantifiable
85 relationship exists between stable caesium (^{133}Cs) contamination (as caesium chloride,
86 CsCl) in *Arabidopsis thaliana* (*A. thaliana*) and reflectance spectra through the utilization
87 of vegetation indices (VI). VI are mathematical combinations of different reflectance
88 spectral bands that attempt to provide semi-analytical measures of vegetation activity.
89 Reflectance spectra were collected at multiple time points because VI may provide
90 better indication of temporal trends in plant status than precise conditions of a plant at
91 a single arbitrary point in time (Berger, Parent, and Tester 2010; Lichtenthaler et al 1998;
92 Viña et al 2011; Van der Meer and de Jong, eds. 2006; Wang and Pingheng 2012). VI
93 may be related to one or more properties of a set of samples (e.g. relative water content
94 or chlorophyll content). Therefore, treatment response variables that can be related
95 back to the reflectance spectra need to be utilized (Agelet and Hurburgh 2010).

96 ***1.2. Recent Studies***

97 Numerous studies have shown that there are shifts in plant reflectance spectra due to
98 metal stress (or simulated metal stress) (Bandaru 2010; Collins et al 1983; Davids and
99 Tyler 2003; Dunagan, Gilmore, and Varekamp 2007; Horler, Barber, and Barringer 1980;
100 Kooistra et al 2004; Maruthi-Sridar et al 2007a, 2007b, 2011; Milton et al 1989, 1991;
101 Schwaller, Schnetzler, and Marshall 1981; Su et al 2007; Woodhouse et al 1994), two of

which consider Cs contaminated plants. Davids and Tyler (2003) reported that Cs and strontium (Sr) contamination within the Chernobyl exclusion zone has a measurable effect on the spectral characteristics of silver birch (*Betula pendula*) and Scots pine (*Pinus sylvestris L.*), and demonstrated the potential of remote reflectance spectroscopy to assess the ecological impact of radionuclide contamination. Su et al (2007) also evaluated accumulation of Cs and Sr, by Indian mustard (*Brassica juncea*), and found morphological changes for Cs treated plants were associated with a shift in the reflectance spectra.

1.3. Caesium toxicity in A. thaliana

A. thaliana is a member of the mustard family that is closely related to various crop plants. It has been the subject of intense study over the past several decades and is considered to be a model organism and ideal for use in the laboratory setting for biological research (NSF 2013).

Caesium is a group I element that exists in nature as a +1 charged cation, and its behaviour in soils resembles that of potassium (K) (White et al 2003; Zhu and Smolders 2000; White and Broadley 2000). However, whereas K is an essential macronutrient (Hampton et al 2004), Cs has no known nutritional role in plant physiology (White and Broadley 2000; White et al 2003) and at excessive levels can become an abiotic oxidative stress factor (Hampton et al 2004; Sahr et al 2005; White and Broadley 2000; White et al 2003). Cs competes with K for binding sites in proteins, and will also inhibit the potassium-induced cellular activities associated with plant nutrition (Hampton et al 2004). The most notable effects of Cs toxicity include reduced growth and

124 photosynthesis (Hampton et al 2004; Sahr et al 2005), and at higher concentrations,
125 necrotic leaf areas have been seen (Sahr et al 2005).

126 Caesium is typically mobile (depending on soil type, environmental conditions,
127 etc.) and easily translocates to aboveground plant parts, with concentrations increasing
128 with soil concentration (Sahr et al 2005). It distributes fairly uniformly within the plant,
129 with increased concentrations in plant stems and veins of leaves (Soudek et al 2004).
130 Once taken up by plants, Cs can enter the terrestrial food chain (Broadly and Willey
131 1997; Hampton et al 2004). This can pose a human health hazard for radioactive
132 isotopes of Cs ("radiocaesium"), in particular ^{134}Cs ($t_{1/2}$ = 2.07 years) and ^{137}Cs ($t_{1/2}$ =
133 30.1 years) (where $t_{1/2}$ is the half-life; ICRP 2008). These isotopes have been released
134 to the environment through the manufacturing and testing of nuclear weapons as well
135 as purposeful or accidental releases from nuclear power plants (Hampton et al 2004;
136 White and Broadley 2000). For example, releases from the Chernobyl nuclear accident
137 were 47 PBq ^{134}Cs and 85 PBq ^{137}Cs and from the Fukushima-Daiichi accident 11.8
138 PBq ^{134}Cs and 12 PBq ^{137}Cs (Steinhouser, Brandl, and Johnson 2014). The naturally
139 occurring isotope of caesium (^{133}Cs) has an environmental concentration of about 0.3 to
140 25 μg g^{-1} dry soil; radiocaesium concentrations in soil are several order of magnitudes
141 lower than this (Broadley et al 1999), although sites remain with long term
142 contamination by radiocaesium (e.g. areas of Belarus and Fukushima; Steinhouser,
143 Brandl, and Johnson 2014). Understanding the behaviour and effects of Cs in plants is
144 important for determination of potential remediation strategies for radiocaesium
145 contamination.

1.4. Consideration of chloride effects

Caesium chloride has been used previously to consider Cs uptake and stress (Broadley et al 2001; Kanter et al 2010; Le Lay et al 2006; Qi et al 2008; Sahr et al 2005), and although chlorine (Cl) is an essential micronutrient for higher plants, at high plant tissue concentrations Cl can be toxic (White and Broadley 2001). However, three weeks after germination on media supplemented with different concentrations of sodium chloride (NaCl), Boyko et al (2010) only saw phenotypic differences in *A. thaliana* plants at concentrations >75 mM NaCl. Effects were attributed primarily to Cl, as experiments were repeated with different salts (NaCl, $MgCl_2$, Na_2SO_4, and $MgSO_4$) to control for the effect of each element. Additionally, Suter and Widmer (2013) saw no major effects on plant fitness below 25 mM NaCl for four different genotypes of *A. thaliana* plants grown in soil and watered once a week with varying concentrations of NaCl. Because the concentrations used in this study are an order of magnitude below concentrations shown to have phenotypic effects on *A. thaliana,* the contribution of Cl to the effects seen here is considered negligible.

2. Materials and Methods

2.1. Plant growth and treatment

The soil mix used was four parts peat-based (Canadian Spahgnum Peak Moss) potting soil mix (Promix PGX, Premier Horticulture Inc., Quakertown, PA) and 1 part Perlite (Hoffman Horticultural Perlite, Good Earth, Lancaster, NY). Soil was mixed and placed in square plastic grow pots (10.8 × 10.8 × 12.7 cm, Kordlok SQL0450 from ITML Horticultural Products, Myers Industries Inc., Akron, OH) with perforated bottoms to allow water seepage; soil was hydrated by placing pots by multiples of twelve in a

169 Nalgene tray (Thermo Scientific, Wilmington, DE) with 3-5 cm deep deionized water.
170 Water level was maintained for at least three days to allow the soil to absorb sufficient
171 moisture for planting. Pots contained an average (wet) soil mass of about 475 g.

172 *A. thaliana* seeds (WT-02-41-01 Columbia [alias Col-0] Wildtype, LEHLE Seeds,
173 Round Rock, TX) were removed from 4°C storage, soaked in 1/32 strength hydroponic
174 (HP) media solution, and exposed to red light for 30 minutes to synchronize germination.
175 Hydroponic media was made with DI water, 1/32 strength Murashige and Skoog basal
176 medium (137.5 mg L^{-1}) (Sigma-Aldrich Cat No M5519, St. Louis MO), and 250 mg L^{-1} MES
177 hydrate (Sigma-Aldrich Cat No M2933), using KOH to pH balance to 5.7. Seeds were
178 subsequently pipetted into a 96 well tray (five seeds per well) to verify number of seeds
179 planted. Seeds were then pipetted from the tray onto potted soil as three sets of five
180 seeds per pot, i.e. 15 seeds per pot, to ensure adequate germination. Following the
181 sowing of the seeds, the 1/32 HP media was further diluted to 1/64 strength for
182 subsequent treatment.

183 After planting, arbitrary sets of 6 pots each were transferred to Sterlite tubs (40
184 × 31.75 × 15.24 cm, Target Corp., Minneapolis, MN). Tubs were placed in rows of up to
185 four on growth shelves, 42 cm beneath growth lights (Four Philips F32T8 TL741 700
186 series 32W ALTO II Fluorescent bulbs, cat. No. 0002904, Philips North America
187 Corporation, Andover, MA). Plants were on a nine hour light : 15 hour dark cycle under
188 ambient laboratory environmental conditions.

189 The bottoms of the pots were submerged in approximately 3 cm distilled water
190 until the plants reached a previously determined treatment date (i.e. day 37 post-
191 planting, rosettes ~30 mm in diameter). At the seedling stage, plants were culled to

192 three per pot, based on size and appearance of health, such that one plant per group
193 remained.

194 Immediately prior to treatment, pots were rearranged between nine tubs (6 pots
195 each, no longer submerged in DI water) such that each tub, now serving as a treatment
196 group, had similar size and quality plants. Spike solution was evenly applied to the top
197 of each pot as 100 mL (25 mL delivered to each quadrant) of the appropriate
198 concentration of CsCl (0.5 mM CsCl or 5 mM CsCl) in 1/64 strength HP media twice
199 weekly, with control plants receiving 100 mL 1/64 HP media only. Each 100 mL
200 treatment of 0.5 mM CsCl corresponds to a Cs concentration of about 27.9 μg g^{-1} soil
201 (279 μg g^{-1} soil for 5 mM treatment); that is, Cs concentration in the soil after 1 week of
202 0.5 mM treatments would be about 55.8 μg g^{-1}. Note that 27.9 μg g^{-1} hydrated soil
203 corresponds to about 195 μg g^{-1} dry soil for the particular soil mix used.

204 Two pots were randomly selected from each treatment group for weekly spectra
205 collection and harvest. After each application of hydroponic media, the plants were
206 rotated within the tubs and the tubs were rearranged among the growth shelves to
207 account for potential variation in lighting or other environmental conditions.

208 ***2.2. Equipment, setup, and collection of spectra***

209 Reflectance spectra were collected using a FieldSpec Pro (FSP 350-2500P; Analytical
210 Spectral Devices (ASD), Boulder, CO) which is a full range (350 nm – 2500 nm) portable
211 spectroradiometer (with sampling intervals/spectral resolutions of 1.4 nm/3 nm and 2
212 nm/10 nm for 350-1000 nm and 1000-2500 nm respectively) (ASD 2002).

213 *2.2.1. Contact probe measurements*

214 Contact probe (CP) spectra were collected using a leaf clip attachment on individual

leaves. The CP provides light (3.825 V, 4.05 W low intensity bulb) and collects reflectance spectra. The leaf clip attachment has both a white (for white reference) and black (to minimize back scatter) background. Triplicate CP spectra were collected on one leaf from each of typically three separate plants per pot.

2.2.2. Field of view measurements

Field of view (FOV) spectra were collected for each sample using an 8° probe (i.e. a viewing angle of 8°) at two different height settings (referred to as "high" and "low" FOV; abbreviated as HFOV and LFOV in summary tables). The investigation into potential differences in height settings stems from the "waist high" or "arm's length" use of a hand held probe in the field (e.g. Filella and Peñuelas 1994), which will vary from person to person. Simulating such a height difference in a laboratory setting gives consideration to whether spectra collected with the same probe at different heights can truly be compared.

Incident light was provided by two halogen lamps (Pro Lamp, 14.5 V, 50W, P/N 145378, ASD, Boulder, CO) angled at 30 degrees from horizontal. The lights were 180° apart at 30.5 cm from the centre of pot on the horizontal and 76.2 cm (high) or 59 cm (low) above the table surface. The fore optics probe was centred between the lights at 66.7 cm (high) or 36.8 cm (low) above the plane of the pot surface (Figure 1). The high and low set ups had spot size diameters of 9.32 cm and 5.15 cm respectively, i.e., viewing areas of 68.3 cm^2 (58.6% of pot surface) and 20.8 cm^2 (17.9% of pot surface).

Reflective surfaces were covered with light-absorbent material to minimize noise and thus variability in spectra, and dark room conditions were approximated by surrounding the lights and fore optics with a black felt canopy. Tripod surfaces were also wrapped in black felt. The white reference was a calibrated Spectralon (25.4 × 25.4

239 cm, LabSphere, North Sutton, NH) panel of 99% reflectance that was elevated to a height
240 equivalent to a grow pot. Grow pots were placed on black paper plates when collecting
241 spectra and the table top was lined with a light-absorbent black rubber. Four spectra
242 were saved for each FOV session. Each of these spectra was collected at a different
243 arbitrary rotation of the pot and then averaged to get an overall assessment of the
244 reflectance of the sample. FOV spectra were always acquired prior to CP because it is
245 possible for the CP to injure the plant and therefore affect subsequent FOV readings.

246 ***2.3. Collection of physical measures***

247 *2.3.1. Relative water content*

248 As metal stress is known to mimic drought stress (Thankabail, Lyon, and Huete 2012),
249 plants were harvested after spectra collection each week to determine relative water
250 content. To determine relative water content, sufficient leaves were removed to obtain
251 between 1000 and 2000 mg of fresh mass for each replicate (i.e. pot). Samples were
252 placed in weigh boats, fresh mass was obtained, samples were dried to a constant mass,
253 and dry mass was obtained. A sample's relative water content (RWC) was then
254 calculated as:

255 $$\mathrm{RWC} = 1 - \frac{m_{\mathrm{dry}}}{m_{\mathrm{fresh}}} \quad (1)$$

256 where m_{dry} is dry mass (mg) and m_{fresh} is fresh mass (mg).

257 *2.3.2. Chlorophyll content*

258 Caesium toxicity has also been associated with an inhibition of the biosynthesis of
259 chlorophyll (Sahr et al 2005), so we consider a representative sample from each plant
260 for destructive determination of chlorophyll content. The concentrations of chlorophyll

261 a (Chl a) and chlorophyll b (Chl b) were determined for each replicate (i.e. pot) (Knudson
262 Tibbitts, and Edwards 1977; Li et al 2009; Papista, Acs, and Boddi 2002); total chlorophyll
263 content (Chl a+b) was taken as the sum of chlorophyll a and chlorophyll b.

264 Four circular leaf subsamples were collected from representative leaves of the
265 plants in a pot using a #3 cork borer (Fisher Scientific, Pittsburgh, PA). Leaf samples were
266 stored in the dark at 4°C in capped 20 mL vials (KG-33 borosilicate glass; Kimble Chase,
267 Vineland, New Jersey) containing 2 mL 100% ethanol for three days before absorbance
268 (*A*) at 665 nm, 649 nm, 629 nm, and 696 nm, with an offset at 750 nm, was determined
269 for 1.5 mL subsamples for each vial using a NanoDrop 2000c UV-Vis spectrophotometer
270 (Thermo Scientific, Wilmington, DE). Disposable methacrylate cuvettes with
271 transmission from 300 to 800 nm > 80% were used with the 1.5 mL subsample (Cole
272 Palmer, Vernon Hills, Illinois). Chlorophyll content was determined using appropriate,
273 previously published equations (Ritchie 2006):

274 $$\text{Chl a} = -5.2007A_{649} + 13.5275A_{665} \quad (2)$$

275 $$\text{Chl b} = 22.4327A_{649} - 7.0741A_{665} \quad (3)$$

276 where Chl a is chlorophyll a content (µg mL^{-1}), Chl b is chlorophyll b content (µg mL^{-1}),
277 A_x is absorbance, and *x* is the relevant wavelength (nm).

278 *2.3.3. Visual assessment*

279 A visual assessment of the proportion of a plot covered by any plant material and any
280 existing chlorotic plant material was performed by overlaying an 8 × 8 (13.5 × 13.5 mm)
281 grid on a computer display of top-down photos of each treatment group at each of three
282 time points, forming 64 squares with 81 evenly-spaced points (grid intersections).
283 Photographs were taken immediately prior to spectra collection, directly above each six-

pot treatment group in the same manner each week. However, to account for any potential change in magnification or alignment, gridlines were laid based on pot dimensions, which were definitively consistent. Using the grid intersections, three additional endpoints were defined as follows, where N_{total} is the total number of points in the grid, N_{leaf} is the number of points on leaf material, and $N_{chlorosis}$ is the number of points on leaf material with visible chlorosis:

- Coarse Leaf Area Index (CLAI) provides an approximate indication of how much of the pot surface is covered by plant material.

$$CLAI = \frac{N_{leaf}}{N_{total}} \quad (4)$$

- Green Factor (GF) provides an approximate indication of the proportion of pot surface that is covered with green plant material.

$$GF = \frac{N_{leaf} - N_{chlorosis}}{N_{total}} \quad (5)$$

- Chlorosis Factor (CF) provides an approximate indication of the proportion of plant material that has visible chlorosis.

$$CF = \frac{N_{chlorosis}}{N_{leaf}} \quad (6)$$

2.4. Data analysis

Fourteen VI (Table 1) are considered for applicable spectra acquisition technique(s) (i.e. FOV and/or CP), including indices from the literature as well as indices selected by the

302 authors through scientific judgement and visual consideration of spectra. Note that R_y
303 represents reflectance at *y* nm. In addition to the fourteen VI listed in Table 1, two
304 transformations were considered (WI/NDVI and (R_{950}/R_{750})/NDVI), as it has been
305 previously suggested that correcting for the effects of NDVI may offer improvement for
306 certain VIs (Peñuelas et al 1997). SAS v9.3 was used for all analyses, and a significance
307 level of 0.05 was used for all tests of significance.

308 A mixed effects model analysis was conducted for each vegetation index to
309 consider the fixed effects of week (1,2,3), treatment (0 mM, 0.5 mM, 5 mM), week by
310 treatment interaction, view (CP, high FOV, low FOV), view by week interaction, and view
311 by treatment interaction with a random effect for the plant within week by treatment.
312 Degrees of freedom were approximated using the Kenward-Rogers approximation to
313 account for variation among the week by treatment combinations except for NDVI
314 where the model did not converge with this approximation. When view or any
315 interactions with view were significant, the subsequent analyses included each of the
316 views. If view was not significant, the average of the views was considered in
317 subsequent analyses. Pearson correlation coefficients were also calculated for each
318 combination of views.

319 The physical measures (i.e. endpoints) were selected to represent plant stress.
320 Therefore, a two-way ANOVA analysis was conducted for each endpoint to test whether
321 means were significantly different by treatment level or week and to determine if
322 significant interaction was present between the treatment level and week. Analyses
323 were then conducted to consider RWC, chlorophyll content, CLAI, GF, and CF as
324 dependent variables in separate analyses whereby vegetation indices at the relevant

field of views from the mixed model analysis were included in the model in order to relate reflectance changes (i.e. changes in certain VI) back to stress indicators (i.e. endpoints). The vegetation indices at the relevant field of views were included in a forward model selection using a predetermined significance level ($\alpha = 0.10$) set above the usual 0.05 level for predictor exploration. When two variables that were transformations of each other were selected, the simplest variable was chosen to remove the effects of multicollinearity (e.g., if both WI and WI/NDVI were selected, only WI would be included in the final model).

3. Results

3.1. Reflectance spectra

Mean reflectance spectra relative to the control for weeks 1 and 3 of 5 mM CsCl treatment are shown for each view in Figure 2 to demonstrate the temporal shift in reflectance at this treatment level. Similar results were seen for 0.5 mM CsCl (not shown), although to a lesser extent. The supplementary online material (Supplemental Figure S1) contains reflectance spectra for each treatment, week, and view. In the visible IR region (400 to 700 nm), FOV differences between the control and 5 mM treatment were much more pronounced than between the control and 0.5 mM treatment. By week 3, differences between all treatment groups were apparent in the near and mid IR regions (700 to 2500 nm) of the spectra in FOV. Differences in CP spectra were more subtle, with the only obvious differences occurring at the 5 mM treatment level.

3.2. Physical measures

The supplementary online material (Supplemental Figures S2-S11) contains weekly pictures of the plants analyzed, along with the grid overlay used for determining the visual assessment factors CLAI, GF, and CF. Necrotic spots were evident on the 5 mM treated plants from week 1, but were not seen on 0.5 mM plants until week 3. There was also obvious growth inhibition in the 5 mM treated plants.

Results from the two-way ANOVA (*p*-values) of the endpoints (physical measures) are shown in Table 2. The changes in endpoints by week and treatment level are shown graphically in Figures 3 - 7. Detailed results from the ANOVA analysis are contained in the supplementary online material (Supplemental Tables S1-S5).

RWC decreased each week for all three treatments, with the control having the highest RWC, followed by 0.5 mM treatment, and then the 5 mM treatment every week; means were significantly different by both week and treatment level, and there was no significant interaction between the two.

Mean values for chlorophyll content at week 1 were similar at the various treatment levels, although variability between the means increased each week. That is, there were no significant differences in the treatment means at week one, but by week 3 the means of all three treatment groups were significantly different, with the control group having the highest chlorophyll content and the 5 mM treatment having the lowest.

Mean CF values increased by week for 0.5 mM and 5 mM CsCl treatments, although the differences were not significant for the 0.5 mM group. For the control plants, mean CF increased slightly from week 2 to week 3, but the average value remained low (0.006). At week 1, both the control and 0.5 mM treatments showed zero

chlorosis, but the 5 mM treatment had an average CF of about 0.55 (or about 55% chlorosis). There was large variation in CF at week 1 for the 5 mM treatment, but variability decreased each week as CF increased. There were no significant differences between the control and 0.5 mM treatment group CF means, nor any significant difference in either of these groups by week. There were, however, significant differences between each of these groups and the 5 mM treatment group. There were also significant differences in the 5 mM treatment group each week. Also note that although the differences were not significant for the 0.5 mM group by week, the general temporal trend suggests an increase in chlorosis with time, similar to but less pronounced than the 5 mM treatment group.

There were no significant differences between the control and 0.5 mM treatment groups for GF in any week. However, the 5 mM treatment group was significantly different from both the control and 0.5 mM treatment group at all three weeks. Additionally there were significant differences between week 1 and week 3 for all three treatment groups. Mean GF decreased each week for the 5 mM treatment group, and mean GF increased between weeks 1 and 3 for the control plants. Mean GF increased slightly from week 1 to week 3 for 0.5 mM treated plants, but a smaller amount than the control plants.

There was a significant increase in the means of all treatment groups from week 1 to week 3 for CLAI. There were again no significant differences between the control group and the 0.5 mM treatment group at any week. At week 1, there were no significant differences between any of the treatment groups. By week 3 there was a significant difference between the 5 mM and both the control and 0.5 mM plants.

3.3. Vegetation indices

Vegetation indices were determined from the reflectance spectra at each week, for each treatment group and view. The detailed results from the mixed model analyses of these VI are included in the supplementary online material (Supplemental Table S6). The averages of each of the vegetation indices significantly differ by field of view, treatment, or an interaction exists between the factors and therefore field of view was considered in the subsequent regression analyses.

Pearson correlation coefficients for each combination of views are shown in Table 3. Values for VI acquired by high FOV and low FOV were all positively correlated, with all but three (PRI, WI, and R_{1390}/R_{1454}) having correlation coefficients more than 0.8. Correlations between high and low FOVs were higher than correlations between either high or low FOV and CP.

Multiple linear regression analysis was used to determine which VI would be the most appropriate predictors of the various endpoints. The results are shown in Table 4, with additional details (*F* test statistics and *p*-values) contained in the supplemental online material (Supplemental Table S7). Table 4 shows the VI and relevant view with which acquired as selected for inclusion in the MLR model. All endpoints had an MLR model that included multiple VIs at a combination of views; that is, no model was obtained that included VIs obtained from only one view. For example, the predictive model for RWC included four different VIs, two obtained using CP, and two using low FOV. CLAI and CF were the only endpoints with models that included multiple views for a single VI; SREP was included in the CLAI model at high FOV and low FOV, and R_{1676}/R_{1933} was included in the CF model at low FOV and CP.

Although GF and CF are similar, complementary variables, with GF providing indication of pot surface covered by green biomass and CF indication of chlorosis, different models were developed for each, with some overlap. R_{1676}/R_{1933} was the first VI included in both of GF and CF models, at low FOV for CF and high FOV for GF. R_{1676}/R_{1933} was followed by YI (at high FOV) and then SREP (at CP) for each model. Other VI included in these models differed.

YI (at high FOV), R_{1676}/R_{1933} (at CP), and R_{1390}/R_{1454} (at CP) were the most frequently included VIs in the models selected, each occurring in the predictive models for three different endpoints. Considering all views, R_{1676}/R_{1933} was the most frequent VI included (six total occurrences) and was the only VI to be included in each endpoint's model. Vegetation indices determined from spectra obtained from the CP were used in the models a total of 11 times, from low FOV a total of 4 times, and from high FOV a total of eight times.

4. Discussion

In this experiment the model plant species *A. thaliana* was treated with a contaminant of interest at two concentrations, in conjunction with a lifetime control. A lifetime control is important, because it has been shown that as plants mature, their reflectance spectra will shift (Horler, Barber, and Barringer 1980; Milton et al. 1989, Eiswerth, and Ager 1991); a lifetime control helps ensure that one can relate spectral changes to the contaminant exposure, without confounding from plant growth stage.

Some vegetation indices proved useful for the assessment of plant characteristics; of the 14 distinct vegetation indices considered, eight were included in the various MLR endpoint models, including all three of the indices selected by the

439 authors. The first vegetation index to be included in the MLR model for each endpoint
440 is the one to account for the largest amount of variability in, and is therefore the best
441 individual predictor of, the relevant endpoint. Generally, single vegetation indices are
442 used to predict various plant characteristics. Inclusion of additional vegetation indices
443 will account for more variability in the endpoint, statistically providing a better
444 prediction. The significance of initial VIs included in the models, with additional
445 consideration of secondary VIs, is discussed below.

446 ***4.1. Relative water content***

447 Of the vegetation indices considered, R_{950}/R_{750} as determined using the CP was the first
448 VI to be included in the MLR model for RWC. The water index (R_{900}/R_{970}) did not prove
449 to be a statistically significant predictor of RWC for any of the spectral views, which is
450 contrary to the findings of other studies (Peñuelas and Inoue 1999). However, the 950-
451 970 nm reflectance band is associated with water absorption (Peñuelas et al 1993) and
452 is common between the chosen index and the water index; the selection of a different
453 reference band (750 nm as opposed to 900 nm) may provide a more appropriate
454 response in some circumstances. Additionally, the ratio WI/NDVI has been used to
455 correct WI for the effect of NDVI (Peñuelas et al 1997). However, consideration of this
456 alternate WI, as well as an alternate R_{950}/R_{750} (i.e. (R_{950}/R_{750})/NDVI), for low and high
457 FOV (NDVI is only appropriate for remote sensing), yielded no improvement in the
458 models.

459 Additional, previously used VI included in the RWC model were PSND (associated
460 with chlorophyll content) and R_{750}/R_{550} (associated with metal content) (Serrano 2008;
461 Davids and Tyler 2003). Although the correlation between RWC and chlorophyll content

462 was weak (0.130) in this study, the former would suggest that RWC is related to both
463 chlorophyll content and metal content. Moreover, RWC likely provided a good
464 indication of Cs stress, as metal stress generally results in plant water imbalance as well
465 as a reduction in total chlorophyll content (Thankabail, Lyon, and Huete 2012), as
466 accounted for in the predictors of RWC.

467 ***4.2. Chlorophyll content***

468 Of the vegetation indices considered, the red edge position (REP) determined by the
469 high FOV set up proved to be the best indicator of total chlorophyll content. However,
470 at the leaf level, REP was not well correlated (-0.128; Pearson correlation coefficient)
471 with chlorophyll content. The latter is inconsistent with findings in the literature;
472 generally REP has been shown to correlate well with total chlorophyll content at the
473 leaf, whole plant, and canopy scales (e.g. Horler et al 1980; Curran, Dungan, and Gholz
474 1990; Filella and Peñuelas 1994; Lichtenthaler, Gitelson, and Lang 1996; Wong and He
475 2013). However, REP acquired by CP was included in the model for CF, which also
476 provides some indication of (the lack of) chlorophyll content. We hypothesize that
477 because of the variability within samples of the same treatment group, acquiring a
478 greater number of representative samples per plant (e.g. acquiring CP spectra and
479 chlorophyll content for all available leaves) would result in a more remarkable
480 relationship between CP spectra and chlorophyll content. However, for this study
481 biomass was needed for determination of water content as well, preventing the use of
482 the entire plant for chlorophyll determination.

483 The yellowness index (YI) (indication of chlorosis; Adams, Philpot, and Norvell
484 1999) was the second predictor included in the model for chlorophyll content, as well

as the second predictor included in both the GF and CF models. In all three instances of use, YI was determined by reflectance spectra acquired by high FOV. Each of these endpoints (chlorophyll content, GF, and CF) is associated with chlorophyll content, so association with YI is consistent with previous work.

4.3. Visual assessment factors

R_{1676}/R_{1933} proved to be the best indicator for both GF and CF, although high FOV was more fitting for GF and low FOV more fitting for CF (although the low FOV was not considered for the GF model because it was highly collinear with other predictors). Because the visual assessment factors were determined using whole-plant photographs, it follows that VI calculated from reflectance spectra acquired by FOV would be more appropriate statistical predictors for these factors than would VI determined from CP acquired spectra; CP only considers individual leaves whereas FOV considers areas of the whole plant. However, although the first two VI included in the models for GF and CF were acquired by FOV, the remaining were acquired by CP. This suggests that although FOV measurements may provide the best indication of GF and CF if using a single VI, inclusion of secondary VI(s) acquired by CP may improve the predictive ability of the model. That is, inclusion of leaf level properties may incorporate characteristics into the model missed by FOV, providing a more complete picture of plant status.

The best indicator of CLAI proved to be SREP, as determined from reflectance spectra acquired by high FOV. CLAI represents the proportion of the pot surface covered by leaf material, i.e., the overall size of the plant relative to the pot. SREP is included a second time (at low FOV) in the CLAI model and is also included in the GF and CF models (at CP). These findings are consistent with prior studies; SREP has previously been shown

to correlate with leaf area index as well as chlorophyll content (e.g. Filella and Penuelas, 1994). These results also suggest that when applying or interpreting SREP, CP may be more appropriate for chlorophyll determination and FOV more appropriate for assessing LAI.

The relationship between visual assessment factors also gives additional insight into plants' general physical condition. For example, GF is similar to CLAI but specifically represents green biomass. Thus, if all biomass is green, then GF will be equal to CLAI. Figure 8 shows GF plotted against CLAI grouped by treatment levels. For control plants, GF was equal to CLAI for all weeks, with very little deviation. For the low (0.5 mM) treatment level, GF decreased below CLAI by week 3, indicating that even though the plant may be increasing in size (significant difference in mean CLAI between week 2 and 3), it may still be experiencing stress. For the high treatment (5 mM) group, GF was less than CLAI for all plants, with this difference being more pronounced with time. Again, this indicates that plants are still growing but are demonstrating significant stress, especially by week 3.

GF appeared to be more useful than CF, especially when taken together with CLAI as above. Because plants initially had little or no chlorosis, early values for CF were predominantly zero. For low treatment levels (control and 0.5 mM) CF was minimal all three weeks. Predictors for GF and CF were very similar, implying that use of both endpoints is likely unnecessary.

In many instances there were no significant differences between control plants and 0.5 mM treated plants' endpoints, although there did appear to be small differences in reflectance (Figure 2). For the visual assessment factors, utilizing a smaller grid overlay or an automated pixel analysis might provide more precise values, if this

technique were to be pursued further. In general, an increased sample size will provide greater statistical power, although the time required to consider more samples should be balanced against the value of the gain in information. Whether or not FOV indication of plant appearance is useful is debatable; on the scale of a few individual plants, a visual assessment is likely less time consuming than acquiring and analysing spectra. However, when applied to a canopy or landscape scale, using vegetation indices would prove more convenient.

4.4. Spectra acquisition technique

Biochemistry may be highly variable within single plants (Bock et al 2010). The distribution of chemical constituents is not uniform because of the organization of cells and organelles; non-uniformity results in micro-differential absorbance and reflectance across a leaf surface. Optical properties of leaves are determined by (1) external leaf structure, (e.g. surface roughness) which controls the reflectance from the upper surface of the leaf, (2) composition, amount and distribution of pigments, which determine the absorption of radiation in the ultraviolet and visible ranges, (3) internal leaf structure, which affects the scattering of incident radiation within the leaf, and (4) water content, which affects the absorption infrared radiation (Knipling 1970; Van der Meer and de Jong, eds. 2006; Peng and Gitelson 2012). While these factors still contribute to reflectance spectra of an entire plant, or multiple plants, trends may be perceived to indicate wilting or decreased vegetative growth. Considering the whole plant may also give indication of leaf properties/orientation in addition to soil properties.

554 The CP acquires reflectance spectra for individual leaves, whereas the FOV
555 probe considers the entire plant, or portion of a plant; reflectance spectra acquired by
556 FOV is a combination of plant and soil reflectance. In theory, FOV is more convenient
557 and can be performed remotely, and although the CP gives cleaner, more consistent
558 spectra (Supplemental Figure S1), FOV was more likely to be the primary indicator of
559 stress conditions in this experiment than the CP (Table 4). However, CP measurements
560 were also included in the MLR predictive models, implying that accounting for both
561 whole plant and leaf properties provides the best indication of plant stress (as opposed
562 to using a single VI or a single acquisition technique).
563 Although most values for VI acquired by high FOV and low FOV were highly
564 correlated, several had significant differences. For example, the correlation coefficient
565 for NDVI between high FOV and low FOV was 0.964, yet the means of the two were
566 significantly different (p<0.001). Field of view readings, both low and high, were
567 calibrated with a white reference panel (as described above), so differences resulting
568 from light attenuation over the distance to the fore optics should not be an issue.
569 However, although light-absorbent material was used on all surfaces, there is still some
570 potential for back-scattering of light to the fore-optics. Because of the greater distance
571 from the sample, we might expect the high FOV to have a larger background
572 contribution than the low FOV. Similarly, although four rotations of the plant sample
573 were used to get an average, representative reflectance, the larger viewing area of high
574 FOV may result in a greater contribution from uncovered soil than low FOV.
575 However, although there are differences between spectra acquired at different
576 heights, the general consistency indicates that acquiring spectra at one height, either
577 high or low, will likely prove sufficiently equivalent as a coarse indication of certain

vegetation indices. Not all VI had high correlations at different views though; in particular, the correlation coefficient for WI acquired by high FOV and low FOV was only 0.311. This implies that at the minimum, height should be consistent when comparing VI.

Additionally, many vegetation indices acquired by CP did not correlate well with the same indices determined by FOV: 20 out of 26 had correlation coefficients below 0.5. That all VI differed significantly by view (Supplementary Table S6) is primarily attributed to these differences in CP-acquired spectra. Note that a portion of the differences between collection techniques (FOV vs CP) in predictive ability may be due to the need for additional samples with the CP (i.e. more readings per sample) to overcome the inherent biological variability between plants.

5. Conclusions

5.1. Limitations

Although certain VI were statistically significant predictors of the corresponding endpoints, these predictors might not necessarily be useful, especially when time, effort, and other resources required for data acquisition are taken into consideration. Additionally, results were not always consistent with the typical findings in the literature, specifically for those indices previously shown to be associated with water and chlorophyll content. For chlorophyll content, results would likely be improved by conducting larger scale experiments with additional plant biomass available for analysis. Also, although water content did not correlate well with the WI, a similar VI (the simple ratio of a reflectance band associated with water to an alternate reference band) did have good predictive ability.

601 It would be statistically beneficial to perform these experiments on a larger scale,
602 but the time required to acquire individual spectra by hand is a limiting factor; the
603 expense of and information gained through data collection by the different types of
604 probes should be considered when developing experiments in the future. Employing
605 techniques to automate, or partially automate, the FOV sampling process (such as
606 placing the sample on a conveyor belt or rotating stand) would reduce the time needed
607 to conduct similar experiments, but these techniques might not necessarily mimic the
608 use of hand-held spectra acquisition in the field.
609 Although positive results were seen in the laboratory, environmental and
610 sampling conditions were controlled; therefore, care should be given if the intent is to
611 extrapolate to field studies. Measurements taken in the field may not be as consistent
612 or informative as measurements taken in the laboratory due to extraneous and
613 potentially unknown environmental factors.

614 ***5.2. Spectra acquisition technique***

615 Care should be given applying VI across views as CP and FOV typically provide different
616 results, depending on the endpoint of concern; different VI should be developed and
617 applied for CP than FOV if utilizing a single reflectance spectra acquisition technique.
618 For certain VI, acquiring spectra at different heights also resulted in statistically
619 significant differences, although correlations between heights were generally high.
620 Consideration should therefore be given to the height with which spectra are acquired,
621 particularly in the laboratory setting or in small scale assessments (e.g. using a hand held
622 probe to assess nearby vegetation). With the natural variability between plants, these
623 differences may become less pronounced with larger samples sizes or additional species

624 of plants. If these differences are relevant on a larger scale or at greater assessment
625 distances is also currently unknown.

626 Neither FOV nor CP proved better than the other overall; the best predictor for
627 RWC was obtained via CP, whereas for the other endpoints the best predictor was
628 obtained via FOV. However, VI for both CP and FOV were included in each model,
629 suggesting the best approximation of plant stress status is made by accounting for both
630 whole plant and leaf optical properties.

631 ***5.3. Vegetation indices***

632 Eight distinct VI were used in the MLR model development for 5 different endpoints, 3
633 of which were previously unused VI selected by the author for consideration. Two of
634 these VI utilized reflectance bands in the mid-infrared region (1300 to 2500 nm),
635 implying that reflectance in the mid-infrared region should be given more attention than
636 it has traditionally. In particular R_{1676}/R_{1933} was the most commonly occurring predictor
637 in these MLR models, and it was the only VI included in each endpoint's model.
638 Additionally, R_{1676}/R_{1933} provided reasonable predictive ability at both the leaf level (CP)
639 and whole plant level (FOV). This VI, or a ratio of similar reflectance bands, may be
640 useful to consider in more detailed studies in the future focusing on Cs stress or general
641 metal stress.

642 A combination of VI and spectra collection techniques and provided the overall
643 best prediction of plant stress indicators. Multi-index use should be given consideration
644 in future studies, with multiple views considered whenever possible.

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Figure Captions:

Figure 1. Schematic of equipment set up for high FOV.

Figure 2. Temporal shift in mean reflectance spectra relative to control for 5 mM CsCl treatments shown for each acquisition technique; Figure 2(*a*) shows high field of view, Figure 2(*b*) shows low field of view, and Figure 2(*c*) shows contact probe. Note that the *y*-axis scale for Figure 2(*c*) is smaller than that of Figures 2(*a*) and 2(*b*).

Figure 3. Plot of relative water content (RWC) vs time for each treatment level. Points represent individual observations, lines connect mean values.

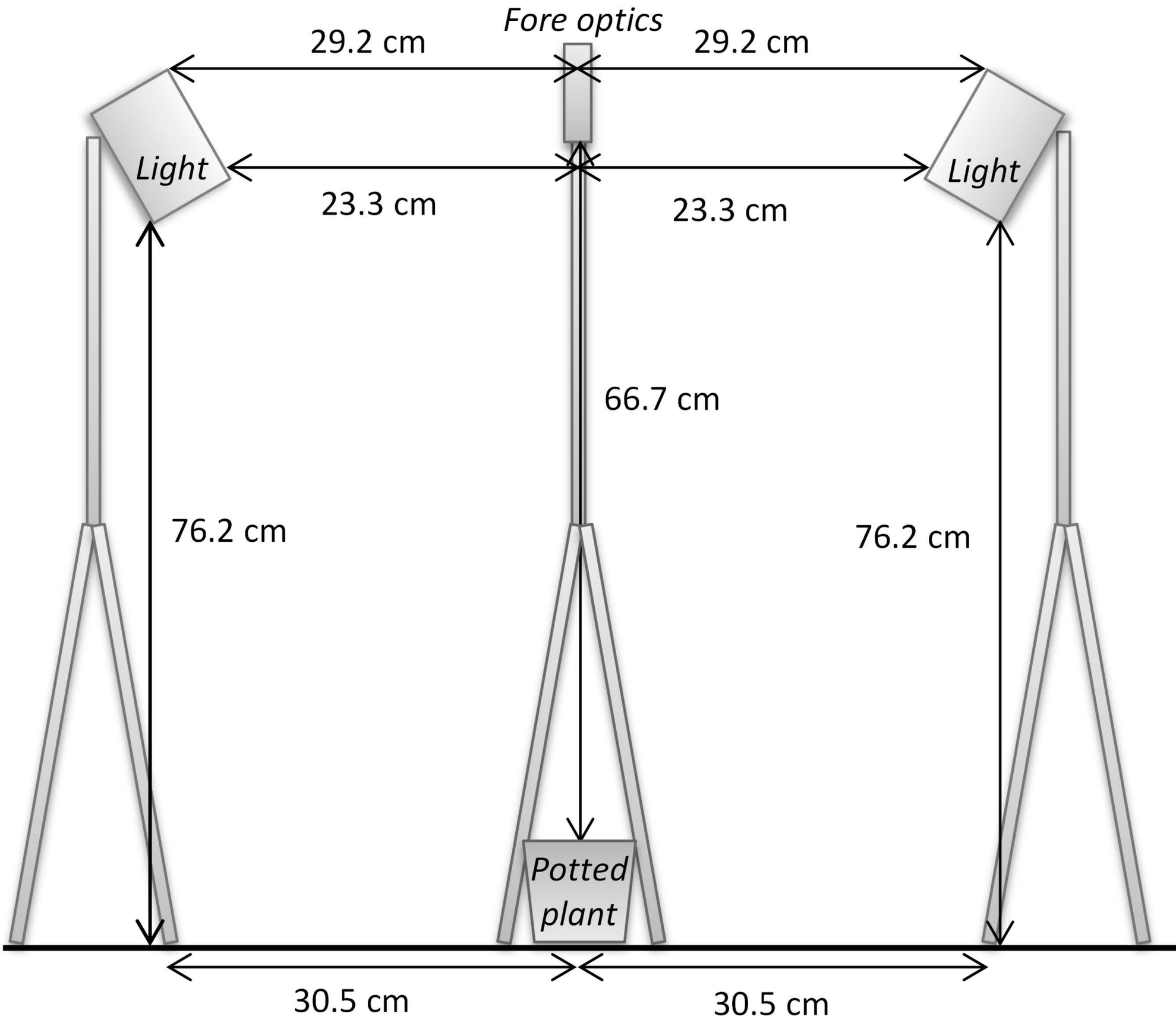
Figure 4. Plot of chlorophyll content (Chl a+b) vs time for each treatment level. Points represent individual observations, lines connect mean values.

Figure 5. Plot of chlorosis factor (CF) vs time for each treatment level. Points represent individual observations, lines connect mean values.

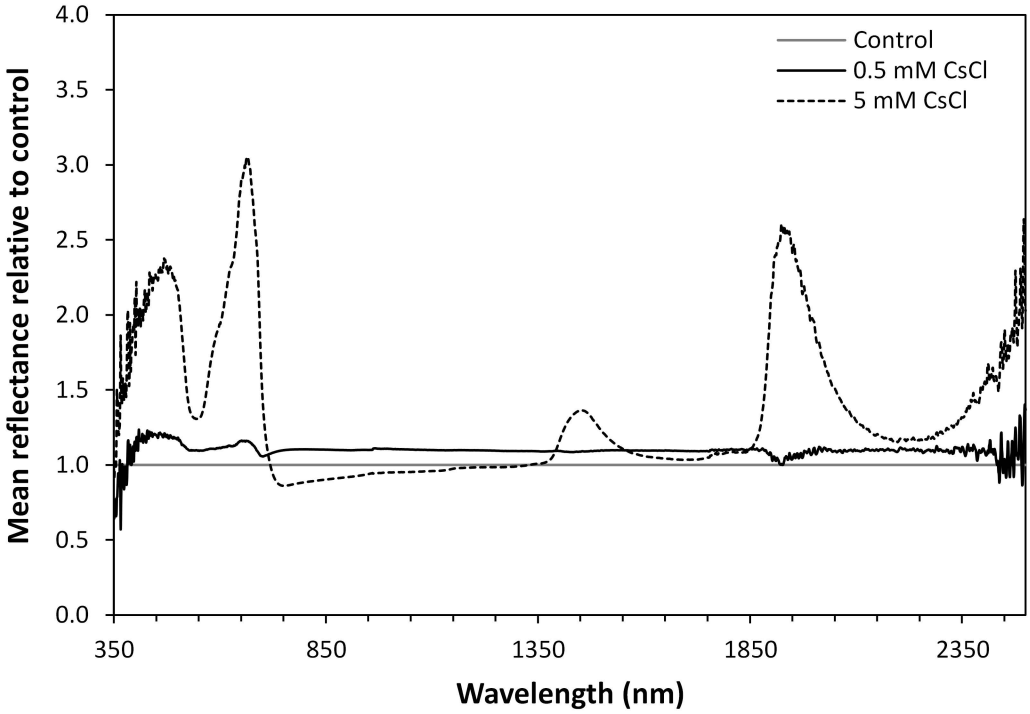
Figure 6. Plot of green factor (GF) vs time for each treatment level. Points represent individual observations, lines connect mean values.

Figure 7. Plot of coarse leaf area index (CLAI) vs time for each treatment level. Points represent individual observations, lines connect mean values.

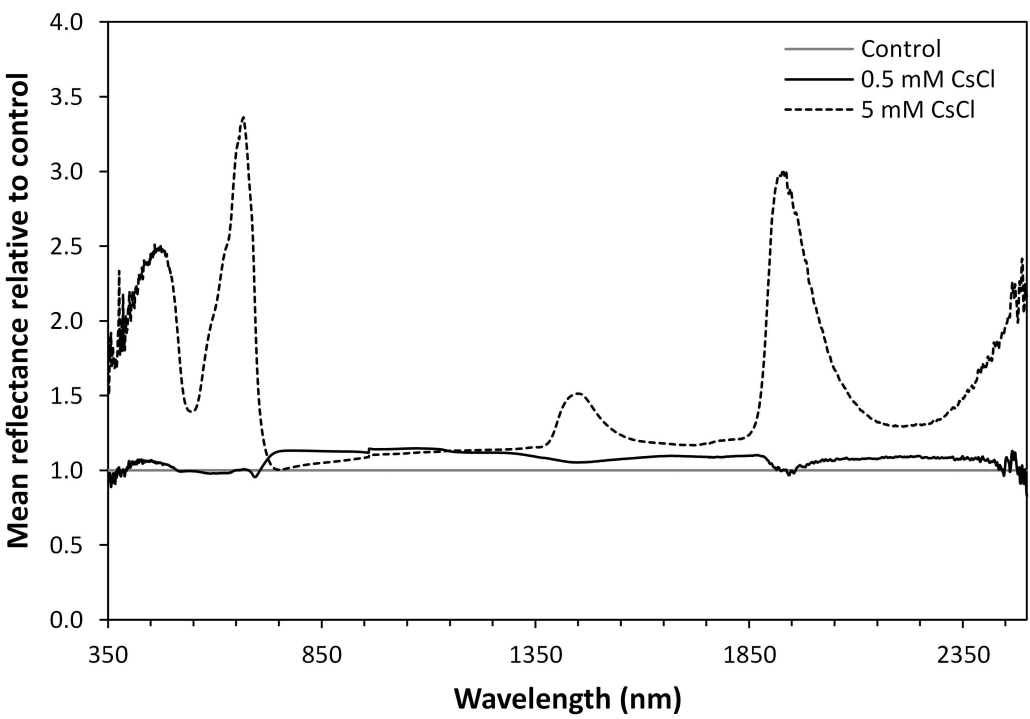
Figure 8. Plot of GF vs CLAI by treatment level and week.


Fore optics
29.2 cm
29.2 cm
Light
Light
23.3 cm
23.3 cm
66.7 cm
76.2 cm
76.2 cm
Potted plant
30.5 cm
30.5 cm

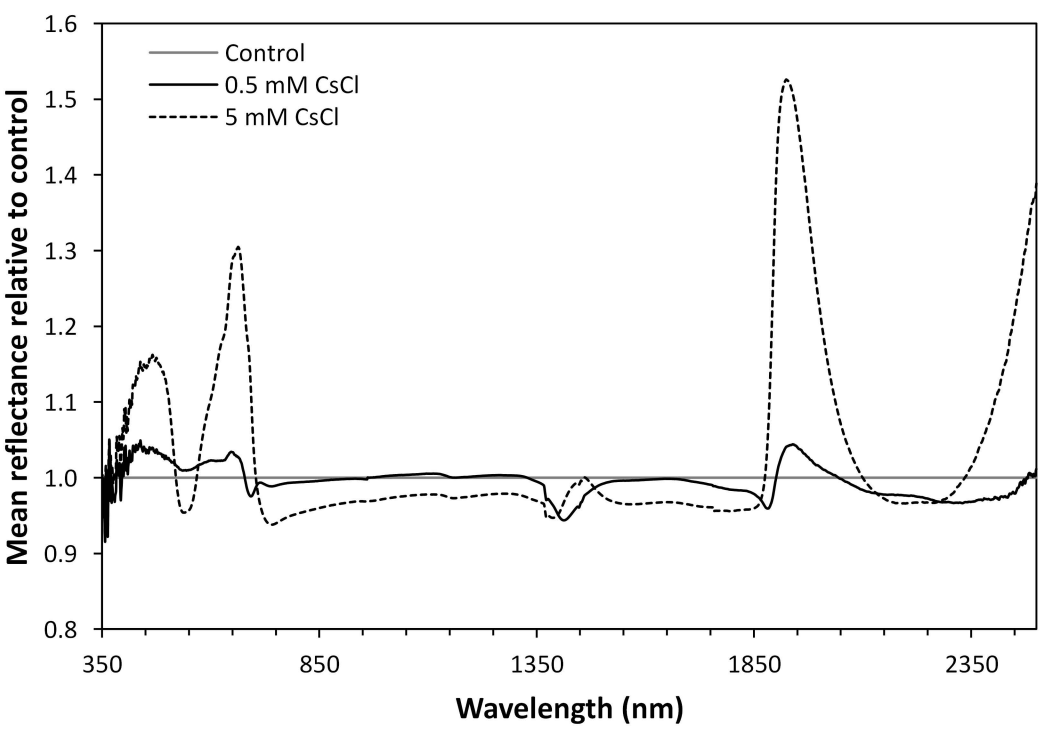
(*a*)

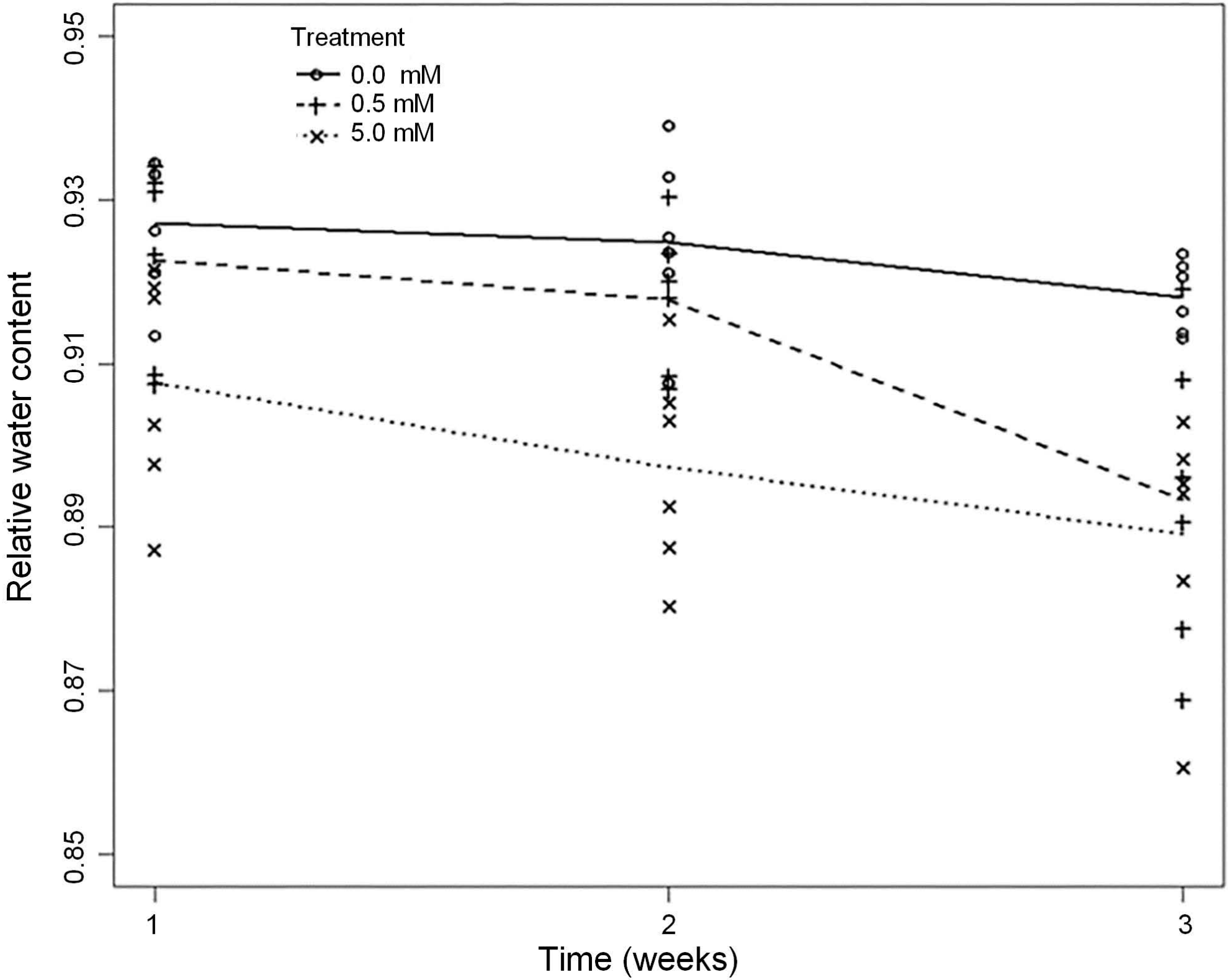

High Field of View
Control
0.5 mM CsCl
5 mM CsCl
Mean reflectance relative to control
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
350
850
1350
1850
2350
Wavelength (nm)

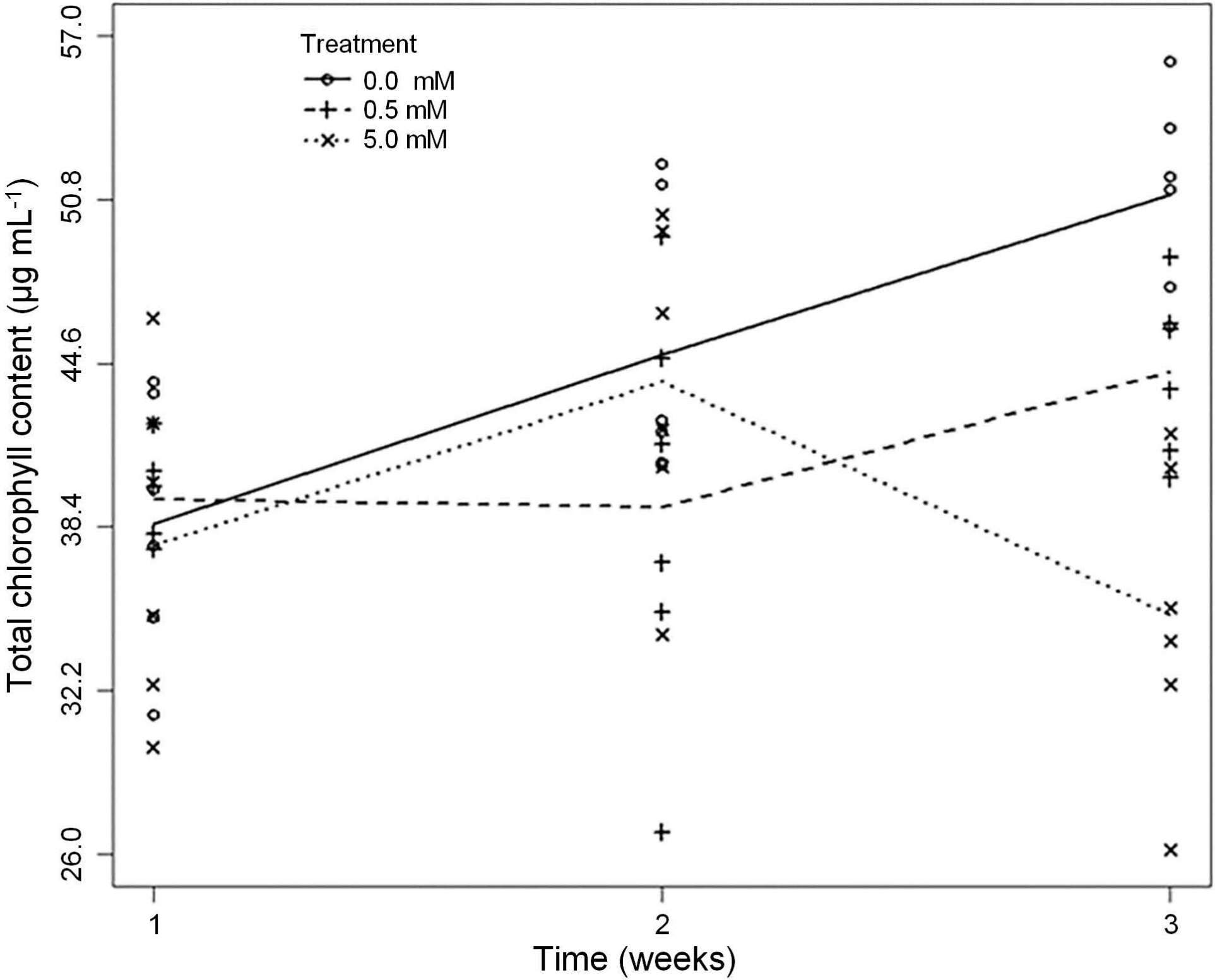
(*b*)

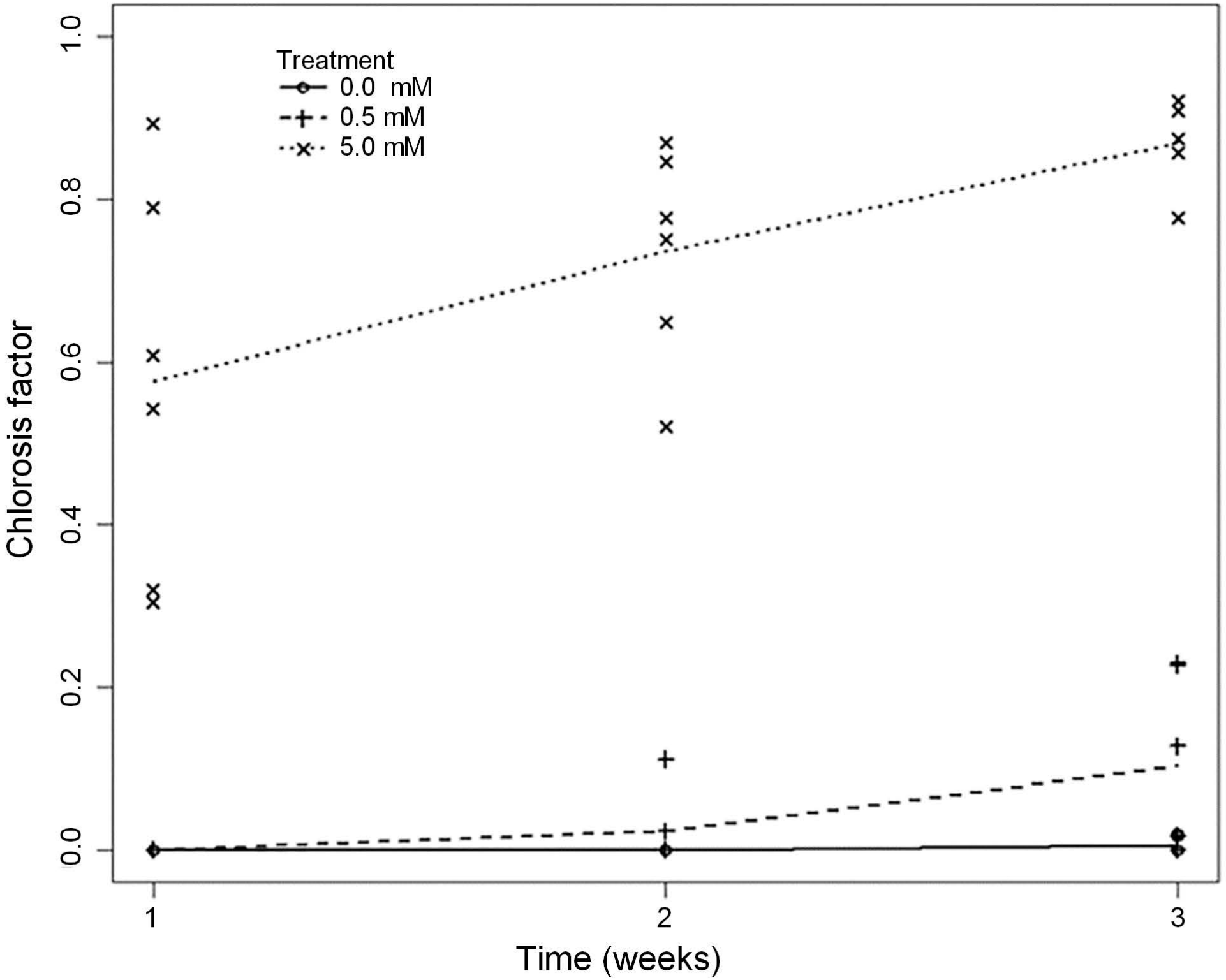

Low Field of View
Control
0.5 mM CsCl
5 mM CsCl
Mean reflectance relative to control
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
350
850
1350
1850
2350
Wavelength (nm)

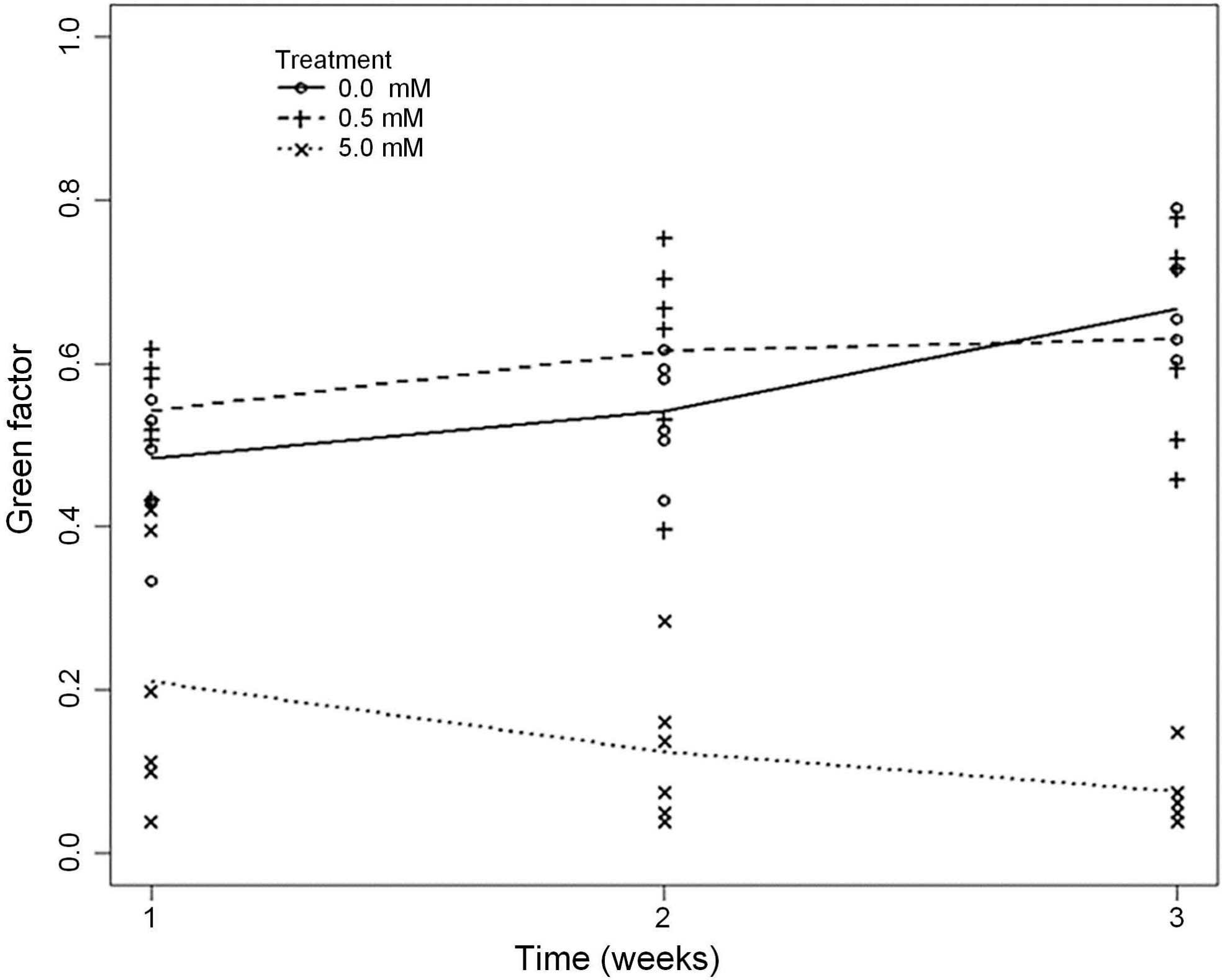
(*c*)

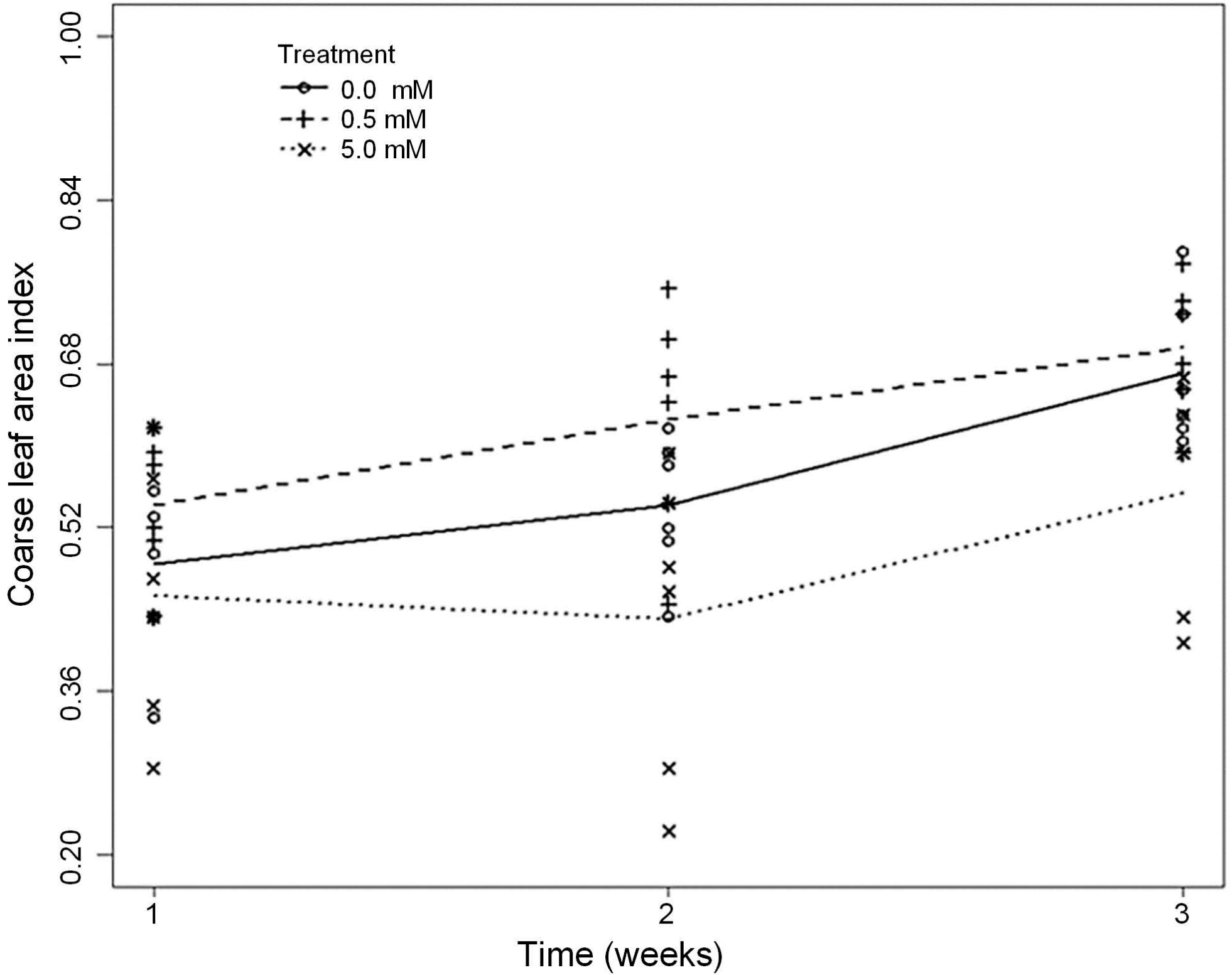

Contact Probe
Control
0.5 mM CsCl
5 mM CsCl
Mean reflectance relative to control
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
350
850
1350
1850
2350
Wavelength (nm)


Treatment
0.0 mM
0.5 mM
5.0 mM
Relative water content
0.95
0.93
0.91
0.89
0.87
0.85
1
2
3
Time (weeks)


Treatment
0.0 mM
0.5 mM
5.0 mM
Total chlorophyll content (µg mL-1)
57.0
50.8
44.6
38.4
32.2
26.0
1
2
3
Time (weeks)


Treatment
0.0 mM
0.5 mM
5.0 mM
Chlorosis factor
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
Time (weeks)


Treatment
0.0 mM
0.5 mM
5.0 mM
Green factor
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
Time (weeks)


Treatment
0.0 mM
0.5 mM
5.0 mM
Coarse leaf area index
1.00
0.84
0.68
0.52
0.36
0.20
1
2
3
Time (weeks)

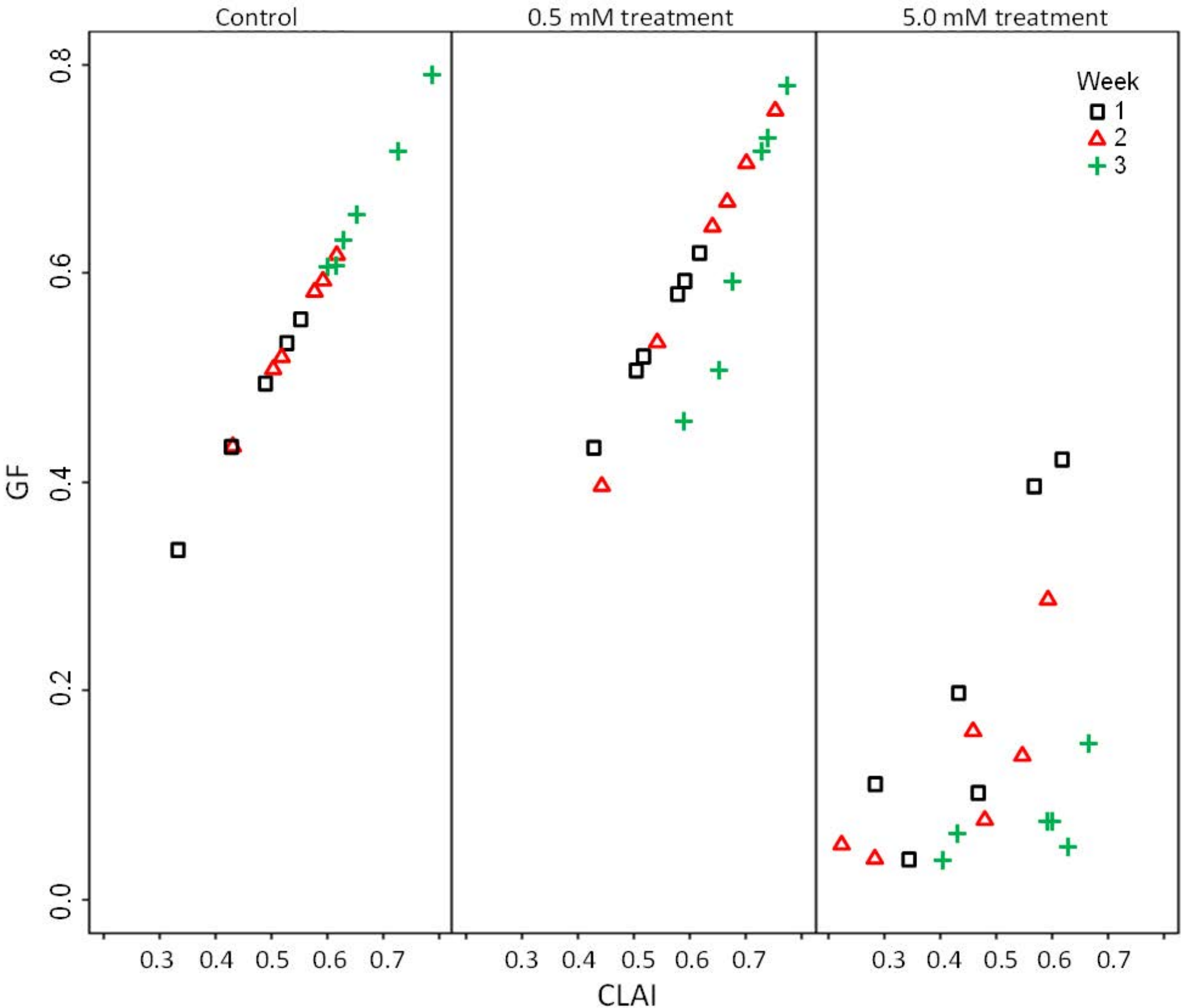

Control
0.5 mM treatment
5.0 mM treatment
Week
1
2
3
GF
0.0
0.2
0.4
0.6
0.8
0.3
0.4
0.5
0.6
0.7
CLAI

Table 1. List of vegetation indices considered, including name and abbreviation if applicable, the relevant acquisition technique(s), formulation, and either potential predictive characteristics along with the corresponding reference or indication that the listed index is newly considered by the authors. FOV represents field of view, CP represents contact probe, and R_y is the reflectance at y nm.

Abbreviation/Name		Relevant acquisition technique	Formulation	Potential indicator of:	Reference
NORMALIZED DIFFERENCES					
NDVI	Non-destructive vegetation index	FOV	$\frac{R_{800}-R_{670}}{R_{800}+R_{670}}$	Green biomass; leaf area	Rouse et al. (1974)
PRI	Photochemical reflective index	FOV CP	$\frac{R_{531}-R_{570}}{R_{531}+R_{570}}$	Photosynthetic radiation-use efficiency	Gamon, Peñuelas, and Field (1992)
SIPI	Structural independent pigment index	FOV CP	$\frac{R_{800}-R_{445}}{R_{800}-R_{680}}$	Carotenoid to chlorophyll a ratio	Peñuelas, Baret, and Filella (1995)
PSND	Pigment specific normalized difference	FOV CP	$\frac{R_{800}-R_{680}}{R_{800}+R_{680}}$	Chlorophyll content	Serrano (2008)
SIMPLE RATIOS					
WI	Water index	FOV CP	$\frac{R_{900}}{R_{970}}$	Plant water content	Peñuelas et al. (1997)
--	--	FOV CP	$\frac{R_{750}}{R_{550}}$	Some correlation with metal content	Davids and Tyler (2003)
--	--	FOV CP	$\frac{R_{1110}}{R_{810}}$	Metal stress	Maruthi-Sridhar et al. (2007a)
--	--	FOV CP	$\frac{R_{725}}{R_{675}}$	Some correlation with chlorophyll content; appeared independent of soil moisture	Davids and Tyler (2003)
--	--	FOV CP	$\frac{R_{950}}{R_{750}}$	*Selected by author*	--
--	--	FOV CP	$\frac{R_{1390}}{R_{1454}}$	*Selected by author*	--
--	--	FOV CP	$\frac{R_{1676}}{R_{1933}}$	*Selected by author*	--
DERIVATIVE ANALYSIS					
YI	Yellowness index	FOV CP	$-0.1\left(\frac{R_{580}-2R_{624}+R_{668}}{\Delta\lambda^2}\right)$	Chlorosis	Adams, Philpot, and Norvell (1999)
REP	Red edge position	FOV CP	Wavelength of inflection point from red to NIR	Chlorophyll content	Horler, Dockray, and Barber (1983)
SREP	Slope at red edge position	FOV CP	First derivative value at the red edge position	Chlorophyll content; leaf area index	Filella and Peñuelas (1994)

Table 2. Results of endpoint analysis for CsCl exposure including P-values from the two-way ANOVA, with significant values (<0.05) shown underlined and in bold.

Source	RWC	Chl a+b	CLAI	GF	CF
Treatment	**<0.001**	**0.006**	**<0.001**	**<0.001**	**<0.001**
Week	**<0.001**	**0.017**	**<0.001**	0.403	**0.001**
Interaction	0.240	**0.002**	0.653	**0.006**	**0.020**

Table 3. Pearson correlation coefficients for VI indices by field of view acquired. HFOV represents high field of view, LFOV represents low field of view, and CP represents contact probe.

VI	LFOV and HFOV	LFOV and CP	HFOV and CP
NDVI	0.964	--	--
PRI	0.602	0.819	0.514
WI	0.311	-0.173	0.075
SIPI	0.900	0.593	0.530
PSND	0.957	0.410	0.447
YI	0.861	0.650	0.664
R_{1110}/R_{810}	0.825	0.181	0.228
R_{950}/R_{750}	0.863	0.484	0.462
R_{750}/R_{550}	0.957	0.295	0.270
R_{1390}/R_{1454}	0.633	0.270	-0.058
R_{1676}/R_{1933}	0.941	-0.219	-0.274
R_{725}/R_{675}	0.953	0.460	0.452
REP	0.894	0.427	0.447
Slope at REP	0.855	0.263	0.334
WI/NDVI	0.893	--	--
(R_{950}/R_{750})/NDVI	0.940	--	--

Table 4. VI indices included in the model (in order of inclusion) for each endpoint, along with appropriate view with which acquired. HFOV represents high field of view, LFOV represents low field of view, and CP represents contact probe.

RWC		Chl a+b		CLAI		GF		CF	
R_{950}/R_{750}	CP	REP	HFOV	SREP	HFOV	R_{1676}/R_{1933}	HFOV	R_{1676}/R_{1933}	LFOV
R_{1676}/R_{1933}	CP	YI	HFOV	R_{1390}/R_{1454}	CP	YI	HFOV	YI	HFOV
PSND	LFOV	R_{1676}/R_{1933}	HFOV	SREP	LFOV	SREP	CP	SREP	CP
R_{750}/R_{550}	LFOV	R_{950}/R_{750}	CP	R_{950}/R_{750}	HFOV	R_{1390}/R_{1454}	CP	R_{1676}/R_{1933}	CP
		R_{1390}/R_{1454}	CP	R_{1676}/R_{1933}	CP			REP	CP

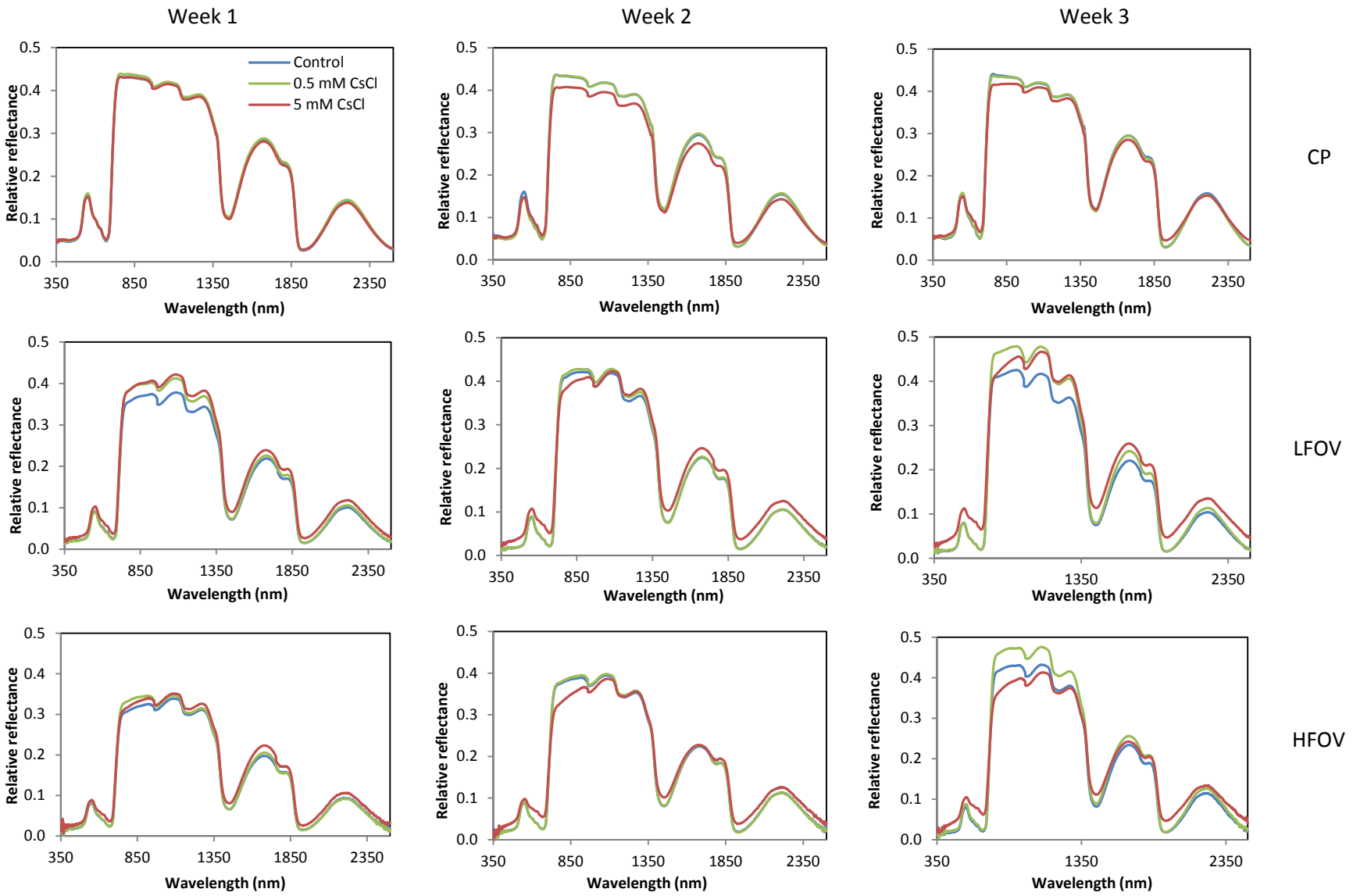


Figure S1: Reflectance spectra for Arabidopsis CsCl treatments, where red is 5 mM CsCl, green is 0.5 mM CsCl, and blue is the control. HFOV represents high field of view, LFOV represents low field of view, and CP represents contact probe.

Treatment group	Week		
	1	2	3
Control			
0.5 mM CsCl			
5.0 mM CsCl			

Figure S2: Pictures of all plant samples by week and treatment group

Figure S3: Control plants at week 1 with grid overlay used for visual assessment comparison

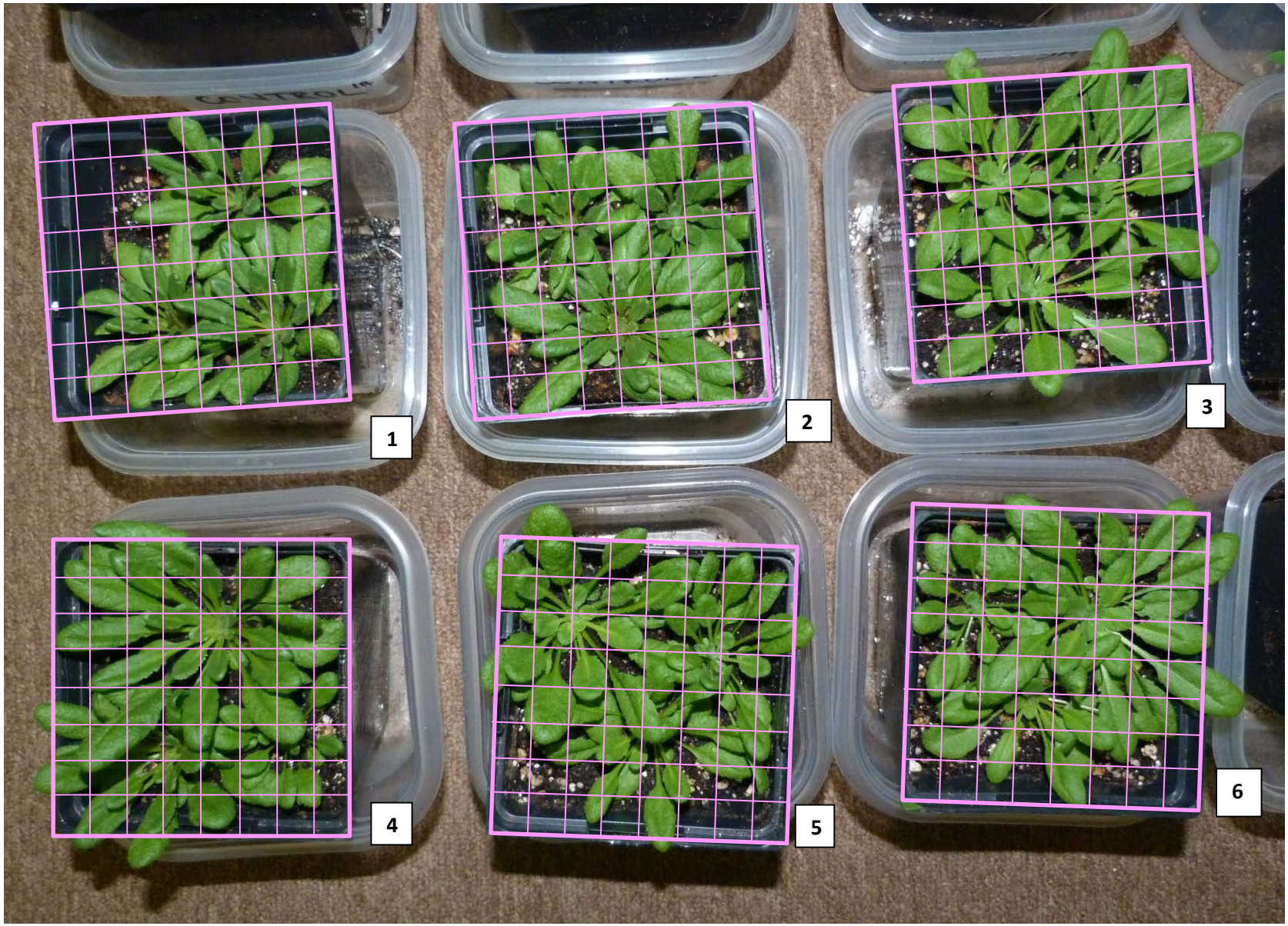


Figure S4: 0.5 mM CsCl treated plants at week 1 with grid overlay used for visual assessment comparison

Figure S5: 5 mM CsCl treated plants at week 1 with grid overlay used for visual assessment comparison

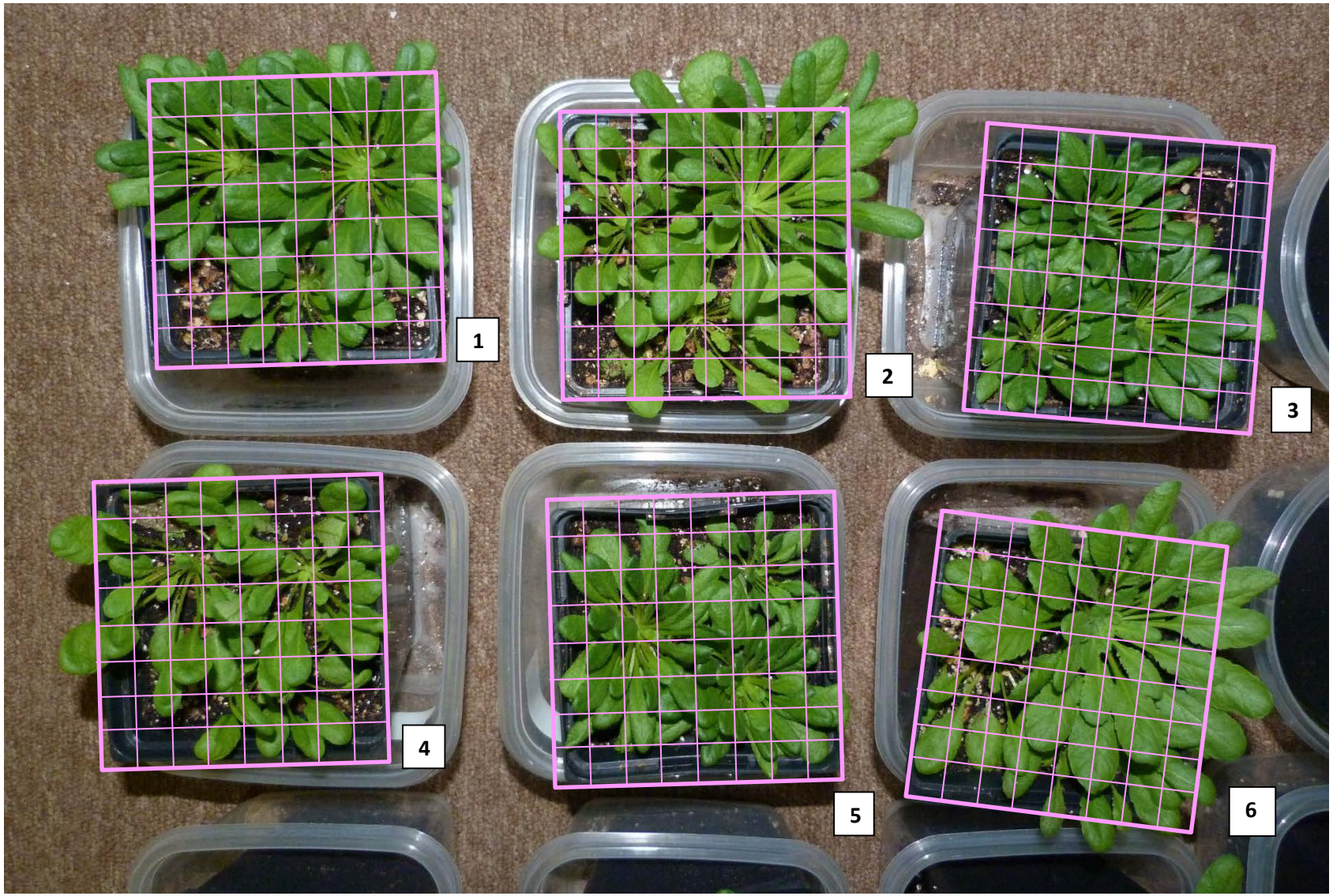


Figure S6: Control plants at week 2 with grid overlay used for visual assessment comparison

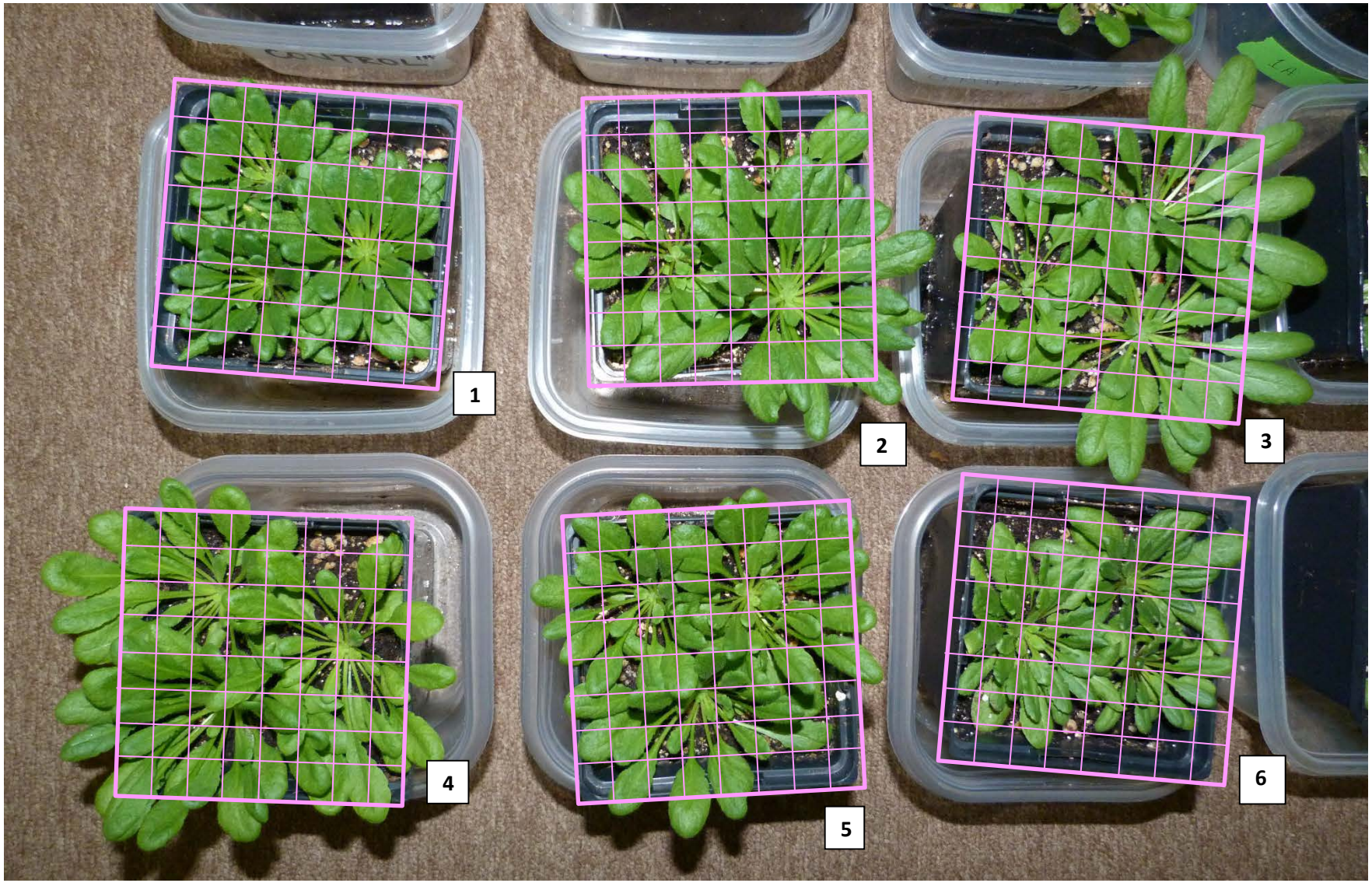


Figure S7: 0.5 mM CsCl treated plants at week 2 with grid overlay used for visual assessment comparison

Figure S8: 5 mM CsCl treated plants at week 2 with grid overlay used for visual assessment comparison

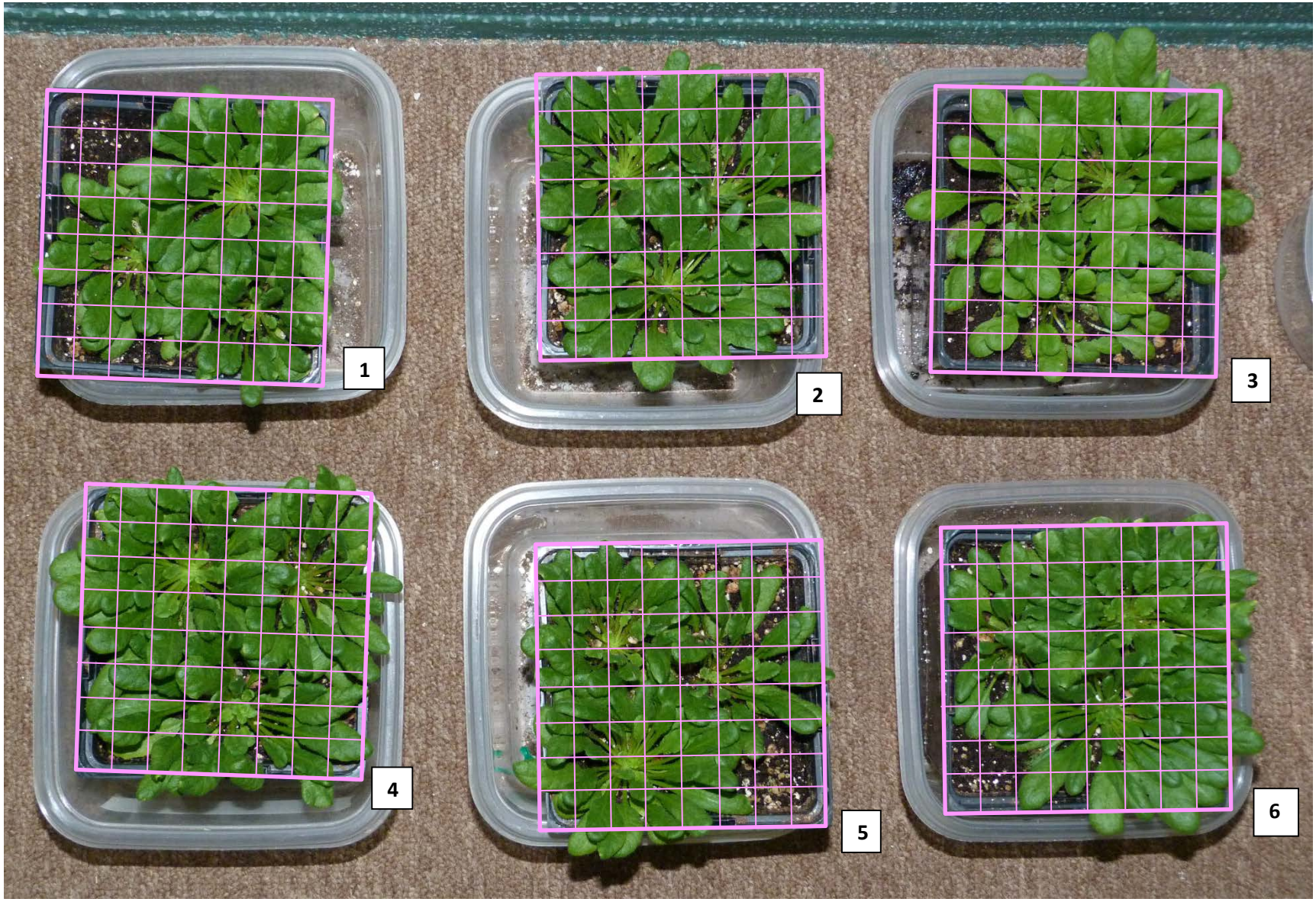


Figure S9: Control plants at week 3 with grid overlay used for visual assessment comparison

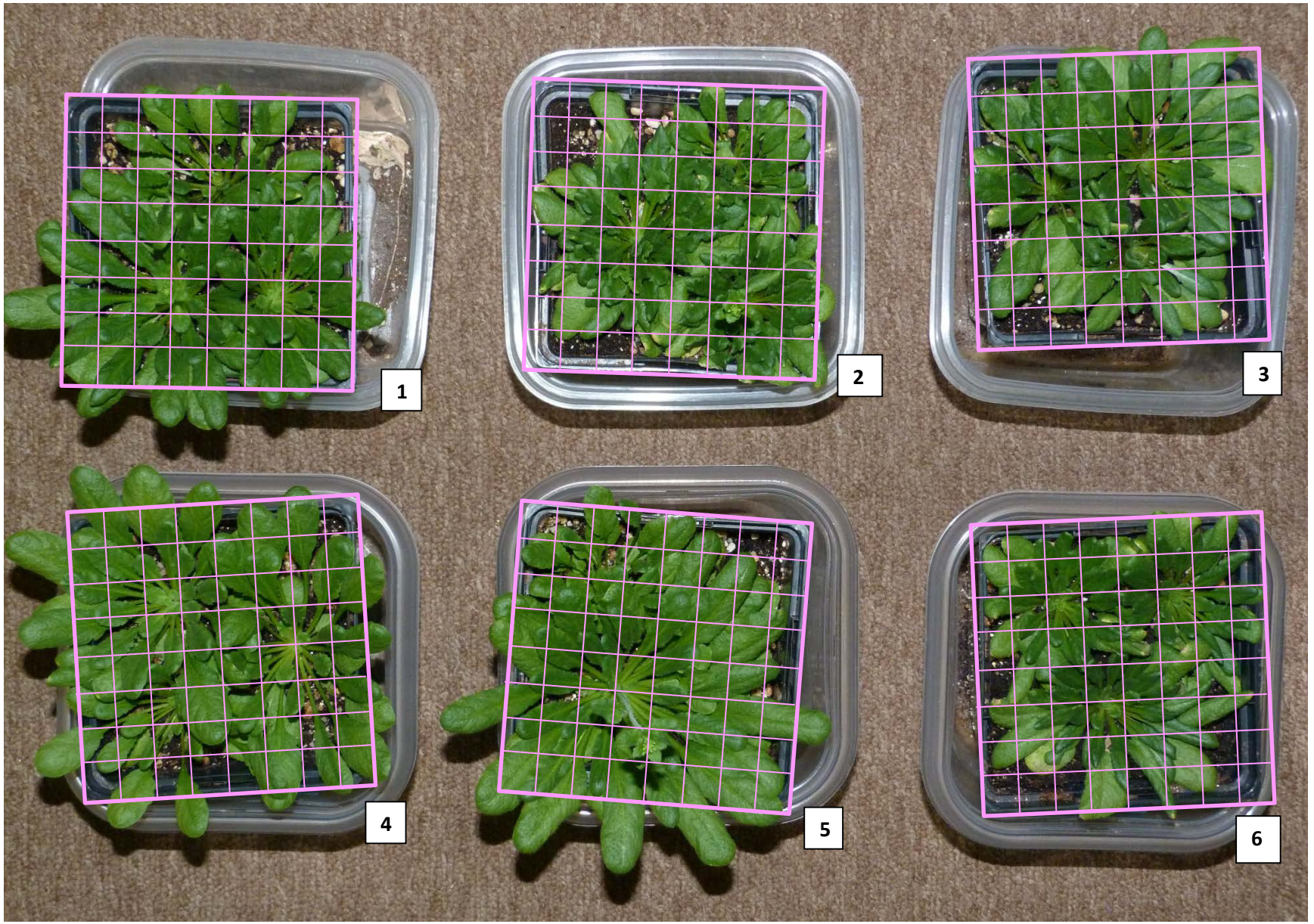


Figure S10: 0.5 mM CsCl treated plants at week 3 with grid overlay used for visual assessment comparison

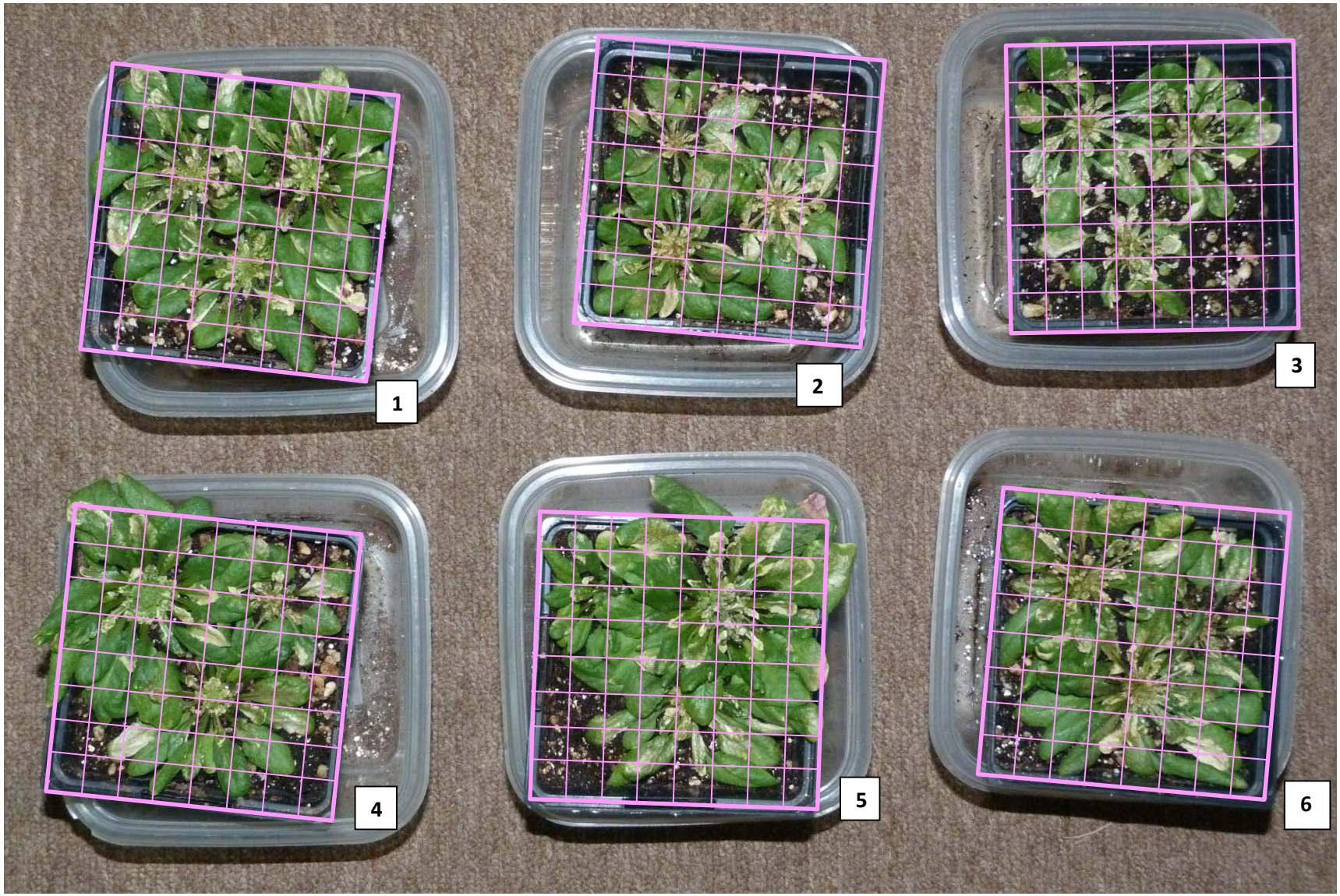


Figure S11: 5 mM CsCl treated plants at week 3 with grid overlay used for visual assessment comparison

Supplementary Tables (S2) – *Assessing the use of reflectance spectroscopy in determining CsCl stress in the model species Arabidopsis thaliana*

Table S1. Week by treatment comparisons of least squares means for relative water content. Results are shown for comparisons of the means at each combination of time point and treatment level. The top number in each cell is the *F* test statistic and the bottom number is the corresponding *p*-value for the comparison of means in the corresponding row/column. For example, comparing the 0.5 mM treatment group at weeks 1 and 3 yielded an *F* test statistic of -4.11 and a *p*-value <0.001.

Time point and treatment level of mean	Week 1, 0.0 mM	Week 1, 5.0 mM	Week 1, 0.5 mM	Week 2, 0.0 mM	Week 2, 5.0 mM	Week 2, 0.5 mM	Week 3, 0.0 mM	Week 3, 5.0 mM
Week 1, 5.0 mM	-2.72							
	0.009							
Week 1, 0.5 mM	-0.62	2.10						
	0.537	0.041						
Week 2, 0.0 mM	-0.32	2.41	0.31					
	0.754	0.020	0.761					
Week 2, 5.0 mM	-4.18	-1.46	-3.56	-3.86				
	<0.001	0.152	<0.001	<0.001				
Week 2, 0.5 mM	-1.31	1.42	-0.69	-0.99	2.87			
	0.198	0.164	0.497	0.327	0.006			
Week 3, 0.0 mM	-1.26	1.47	-0.63	-0.94	2.92	0.05		
	0.216	0.149	0.529	0.352	0.005	0.960		
Week 3, 5.0 mM	-5.32	-2.60	-4.70	-5.01	-1.14	-4.02	-4.07	
	<0.001	0.013	<0.001	<0.001	0.258	<0.001	<0.001	
Week 3, 0.5 mM	-4.73	-2.01	-4.11	-4.42	-0.55	-3.43	-3.48	0.59
	<0.001	0.050	<0.001	<0.001	0.582	0.001	0.001	0.558

Table S2. Week by treatment comparisons of least squares means for chlorophyll content. Results are shown for comparisons of the means at each combination of time point and treatment level. The top number in each cell is the *F* test statistic and the bottom number is the corresponding *p*-value for the comparison of means in the corresponding row/column.

Time point and treatment level of mean	Week 1, 0.0 mM	Week 1, 5.0 mM	Week 1, 0.5 mM	Week 2, 0.0 mM	Week 2, 5.0 mM	Week 2, 0.5 mM	Week 3, 0.0 mM	Week 3, 5.0 mM
Week 1, 5.0 mM	-0.27							
	0.786							
Week 1, 0.5 mM	0.29	0.56						
	0.774	0.577						
Week 2, 0.0 mM	2.09	2.37	1.80					
	0.042	0.022	0.078					
Week 2, 5.0 mM	1.76	2.03	1.47	-0.34				
	0.086	0.048	0.149	0.738				
Week 2, 0.5 mM	0.19	0.47	-0.09	-1.90	-1.56			
	0.847	0.643	0.925	0.064	0.125			
Week 3, 0.0 mM	4.08	4.35	3.79	1.98	2.32	3.88		
	<0.001	<0.001	<0.001	0.054	0.025	<0.001		
Week 3, 5.0 mM	-1.13	-0.86	-1.42	-3.22	-2.89	-1.32	-5.20	
	0.265	0.396	0.163	0.002	0.006	0.192	<0.001	
Week 3, 0.5 mM	1.88	2.15	1.59	-0.21	0.13	1.69	-2.20	3.01
	0.066	0.037	0.118	0.833	0.901	0.099	0.033	0.004

Table S3. Week by treatment comparisons of least squares means for coarse leaf area index (CLAI). Results are shown for comparisons of the means at each combination of time point and treatment level. The top number in each cell is the *F* test statistic and the bottom number is the corresponding *p*-value for the comparison of means in the corresponding row/column.

Time point and treatment level of mean	Week 1, 0.0 mM	Week 1, 5.0 mM	Week 1, 0.5 mM	Week 2, 0.0 mM	Week 2, 5.0 mM	Week 2, 0.5 mM	Week 3, 0.0 mM	Week 3, 5.0 mM
Week 1, 5.0 mM	-0.54 0.593							
Week 1, 0.5 mM	1.00 0.321	1.54 0.130						
Week 2, 0.0 mM	1.00 0.321	1.54 0.130	0.00 1.000					
Week 2, 5.0 mM	-0.93 0.356	-0.39 0.695	-1.94 0.059	-1.94 0.059				
Week 2, 0.5 mM	2.48 0.017	3.01 0.004	1.47 0.148	1.47 0.148	3.41 0.001			
Week 3, 0.0 mM	3.26 0.002	3.80 <0.001	2.26 0.029	2.26 0.029	4.20 <0.001	0.79 0.434		
Week 3, 5.0 mM	1.22 0.229	1.76 0.086	0.22 0.831	0.22 0.83	2.15 0.037	-1.26 0.216	-2.04 0.047	
Week 3, 0.5 mM	3.70 0.001	4.23 <0.001	2.69 0.010	2.69 0.010	4.63 <0.001	1.22 0.229	0.43 0.669	2.48 0.017

Table S4. Week by treatment comparisons of least squares means for green factor (GF). Results are shown for comparisons of the means at each combination of time point and treatment level. The top number in each cell is the *F* test statistic and the bottom number is the corresponding *p*-value for the comparison of means in the corresponding row/column.

Time point and treatment level of mean	Week 1, 0.0 mM	Week 1, 5.0 mM	Week 1, 0.5 mM	Week 2, 0.0 mM	Week 2, 5.0 mM	Week 2, 0.5 mM	Week 3, 0.0 mM	Week 3, 5.0 mM
Week 1, 5.0 mM	-4.67 <0.001							
Week 1, 0.5 mM	0.98 0.331	5.65 <0.001						
Week 2, 0.0 mM	0.98 0.331	5.65 <0.001	0.00 1.000					
Week 2, 5.0 mM	-6.14 <0.001	-1.47 0.148	-7.12 <0.001	-7.12 <0.001				
Week 2, 0.5 mM	2.25 0.030	6.91 <0.001	1.26 0.213	1.26 0.213	8.39 <0.001			
Week 3, 0.0 mM	3.12 0.003	7.79 <0.001	2.14 0.038	2.14 0.038	9.26 <0.001	0.88 0.385		
Week 3, 5.0 mM	-6.98 <0.001	-2.32 0.025	-7.97 <0.001	-7.97 <0.001	-0.84 0.404	-9.23 <0.001	-10.11 <0.001	
Week 3, 0.5 mM	2.49 0.017	7.16 <0.001	1.51 0.138	1.51 0.138	8.63 <0.001	0.25 0.807	-0.63 0.531	9.47 <0.001

Table S5. Week by treatment comparisons of least squares means for chlorosis factor (CF). Results are shown for comparisons of the means at each combination of time point and treatment level. The top number in each cell is the *F* test statistic and the bottom number is the corresponding *p*-value for the comparison of means in the corresponding row/column.

Time point and treatment level of mean	Week 1, 0.0 mM	Week 1, 5.0 mM	Week 1, 0.5 mM	Week 2, 0.0 mM	Week 2, 5.0 mM	Week 2, 0.5 mM	Week 3, 0.0 mM	Week 3, 5.0 mM
Week 1, 5.0 mM	9.94 <0.001							
Week 1, 0.5 mM	0.00 1.000	-9.94 <0.001						
Week 2, 0.0 mM	0.00 1.000	-9.94 <0.001	0.00 1.000					
Week 2, 5.0 mM	12.69 <0.001	2.74 0.009	12.69 <0.001	12.69 <0.001				
Week 2, 0.5 mM	0.38 0.702	-9.56 <0.001	0.38 0.702	0.38 0.702	-12.30 <0.001			
Week 3, 0.0 mM	0.11 0.916	-9.84 <0.001	0.11 0.916	0.11 0.916	-12.58 <0.001	-0.28 0.782		
Week 3, 5.0 mM	14.99 <0.001	5.05 <0.001	14.99 <0.001	14.99 <0.001	2.31 0.026	14.61 <0.001	14.89 <0.001	
Week 3, 0.5 mM	1.77 0.0831	-8.17 <0.001	1.77 0.0831	1.77 0.0831	-10.91 <0.001	1.39 0.172	1.67 0.103	-13.22 <0.001

Table S6: Results (*F* test statistics and *p*-values) from the mixed model effects analysis of vegetation indices

	NDVI		PRI		WI		SIPI	
Source	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value
Week	*F*(2,45)=0.32	0.730	*F*(2,39.8)=16.61	<0.001	*F*(2,50.3)=16.61	0.108	F(2,35.2)=4.57	0.017
Treatment	*F*(2,45)=57.11	<0.001	*F*(2,39.9)=134.41	<0.001	*F*(2,50.3)=134.41	<0.001	F(2,41.6)=37.70	<0.001
Week* Treatment	*F*(4,45)=5.02	0.002	*F*(4,39.9)=9.25	<0.001	*F*(4,42.0)=9.25	0.178	F(4,35.1)=6.60	0.001
View	*F*(1,49)=86.71	<0.001	*F*(2,55.1)=80.90	<0.001	*F*(2,83.6)=80.90	<0.001	F(2,21.4)=19.80	<0.001
View*Week	*F*(2,49)=3.82	0.029	*F*(4,29.3)=217.80	<0.001	*F*(4,77.2)=217.80	<0.001	F(4,36.9)=8.03	<0.001
View* Treatment	*F*(2,49)=5.46	0.007	*F*(4,52.0)=16.47	<0.001	*F*(4,74.3)=16.47	0.001	F(4,37.3)=5.53	0.001

	PSND		YI		R_{1110}/R_{810}		R_{950}/R_{750}	
Source	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value
Week	*F*(2,46.1)=0.61	0.547	*F*(2,41.6)=1.31	1.310	*F*(2,49.8)=0.96	0.391	F(2,48.0)=0.56	0.572
Treatment	*F*(2,44.2)=66.98	<0.001	*F*(2,37.2)=74.55	<0.001	*F*(2,47.4)=21.11	<0.001	F(2,49.1)=31.20	<0.001
Week* Treatment	*F*(4,39.3)=6.17	0.001	*F*(4,34.2)=4.45	0.005	*F*(4,39.2)=3.60	0.014	F(4,43.5)=5.45	0.001
View	*F*(2,33.1)=85.75	<0.001	*F*(2,63.5)=30.09	<0.001	*F*(2,40.2)=156.16	<0.001	F(2,34.4)=216.51	<0.001
View*Week	*F*(4,29.4)=190.91	<0.001	*F*(4,47.8)=8.58	<0.001	*F*(4,49.2)=4.89	0.002	F(4,34.6)=15.45	<0.001
View* Treatment	*F*(4,35.4)=9.45	<0.001	*F*(4,56.9)=2.63	0.043	*F*(4,49.8)=5.55	0.001	F(4,49.4)=5.66	0.001

	R_{750}/R_{550}		R_{725}/R_{675}		REP		Slope at REP	
Source	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value
Week	*F*(2,42.9)=7.70	0.001	*F*(2,47.1)=3.12	0.053	*F*(2,37.2)=10.05	<0.001	*F*(2, 40.5)=4.63	0.015
Treatment	*F*(2,42.9)=14.18	<0.001	*F*(2,47.9)=138.05	<0.001	*F*(2,37.7)=10.65	<0.001	*F*(2, 38.5)=23.27	<0.001
Week* Treatment	*F*(4,41.5)=2.53	0.055	*F*(4,39.4)=10.24	<0.001	*F*(4,35.7)=1.47	0.231	*F*(4,34.7)=5.26	0.002
View	*F*(2,78.3)=223.02	<0.001	*F*(2,67.2)=142.62	<0.001	*F*(2,73.0)=64.34	<0.001	*F*(2,60.4)=191.41	<0.001
View*Week	*F*(4,60.4)=12.05	<0.001	*F*(4,39.0)=27.82	<0.001	*F*(4,64.1)=7.88	<0.001	*F*(4,59.5)=17.47	<0.001
View* Treatment	*F*(4,64.5)=9.56	<0.001	*F*(4,45.1)=40.01	<0.001	*F*(4,62.5)=6.86	<0.001	*F*(4,56.2)=3.27	0.018

	R_{1676}/R_{1933}		R_{1390}/R_{1454}		WI/NDVI		(R_{950}/R_{750})/NDVI	
Source	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value	*F* test statistic	*p*-value
Week	*F*(2,51.2)=10.72	<0.001	*F*(2,53.7)=0.96	0.391	*F*(2,24.4)=3.30	0.054	*F*(2,21.2)=4.57	0.286
Treatment	*F*(2,50.8)=125.79	<0.001	*F*(2,51.5)=53.43	<0.001	*F*(2,44.0)=42.20	<0.001	*F*(2,37.0)=37.70	<0.001
Week* Treatment	*F*(4,42.8)=2.17	0.089	*F*(4,45.5)=3.93	0.008	*F*(4,34.3)=7.65	<0.001	*F*(4,30.5)=6.60	0.001
View	*F*(2,73.3)=69.45	<0.001	*F*(2,58.4)=40.86	<0.001	*F*(1,27.6)=3.08	0.090	*F*(1,12.8)=8.48	0.012
View*Week	*F*(4,62.0)=2.08	0.094	*F*(4,42.2)=40.88	<0.001	*F*(2,27.1)=4.28	0.024	*F*(2,9.04)=13.05	0.002
View* Treatment	*F*(4,51.0)=95.76	<0.001	*F*(4,56.0)=15.51	<0.001	*F*(2,27.5)=3.34	0.050	*F*(2,13.1)=4.02	0.044

Table S7: Results (*F* test statistics and *p*-values) from multiple linear regression analysis.* HFOV represents high field of view, LFOV represents low field of view, and CP represents contact probe.

		RWC		Chl a+b		CLAI		GF		CF	
VI	View	*F*(1,38)	*p*-value	*F*(1,48)	*p*-value	*F*(1,49)	*p*-value	*F*(1,49)	*p*-value	*F*(1,47)	*p*-value
PSND	LFOV	9.61[3]	0.003	--	--	--	--	--	--	--	--
R_{1390}/R_{1454}	CP	--	--	6.39[5]	0.015	7.80[2]	0.007	9.40[4]	0.004	--	--
R_{1676}/R_{1933}	CP	8.42[2]	0.006	--	--	3.05[5]	0.087	--	--	9.64[4]	0.003
	LFOV	--	--	--	--	--	--	--	--	340.26[1]	<0.001
	HFOV	--	--	4.66[3]	0.036	--	--	190.31[1]	<0.001	--	--
R_{750}/R_{550}	LFOV	22.49[4]	<0.001	--	--	--	--	--	--	--	--
R_{950}/R_{750}	CP	25.67[1]	<0.001	6.53[4]	0.014	--	--	--	--	--	--
	HFOV	--	--	--	--	6.65[4]	0.013	--	--	--	--
REP	HFOV	--	--	16.98[1]	<0.001	--	--	--	--	--	--
	CP	--	--	--	--	--	--	--	--	5.39[5]	0.025
Slope at REP	CP	--	--	--	--	--	--	4.49[3]	0.039	13.53[3]	0.001
	LFOV	--	--	--	--	4.88[3]	0.032	--	--	--	--
	HFOV	--	--	--	--	69.65[1]	<0.001	--	--	--	--
YI	HFOV	--	--	3.25[2]	0.077	--	--	14.06[2]	0.001	5.17[2]	0.027

* Superscripts denote the order in which the predictor entered into the model. All F-values are sequential. For RWC, NDVI at low field of view was highly collinear with other predictors. The model selection procedure was run without NDVI at low field of view and this is the resulting model selected. For GF, R_{1676}/R_{1933} at low field of view was highly collinear with other predictors. The model selection procedure for GF was run without R_{1676}/R_{1933} at low field of view and this is the resulting model selected.