Physiological response of Miscanthus x giganteus grown in nutritionally poor post-military soil

Hana Malinská (malinska@gmail.com)
Univerzita Jana Evangelisty Purkyne v Usti nad Labem
Valentina Pidlisnyuk
Univerzita Jana Evangelisty Purkyne Fakulta zivotniho prostredi
Diana Nebeská
Univerzita Jana Evangelisty Purkyne Fakulta zivotniho prostredi
Anna Erol
Univerzita Jana Evangelisty Purkyne
Andrea Medžová
Univerzita Jana Evangelisty Purkyne v Usti nad Labem
Josef Trögl
Univerzita Jana Evangelisty Purkyne Fakulta zivotniho prostredi

Research article

Keywords: Miscanthus x giganteus, leaf fluorescence, nutritionally poor post-military soil, plant physiology, plant stress

Posted Date: October 22nd, 2019

DOI: https://doi.org/10.21203/rs.2.16314/v1

Abstract

Background: To determine the level of plant stress of second-generation biofuel crop Miscanthus x giganteus (Mxg) grown in poor quality post-military soils using physiological parameters; to identify impact of plant growth regulators (PGRs) on the stress reduction and to select the fluorescence indicators most suitable for quantification of stress. Plant fitness was quantified with non-invasive measurement of leaf fluorescence using 16 different indexes. Simultaneously visible stress signs were observed on stems and leaves and differences were revealed by microscopy of leaf sections.

Results: Leaf fluorescence analysis, visual observation and changes of leaf anatomy revealed significant stress in all tested subjects . Besides commonly used F v /F m and P.I. (performance index), the fluorescence parameter T fm (time to achieve maximum fluorescence) seems to be rather sensitive and applicable for revealing finer differences. However, none of investigated parameters proved significant positive effect of PGRs on stress reduction.

Conclusions: Measuring F v /F m , P.I., and T fm is a suitable method for revealing stress affecting Mxg in poor post-military soils. Since PGRs application did not reduce the stress level, direct application of soil amendments should be considered for stress reduction and improving the biomass quality.

Background

Plant biomass is a renewable source of energy and feedstock for bio products which ensures achieving of given sustainability goals [1]. However, its competition with food crops on the high quality agricultural land is against these objectives. Thus, different types of marginal lands are under consideration for biomass production with proper selection of suitable crops. According to Gerwin et al., 46 % of area in Europe were identified as marginal for different reasons (low fertility, texture, pH, salinity, wetting or contamination) and 22.6 % of these sites were determined to be suitable for biomass production [2].

Second generation biofuel crop *Miscanthus x giganteus* (*Mxg*)„ a triploid hybrid ($2n = 3x = 57$) derived from two parental species *Miscanthus sinensis* and *Miscanthus sacchariflorus* [3], is a perspective plant for biomass production at the marginal lands [4, 5, 6]. Its closest relatives are other important crops like sorghum, sugarcane or worldwide produced crop, maize. Production of these crops is highly affected by climate [7]. In current, rapidly changing weather condition, *Mxg* showed 59% increase of yield compared to maize in field trials [8]. Also, compared to other C4 cold tolerant biomass crops like switchgrass, *Mxg* is more than twice as productive [9].

Different miscanthus species vary in cold tolerance. According to physiological measurements (F_v/F_m), *Mxg* displays the highest vitality among other miscanthus species [10]. This perennial grass proved to be very adaptable to different climate zones, its efficient C4 metabolism contributes to high yields of biomass within single vegetation season. Production is stable for more than 20 years, except for the first and second years after planting [11]. Due to the high content of lignin and cellulose [12], it can also serve as feedstock for bio products. The hybrid is sterile and does not represent any danger of invasion being

reproduced through vegetative rhizomes only. Growing *Mxg* can be beneficial in different ways simultaneously: it can serve as fast growing source of biomass, it is able to improve soil quality and to prevent soil erosion, to retain moisture and it is tolerant to different types of contaminants [13, 14].

In our previous study [15], the effect of two plant growth regulators (PGRs) on *Mxg* biomass parameters was tested. Unusually high uptake of biogenic metals Mn and Zn was determined in *Mxg* above-parts if grown in poor post-military soil with low metals concentrations. It was assumed that this fact was connected with stress caused by poor soil characteristics and insufficient nutrients supply.

Many types of stress can directly or indirectly influence the level of plant fitness. As a result, main metabolic functions can be altered or suppressed and even green vigorously growing plants can undergo severe stress [16] and the most important process, photosynthesis, can be influenced.

The common approaches for investigation of plant fitness level include analyses of secondary metabolites [17], measuring level of plant hormones [18] and extraction of leaf pigments [19]. While using these methods, certain amount of plant tissue has to be destructed and analysed. Furthermore, these methods are time-consuming, costly and may create an additional stress while harming the plant. As an alternative, non-destructive measurement of leaf fluorescence can give broader picture about different stages of photosynthesis process throughout vegetation season [20]. This approach is fast, less laborious and minimizes additional stress to the plant.

It is known [21], that yield of leaf fluorescence can give a valuable information about efficiency of primary photochemistry and plant fitness. Different plant physiology indexes are applied for identification and quantification of plant stress. These “vitality indexes” usually focus on processes within primary photochemistry, however, they can result in slightly different cipher. There is a little data about the most appropriate type of measurement for identification of stress in plants while growing in the nutritionally poor soils, so it is not clear, which of these numerous indexes is the most appropriate.

The use of microscopy for measurement of leaf sections, thickness of leaves and stomatal density can give broader idea about understanding the tissue structure [22]. Quantification of certain specific structures, like sclerenchyma cells, can also reveal substantial changes between plants. Sclerenchyma cells have very thick secondary walls, usually lignified or incrusted by silicon oxides. In monocotyledonous plants, they are aggregated in clusters forming “caps” around vascular bundles. These cells provide mechanical support, and due to the thick walls, they do not possess living content therefore they are not capable of mitotic division [23], therefore, their number is final. However, there are only a few data when microscopy research was used for studying of miscanthus leaves [24]. In that study, only differences of leaves structure of miscanthus varieties in response to cold, were measured and authors did not elaborate any stress factor related to the soil in which the plant grew.

In the current study, the complex approach was applied which united non-destructive method of measurement of leaf fluorescence with supplementary microscopic measuring at harvest. The evaluation of the common plant physiology values/indexes F_v/F_m and Performance index were combined with less

used indicators: ET_0/RC, TR_0/RC, V_j and M_0, F_0, F_m, F_v, ABS/CS, T_{fm}, TR_0/CS, ET_0/CS, DI_0/CS, RC/CS_0, RC/CR_m which can help to detect minor changes in primary phase of photosynthesis. That combination permitted to study the effect more deeply and to evaluate impact of soil properties and treatment of crop by PGRs on the physiology parameters during plant growth in nutritionally poor post-military marginal soils.

Methods

Soils

Soils for the experiment were taken from two points at the former military airport Hradcany, marked as Hradcany 1 (H1), 50°37’31”N, 14°43’23”E and Hradcany 2 (H2), 50°37’26”N, 14°44’49”E. More detailed explanation of site location and sampling procedure was done previously [15]. Generally, soil samples were taken from upper 30 cm and homogenized according to the standard procedure [25].

Both soils were regosols, sandy types of soil with low water retention capacity and prone to acidification. According to online BPEJ (estimated pedologic-ecological unit) catalogue [26] production potential of this area is very low (code 5.21.10). Agrochemical parameters of soils were examined in accordance with methodology compatible with ISO or CEN standards (Table 1). Data confirmed low fertility and slight acidity of both tested soils. Slightly higher concentration of available nutrients was detected for H2 but H1 had higher content of organic matter.

The low quality of soil was reflected also in the state of soil microorganisms which play important role in soil functions and plant growth. The soil microbial communities were characterized using phospholipid fatty acids (PLFA), method compliant with ISO/TS 29843–2 [27] used in our recent studies [28, 29, 30]. Microbial activity was assessed via basal soil respiration as described previously [31]. The value of $PLFA_{tot}$, representing total living microbial biomass was very low, comparable with recent spoil heaps after brown coal mining [32]. The other PLFA parameters also indicated the influence of stress conditions: low ratios fungi/bacteria (F/B PFLA) and grampositive bacteria/gramnegative bacteria (G+/G- PLFA) [33]. All tested parameters were more favourable in H1 suggesting that content of organic matter was more limiting factor than N, P, K accessibility for microorganisms.

One plant was grown in compost (marked as C soil) as representative of good quality standard soil (Table 1). This plant was used in microscopy experiments.

Experiment design

The experiment design was the same as described earlier [15]. Two rhizomes of *Mxg "*Rankova Zorya”(three years old), produced by the Institute of Bioenergy Crops and Sugar Beet, Ukraine, with

average weight 20±2g, were planted in pots with 1 kg of sand, 1 kg of ceramzite drainage and 10 kg of soil. The depth was about 10 cm.

Two PGRs, Stimpo and Regoplant were provided by Agrobiotech, Ukraine (http://www.agrobiotech.com.ua/). PGR treatment was performed in two or three replicates. These PGRs previously positively affected production of several first generation energy crops [34], were applied to miscanthus rhizomes before planting and during vegetation. Those substances include essential micronutrients, phytohormones and natural extracts that promote growth of bacteria in the soil. Detailed characteristics of selected PGRs are shown in Table 2.

Part of the rhizomes was treated before planting by pre-soaking in 10 L of PGR solution of different concentrations for 12 hours (Table 3). The control rhizomes were soaked in distilled water for the same time. The pre-soaking concentrations were 10 mL/10 L; 25 mL/10 L; 50 mL/10 L for treatment by Stimpo and 250 mL/10 L for Regoplant.

Above part biomass of selected plants was additionally sprayed with PGR solution in given concentration during vegetation. First spraying was performed when 3 - 4 leaves appeared, the second spraying was performed at full vegetation. *Mxg* was grown one vegetation season in real conditions and irrigated by tap water while necessary (2—3 times per week).

Physiological parameters

Measurement of chlorophyll a fluorescence was performed throughout vegetation season (in the same time of the day) using portable fluorimeter Handy PEA (Hansatech Instruments, UK). After 15 minutes of dark adaptation, 650 nm saturation pulse with intensity of 3500 μmol/m^2/s was emitted by 3 LED diodes. Three youngest fully developed leaves were measured for each plant.

Fluorescence parameters: F_0, minimal fluorescence intensity, initial fluorescence after application of actinic light when all reaction centers of PSII are open, were measured. F_m, maximal level of fluorescence measured when all PSII reaction centers are closed. In addition, values in between these stages, F_j equal to fluorescence at 2ms, F_i corresponding to fluorescence at 30 ms and F_k describing fluorescence after 300 μs were analysed.

Based on these values, other physiological parameters were calculated: V_j, fluorescence intensity at 2 ms. M_0, approximated initial slope of the fluorescence transient, F_v/F_m ratio, maximum quantum yield of primary photochemistry, ET_0 (electron transported), DI_0 (energy dissipated) and TR_0 (energy trapped) per reaction centre (RC) or cross section (CS). The absorption per cross section (ABS/CS) and density of reaction centers per excited cross section at time t = 0 (RC/CS_0) or t = m (RC/CS_m) were evaluated as well. The detailed description of indexes is done at Abbreviations and formulas.

Microscopy

Microscopy analysis was performed at the end of vegetation season for avoiding additional plant stress. Second fully developed leaf from one plant grown in soil H1 and one from soil H2 was extracted 5 cm from leaf tip and compared with same material harvested from plant grown in soil C. 3 x 4 mm blocks of leaves were frozen and sliced using Leica CM 1100 freezing microtome to 12μm thick slices, mounted in water and observed under inverted fluorescence microscope NIB–100F, Novel. Autofluorescence was recorded at excitation of 400–410 nm with barrier filter 455 nm using camera Eurekam 3.0 PLUS, BEL. Images were processed for brightness and contrast only using Image J software. Measurements of anatomical traits were performed using Scopelmage 9.0.

Number of stomata per square cm was counted on bottom side of leaf (3 measurements per each leaf). Bundle size was measured using “radius” tool in Scopelmage software, measuring three biggest vascular bundles. Sclerenchyma cells above and under these largest bundles were counted. Only cells with less than 50% of inner content were counted as “stone cells”. Leaf thickness and small bundle distance were measured multiple times (40 times per each leaf).

Statistical analysis

The fluorescence data were processed using Microsoft Excel and Statistica for basic analysis. The differences among varieties and treatments were analysed by the Student t-test at $\alpha = 0.05$ significance level. Further statistical analysis was carried out using R Software Version 3.4.1–”Single Candle” [35]. There were 12 or less data points for each group available, so can not be assumed. That is why non-parametrical Kruskal-Wallis test was carried out [36], For the 95% significant results of Kruskal-Wallis test, a post-hoc test - pairwise comparisons using Wilcoxon rank sum test [37] were conducted. The adjustment method for multiple comparison after Benjamini-Hochberg was used [38]. The plots were prepared using “ggplot2”, “tidyverse” and “extrafont” packages [39].

Results

Soil type effect

F_v/F_m ratio and performance index (P. I.) were used for basic characteristics of non-treated plants. Results presented at Fig. 1a illustrate that plants growing in H2 soil were a little more successful in performing primary phase of photosynthesis (mainly photosystem II) expressed by F_v/F_m ratio. For P. I. which describes functionality of both photosystem I and photosystem II, difference in groups was not so obvious (Fig. 1 b). Nevertheless both groups display much lower F_v/F_m than expected in healthy plants with value 0.85 [40].

Despite the fact that F_v/F_m ratio in all individuals implies severe stress in both groups, plants look green and vigorous (Fig. 2). However, plants in H1 and H2 soil are light green with purple veins and lower leaves compared to same-aged individuals in C soil (Fig. 2 a). Plants in high-nutritional soil also lacked dry ends

and dry spots on leaves, looked more “dark green” and produced more/stronger stems compared to H1 and H2 plants.

PGRs treatments effect

Boxplot of F_v/F_m and P. I. values of Stimpo and Regoplant treated plants are illustrated in Fig. 3. The slight difference could be seen in fitness among plants treated by Stimpo. In comparison to control, some groups showed increase of F_v/F_m (such as H1_S25x25, H1_S25x50), another (H2_S10x0, H2_S25x0) showed decrease of that value. However, none of these differences was statistically significant at 95% level of significance (Table 4).

For evaluation of Regoplant treatment effect, pairwise comparisons using Wilcoxon rank sum test was provided, and only treatment H2_R250x250 (highest concentration of Regoplant, 250 mL/10 L) gave statistically different result in comparison to untreated control plant in both parameters. For F_v/F_m, H2_R250x250 showed statistical significance not only to control H2, but this index was also significantly lower than control plants grown in H1 soil.

Since different concentrations of PGRs (except from group H2_R250x250) did not show significant impact to the stress factors, further evaluation was focused on type of PGR application. Mean values for 16 physiological parameters were calculated for non-treated plants and plants treated by Stimpo and Regoplant. Different ways of PGRs application, soaking (So) and soaking combined with spraying (Sp) were compared (Fig 4). The worst physiological states were found for plants treated by spraying of above biomass by high concentration of Regoplant, the effect was mainly determined in plants grown in H2. Treatment by Stimpo in high concentrations did not have such dramatic effect neither on plant physiology, either on biomass production [15]. It was comparable to non-treated variants.

Besides F_v/F_m and P. I. value, which showed partial differences between variants, another measured parameter - T_{fm} showed differences in individual groups. Increase of T_{fm} was observed after application of Regoplant in both soil types, the same picture was observed for treatment by Stimpo in H2 variants. On contrary, Stimpo, even in the highest concentrations, did not affect T_{fm} for H1. That trend in value of T_{fm} for Stimpo sprayed plants suggests that this parameter is highly sensitive and may be considered for detailed analyses.

The similar results were observed for other measured parameters: TR_0/CS, RC/CS or $ET_0/$ CS, where application of Regoplant in all variants decreased trapping time, electron transport and number of reaction centres per cross section of the leaf. On the other hand, additional spraying with Stimpo increased these parameters independently on soil type and revealed some stimulatory effect of this PGR.

Effect on morphology

The autofluorescence of leaf surface and transversal leaf sections confirmed severe changes in morphology in plants grown in the different soil types. The comparison of H2 plants with H1 plants and C plant (grown in nutritionally rich compost) was presented in Fig. 2. Leaves of C plants were green (Fig. 2 a), vigorous, had well organized stomata in one row, as seen from bottom of the leaf (Fig. 5 e) and, were rich in sclerenchyma cells as seen on transversal section (Fig. 6). Considering, that C plant was incubated under the same conditions as H1 and H2 plants and the only difference was much better nutrition, that plant can be handled as “standard phenotype”.

Closest phenotype to “standard” could be observed in plants grown in H2 soil, where leaves were green with light purple edges (Fig. 2 c), they lack some sclerenchyma, compared to C, but resemble developmental stage of standard plant (Fig. 5 c, d, Fig. 6). Its stomata were organized in two rows or one and two rows (Fig. 5 g, h). In H1 plants, alterations of xylem could be seen (Fig. 5 b, red arrow), according to shape and number of bundles, and seems to be underdeveloped. Size and number of motor cells was much lower than in C plants and plants cultivated in soil H2. Overall colour of the leaf was faint with dark purple edges (Fig. 2 b) which is typical for undernourished leaves.

The measurement of anatomical traits (Fig. 6) showed changes in certain plants. It could be concluded that stomatal density and leaf thickness increased in plants grown in soil type H2, on the other hand size of sclerenchyma regions above and under big bundles in H2 and H1 plants decreased along with distance between small vascular bundles. Main vein size remained almost unchanged.

Discussion

Physiological status and changes in leaf fluorescence

The differences in physiological and morphological parameters observed among plants grown in H1 and H2 soil can be explained by different soil properties. According to Strašil et al., the appropriate soil pH for growing miscanthus is 5.5–6.5 [41]. This is optimal for most plants because slightly acidic soil pH ensures good availability of most micronutrients. In our case, both types of soil were in optimal pH range, therefore changes in fitness had to be caused by another reason. Andrejić et al. observed decrease of F_v/F_m after one month of exposure to zinc contaminated soil [42]. In that research miscanthus did not display such a low value of maximum quantum yield of PSII photochemistry as in our study, contrary, plants showed chlorotic spots and dry margins on their leaves, Rapid drop of F_v/F_m was observed after exposure to physical stress rather than chemical. In experiment of Jiao et al. [10], various genotypes of *Mxg* were grown at different temperatures (24, 16, 10, 6°C). Subjects exposed to lower temperatures displayed much lower F_v/F_m. Similar results were obtained by Ings et al. [43] during measurement of F_v/F_m and P. I. value in drought stressed *Mxg* plants. After four weeks both parameters dropped rapidly. In experiment of Bilska-Kos et al. with *Mxg* after three days cold treatment no significant differences were found in F_v/F_m values [24].

In the current study 16 physiological parameters were evaluated in order to detect the possible positive effect of PGRs for stress reduction in the system. As it is seen in Fig. 3, some parameters remained almost unchanged in treated and non-treated plants (TR_0/RC), some parameters changed within one type of treatment: for T_{fm} parameter changes between concentrations of applied PGR can be found, implying that this parameter can be used as highly sensitive marker to minor changes in plant photochemistry. However, all available instruments did not provide this type of measurement. Therefore the common F_v/F_m parameter [16, 20] was included in this study.

Some authors consider F_v/F_m ratio as parameter with low sensitivity [45]. However, in our case F_v/F_m displayed bigger changes than widely used performance index (P. I.). This may be explained by the fact that main changes occur within PSII, however, it is necessary to stress that high variability of measured data was received. Because F_v/F_m and other fluorescence-based parameters are not stress-specific, the investigation of plant leaf structure by independent, but invasive, method was performed.

Changes in leaf anatomy

The various changes in leaf anatomy were observed starting with increased number of stomata in H1 and H2 plants. As known [46], increase in stomatal density is often connected to influence of drought and environmental factors. In our case, low water retention rate of H1 and H2 soil, might cause some anatomical changes rather than impact of differences in soil nutritional qualities.

The other typical feature for H1 and H2 plants was lower amount of sclerenchyma cells in comparison with C plant. Moreover, H1 plants differ in sclerenchymatic tissues from H2 plants. It was proved, that lack of potassium in rice can lead to decreased amount of sclerenchyma [47]. That peculiarities were observed in the current study, when H1 soil with lower concentration of available potassium showed lower rate of sclerenchyma cells. Significant role of potassium for synthesis of stability tissues (like sclerenchyma) was observed for other crops as wheat [48] and oilseed rape [49].

H2 soil was generally more nutritious in some inorganic elements and H1 soil contained more organic particles.

In that case, beside lower concentration of nutrients, visible signs of undernourishment like purple leaves, signs of stress (low F_v/F_m, P. I.) were not only caused by low concentration of nutrients, but could be amplified by low retention of the soil that caused problematic accessibility of nutrients to plants.

The anatomical changes were reported after exposure to abiotic stress as well. Pitman et al. observed increase in sclerenchyma cells in kleingrass subjected to water stress [50]. Some other changes in anatomical traits were observed in plants being exposed to stress. Bilska-Kos et al. applied cold treatment to young *Mxg* plants and after three days [24], leaf thickness as well as bundle sheath area increased. Bundle sheath distance varied between experimental variants. Similarly to our plants, Makbul et al. observed decrease in amount of sclerenchyma in soybean stems in drought stressed plants [51].

Effect of PGRs

Overall, the satisfactory stimulatory effect of PGRs was not evident in our system. The partial increase of certain parameters was observed with treatment by Stimpo while plant grew in both types of soils H1 and H2, but generally influence on fitness was rather negative and mainly evident for high concentration of applied Regoplant. The reason for this effect was not clear and requires additional research, nevertheless all of studied plants (treated and also non-treated) displayed certain dose of stress (F_v/F_m below 0.85). Results from our previous study on biomass [15] implies that their growth is retarded. This could be caused by various environmental factors or experiment itself (incubation in pots).

Both PGRs contain various compounds and extracts which were expected to stimulate plant growth. As reported by Ponomarenko et al. (2010), when energy crops grew in nutritionally rich soil, these PGRs stimulated nutrient uptake, plants grew well and used strong photosynthetic apparatus for production of large amount of biomass [34]. However, as it was observed in the current study when *Mxg* grew in nutritionally poor soil, stimulation by PGRs did not result in better growth like due to lack of available nutrients. The situation was worsened by Regoplant, which contains synthetic analogue of plant auxin (1-NAA) and as a result, the stimulatory effect of this hormone for growth could not be fulfilled (because of lack of nutrients) and depleted plant even more. The results clearly showed that for improving *Mxg* biomass parameters during plant growth in poor soil, other agronomic technique can be proposed, including incorporation of soil amendments [52], which positively influenced biomass parameters of *Mxg* when the crop was grown at post-mining soil.

Conclusions

Application of two PGRs Stimpo and Regoplant was tested for reduction of stress level of energy crop *Mxg* when the plant grew in nutritionally poor post-military soil. It was verified that measurement of plant leaf fluorescence can serve as powerful tool to detect plant stress *in vivo.* It was shown that determination of common parameters F_v/F_m and P. I. was a suitable method for identification of major stress affecting *Mxg* in the research system. Finer changes in plant fitness were usually hidden from these two parameters, however the minor alterations can be revealed using other indexes. T_{fm} parameter was proposed as highly sensitive marker for detection of the minor changes in plant photochemistry.

The substantial changes in leaf morphology, i.e.: increased number of stomata and lower amount of sclerenchyma cells in plants grown in poor soils were found. Since it was established that application of PGRs Stimpo and Regoplant did not reduce the stress level of *Mxg,* the direct improvement of soil by soil amendments has to be considered for stress reduction.

Declarations

Ethics approval and consent to participate: The use of plants in the present study comply with international, national and/or institutional guidelines.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was funded by NATO Science for Peace and Security Programme MYP G4687, internal university grant UJEP-IGA-TC–2019–44–01–2 and grant UJEP-SGS–2017–44–003–3 within student grant competition at Jan Evangelista Purkyně University in Ústí nad Labem.

Authors' contributions: HM performed microscopy, analysed fluorescence data, wrote manuscript, VP conceived and designed the experiment, participated on drafting of the manuscript, DN performed soil analyses, analysed data, prepared manuscript figures, participated on drafting of the manuscript, AE conducted statistical analyses, AM performed fluorescence measurements, JT supervised the experiment, participated on drafting of the manuscript. All authors critically revised, commented and contributed to the final version of the manuscript. All authors read and approved the final manuscript.

Acknowledgements: We would like to thank Agrobiotech for providing us with Stimpo and Regoplant.

Permissions: We have permission to collect soil in research area. For pot experiments, no permission is required for collection of plant material. Since *Miscanthus x giganteus* is a sterile hybrid, it does not propagate through seeds, therefore it does not make threat for environment.

Abbreviations, Formulas (Adapted From Hansatech Instruments 2006)

F_o- zero fluorescence, fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidised; these acceptors are available to receive light energy due to dark adaptation.

F_m - maximum fluorescence, fluorescence level when all electron acceptors are fully reduced, no longer available for photochemistry

F_v - variable fluorescence (F_m-F_o)

CS - cross section of the leaf tissue

RC - reaction centre

F_j - fluorescence intensity at 2 ms

F_i - fluorescence intensity at 30 ms

F_k - fluorescence intensity at 300 μs

Parameters

F_v/F_m - maximum quantum efficiency of Photosystem II; maximum quantum yield of primary photochemistry; F_v/F_m - $TR_0/$ABS = $(F_m-F_o)/F_m$; typical value for non-stressed plant is 0,85 [40], can be decreased by biotic or abiotic stress

T_{fm} - time to achieve maximum fluorescence; shorter T_fm can indicate stress of the plant

M_0 - slope at the origin of the fluorescence rise; $M_0 = (F_k - F_{o)} / (F_m - F_{o)}$

Vj - relative variable fluorescence at 2 ms; $Vj = (F_j$ /Fo) / (F_m -Fo)

ABS/RC - absorption per RC; ABS/RC = $(M_{0/} Vj)$ / ((1- F_o / $F_{m))}$)

TR_0/RC - trapping at time zero per RC $TR_0/$RC = $M_{0/} Vj$

DI_0/RC - dissipation at time zero, per RC; $DI_0/$RC = (ABS/RC) - ($TR_0/$ABS)

ET_0/RC - electron transport at time zero per RC; $ET_0/$RC = $(M_{0/} Vj)$ (1- Vj)

ABS/CS - absorption per CS; ABS/CS = ($TR_0/$ABS) / (ABS/CS)

TR_0/CS - trapping at time zero per CS; $TR_0/$CS = ($TR_0/$ABS) (ABS/CS)

DI_0/CS - dissipation at time zero per CS; $DI_0/$CS = (ABS/CS) - ($TR_0/$CS)

ETo/CS - electron transport at time zero per CS; $ET_0/$CS = $(M_{0/} Vj)$ (1- Vj)

P. I.- performance index, vitality index; PI ABS = ((1-(F_o / $F_{m))/(}$/($M_{0/} Vj$))*(()*((F_m - $F_{o)/}$/ $F_{o)*((}$*((1- Vj)// Vj)

RC/CS_m - density of reaction centers per CS at F_m

RC/CS_0 - density of reaction centers per CS at F_o

References

[1]United Nations, “Sustainable development knowledge platform.”.

[2]W. Gerwin *et al.,* “Assessment and quantification of marginal lands for biomass production in Europe using soil-quality indicators,” vol. 4, pp. 267–290, 2018.

[3]T. R. Hodkinson and S. Renvoize, “Nomenclature of Miscanthus xgiganteus (Poaceae),” *Kew Bull.,* vol. 56, no. 3, p. 759, 2001.

[4]F. Nsanganwimana, B. Pourrut, M. Mench, and F. Douay, “Suitability of Miscanthus species for managing inorganic and organic contaminated land and restoring ecosystem services. A review,” *J. Environ. Manage.,* vol. 143, pp. 123–134, Oct. 2014.

[5]V. Pidlisnyuk, L. Erickson, S. Kharchenko, and T. Stefanovska, “Sustainable Land Management: Growing Miscanthus in Soils Contaminated with Heavy Metals,” *J. Environ. Prot. (Irvine,. Calif).,* vol. 05, no. 08, pp. 723–730, 2014.

[6]S. Rusinowski, J. Krzyżak, K. Sitko, H. M. Kalaji, E. Jensen, and M. Pogrzeba, “Cultivation of C4 perennial energy grasses on heavy metal contaminated arable land: Impact on soil, biomass, and photosynthetic traits,” *Environ. Pollut.,* vol. 250, pp. 300–311, Jul. 2019.

[7]C. V. Beale and S. P. Long, “Can perennial C4 grasses attain high efficiencies of radiant energy conversion in cool climates?,” *Plant, Cell Environ.,* vol. 18, no. 6, pp. 641–650, Jun. 1995.

[8]F. G. Dohleman and S. P. Long, “More Productive Than Maize in the Midwest: How Does Miscanthus Do It?,” *PLANT Physiol.,* vol. 150, no. 4, pp. 2104–2115, Aug. 2009.

[9]F. G. Dohleman, E. A. Heaton, A.D. B. Leakey, and S. P. Long, “Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of Miscanthus relative to switchgrass?,” *Plant. Cell Environ.,* vol. 32, no. 11, pp. 1525–1537, Nov. 2009.

[10]X. Jiao *et al.,* “Can miscanthus C_4 photosynthesis compete with festulolium C_3 photosynthesis in a temperate climate?,” *GCB Bioenergy,* vol. 9, no. 1, pp. 18–30, Jan. 2017.

[11]D. G. Christian, A. B. Riche, and N. E. Yates, “Growth, yield and mineral content of Miscanthus × giganteus grown as a biofuel for 14 successive harvests,” *Ind. Crops Prod.,* vol. 28, no. 3, pp. 320–327, Nov. 2008.

[12]T. Devrije, “Pretreatment of Miscanthus for hydrogen production by Thermotoga elfii,” *Int. J. Hydrogen Energy,* vol. 27, no. 11–12, pp. 1381–1390, Nov. 2002.

[13]N. Wanat *et al.,* “Potentials of Miscanthus×giganteus grown on highly contaminated Technosols,” *J. Geochemical Explor.,* vol. 126–127, no. 126–127, pp. 78–84, Mar. 2013.

[14]M. Pogrzeba *et al.,* “Relationships between soil parameters and physiological status of Miscanthus x giganteus cultivated on soil contaminated with trace elements under NPK fertilisation vs. microbial inoculation,” *Environ. Pollut.,* vol. 225, pp. 163–174, Jun. 2017.

[15]D. Nebeská *et al.,* “Impact of plant growth regulators and soil properties on Miscanthus x giganteus biomass parameters and uptake of metals in military soils,” *Rev. Environ. Health,* vol. 34, no. 3, pp. 283–291, Sep. 2019.

[16]N. R. Baker, “A possible role for photosystem II in environmental perturbations of photosynthesis,” *Physiol. Plant.,* vol. 81, no. 4, pp. 563–570, Apr. 1991.

[17]V. Shulaev, D. Cortes, G. Miller, and R. Mittler, “Metabolomics for plant stress response,” *Physiol. Plant.,* vol. 132, no. 2, pp. 199–208, Jan. 2008.

[18]V. Verma, P. Ravindran, and P. P. Kumar, “Plant hormone-mediated regulation of stress responses.,” *BMC Plant Biol.,* vol. 16, p. 86, Apr. 2016.

[19]L. Barton and B.C. Hemming, *Iron chelation in plants and soil microorganisms.* Academic Press, 1993.

[20]G. H. Krause and E. Weis, “Chlorophyll Fluorescence and Photosynthesis: The Basics,” *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* vol. 42, no. 1, pp. 313–349, Jun. 1991.

[21]W. L. Butler, “Energy Distribution in the Photochemical Apparatus of Photosynthesis,” *Annu. Rev. Plant Physiol.,* 2003.

[22]C.-D. Jiang, X. Wang, H.-Y. Gao, L. Shi, and W. S. Chow, “Systemic Regulation of Leaf Anatomical Structure, Photosynthetic Performance, and High-Light Tolerance in Sorghum,” *Plant Physiol.,* vol. 155, no. 3, pp. 1416–1424, Mar. 2011.

[23]F. B. Lopez and G. F. Barclay, “Plant Anatomy and Physiology,” *Pharmacognosy,* pp. 45–60, Jan. 2017.

[24]A. Bilska-Kos, P. Panek, A. Szulc-Głaz, P. Ochodzki, A. Cisło, and J. Zebrowski, “Chilling-induced physiological, anatomical and biochemical responses in the leaves of Miscanthus × giganteus and maize (Zea mays L.),” *J. Plant Physiol.,* vol. 228, pp. 178–188, Sep. 2018.

[25]DSTU, *DSTU ISO 11464–2001: Soil quality. Preliminary preparation of samples for physicalchemical analysis.* Kyiv, Ukraine, 2001.

[26]Research Institute for Soil and Water Conservation, “eKatalog BPEJ,” 2019..

[27]J. Trögl *et al.,* “Estimation of the quantity of bacteria encapsulated in Lentikats Biocatalyst via phospholipid fatty acids content: a preliminary study,” *Folia Microbiol. (Praha).,* vol. 58, no. 2, pp. 135–140, Mar. 2013.

[28]D. Nebeská *et al.,* “Effect of Growing Miscanthus x giganteus on Soil Microbial Communities in Post-Military Soil,” *Sustainability,* vol. 10, no. 11, p. 4021, Nov. 2018.

[29]J. Kukla *et al.,* “Tourist Traffic Significantly Affects Microbial Communities of Sandstone Cave Sediments in the Protected Landscape Area ‘Labské Pískovce’ (Czech Republic): Implications for Regulatory Measures,” *Sustainability,* vol. 10, no. 2, p. 396, Feb. 2018.

[30]E. Kakosová *et al.,* “Effect of various chemical oxidation agents on soil microbial communities,” *Chem. Eng. J.,* vol. 314, pp. 257–265, Apr. 2017.

[31]P. Kuráň *et al.,* “Biodegradation of Spilled Diesel Fuel in Agricultural Soil: Effect of Humates, Zeolite, and Bioaugmentation,” *Sci. World J.,* vol. 2014, pp. 1–8, 2014.

[32]P. Baldrian *et al.,* “Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining,” *Soil Biol. Biochem.,* vol. 40, no. 9, pp. 2107–2115, Sep. 2008.

[33]A. Kaur, A. Chaudhary, A. Kaur, R. Choudhary, and R. Kaushik, “Phospholipid fatty acid—A bioindicator of environment monitoring and assessment in soil ecosystem,” *Curr. Sci.,* 2005.

[34]S. P. Ponomarenko *et al.,* “Bioregulation of growth and development of plants: Plant growth regulators in crop science,” in *Bioregulyatsiya mikrobnorastitel’nykh system [Bioregulation of microbial-plant systems],* S. P. Ponomarenko and H. O. Iutynska, Eds. Kiev, Ukraine: Nichlava, 2010, pp. 251–291 [in Russian].

[35]R. Team, “R: A language and environment for statistical computing,” 2013.

[36]W. H. Kruskal and W. A. Wallis, “Use of Ranks in One-Criterion Variance Analysis,” *J. Am. Stat. Assoc.,* vol. 47, no. 260, pp. 583–621, Dec. 1952.

[37]F. Wilcoxon, “Individual Comparisons by Ranking Methods,” *Biometrics Bull.,* vol. 1, no. 6, p. 80, Dec. 1945.

[38]Y. Benjamini and Y. Hochberg, “Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing,” *Journal of the Royal Statistical Society. Series B (Methodological),* vol. 57. WileyRoyal Statistical Society, pp. 289–300, 1995.

[39]H. Wickham, *ggplot2.* New York, NY: Springer New York, 2009.

[40]O. Björkman and B. Demmig, “Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins,” *Planta,* vol. 170, no. 4, pp. 489–504, Apr. 1987.

[41]Z. Strašil, J. Weger, P. Hutla, and J. Kára, “The cultivation of Miscanthus determined for energy use (a summary of long-term monitoring),” in *Biopaliva z pohledu energetiky a vlivu na životní prostředí,* 2015, pp. 29–41.

[42]G. Andrejić, G. Gajić, M. Prica, Ž. Dželetović #, and T. Rakić, “Zinc accumulation, photosynthetic gas exchange, and chlorophyll a fluorescence in Zn-stressed Miscanthus × giganteus plants.”

[43]J. Ings, L. A. J. Mur, P. R. H. Robson, and M. Bosch, “Physiological and growth responses to water deficit in the bioenergy crop Miscanthus x giganteus,” *Front. Plant Sci.,* vol. 4, p. 468, 2013.

[44]R. Strasser, A. Srivastava, and M. Tsimilli-Michael, “The fluorescence transient as a tool to characterize and screen photosynthetic samples,” in *Probing Photosynthesis: Mechanism, Regulation & Adaptation,* 1st Editio., M. Yunus, U. Pathre, and M. Prasann, Eds. 2000, pp. 445–483.

[45]A. Oukarroum, S. Sa¨ıd, E. Madidi, G. Schansker, and R. J. Strasser, “Probing the responses of barley cultivars (Hordeum vulgare L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering,” *Environ. Exp. Bot.,* vol. 60, pp. 438–446, 2007.

[46]I. Gindel, “Stomata Constellation in the Leaves of Cotton, Maize and Wheat Plants as a Function of Soil Moisture and Environment,” *Physiol. Plant.,* vol. 22, no. 6, pp. 1143–1151, Jun. 1969.

[47]B. M. Vaithilingam C, “Effect of potash on sclerenchyma thickness and silica content in rice,” *Indian Potash J.,* vol. 1, pp. 17–23, 1976.

[48]R. D. Munson, J. D. Beaton, and G. S. Sekhon, “Potassium Nutrition of Wheat and Other Small Grains,” 1985.

[49]S. R. Sharma and S. J. Kolte, “Effect of soil-applied NPK fertilizers on severity of black spot disease (Alternaria brassicae) and yield of oilseed rape,” *Plant Soil,* vol. 167, no. 2, pp. 313–320, Dec. 1994.

[50]W. D. Pitman, E. C. Holt, B. E. Conrad, and E. C. Bashaw, “Histological Differences in Moisture-Stressed and Nonstressed Kleingrass Forage1,” *Crop Sci.,* vol. 23, no. 4, p. 793, 1983.

[51]S. Makbul, N. Saruhan Güler, and N. Durmuş, “Changes in anatomical and physiological parameters of soybean under drought stress,” *Turk J Bot,* vol. 35, pp. 369–377, 2011.

[52]M. Kharytonov, V. Pidlisnyuk, T. Stefanovska, M. Babenko, N. Martynova, and I. Rula, “The estimation of Miscanthus×giganteus’ adaptive potential for cultivation on the mining and post-mining lands in Ukraine,” *Environ. Sci. Pollut. Res.,* vol. 26, no. 3, pp. 2974–2986, Jan. 2019.

[53]J. Zbíral *et al., Jednotné pracovní postupy: Analýza půd I.* Brno: Central Institute for Supervising and Testing in Agriculture, 2016.

[54]J. Zbíral *et al., Jednotné pracovní postupy: Analýza půd III.* Brno: Central Institute for Supervising and Testing in Agriculture, 2011.

[55]D. Nebeská *et al.,* “Impact of plant growth regulators and soil properties on Miscanthus x giganteus biomass parameters and uptake of metals in military soils,” *Rev. Environ. Health,* vol. 0, no. 0.

Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

Figures

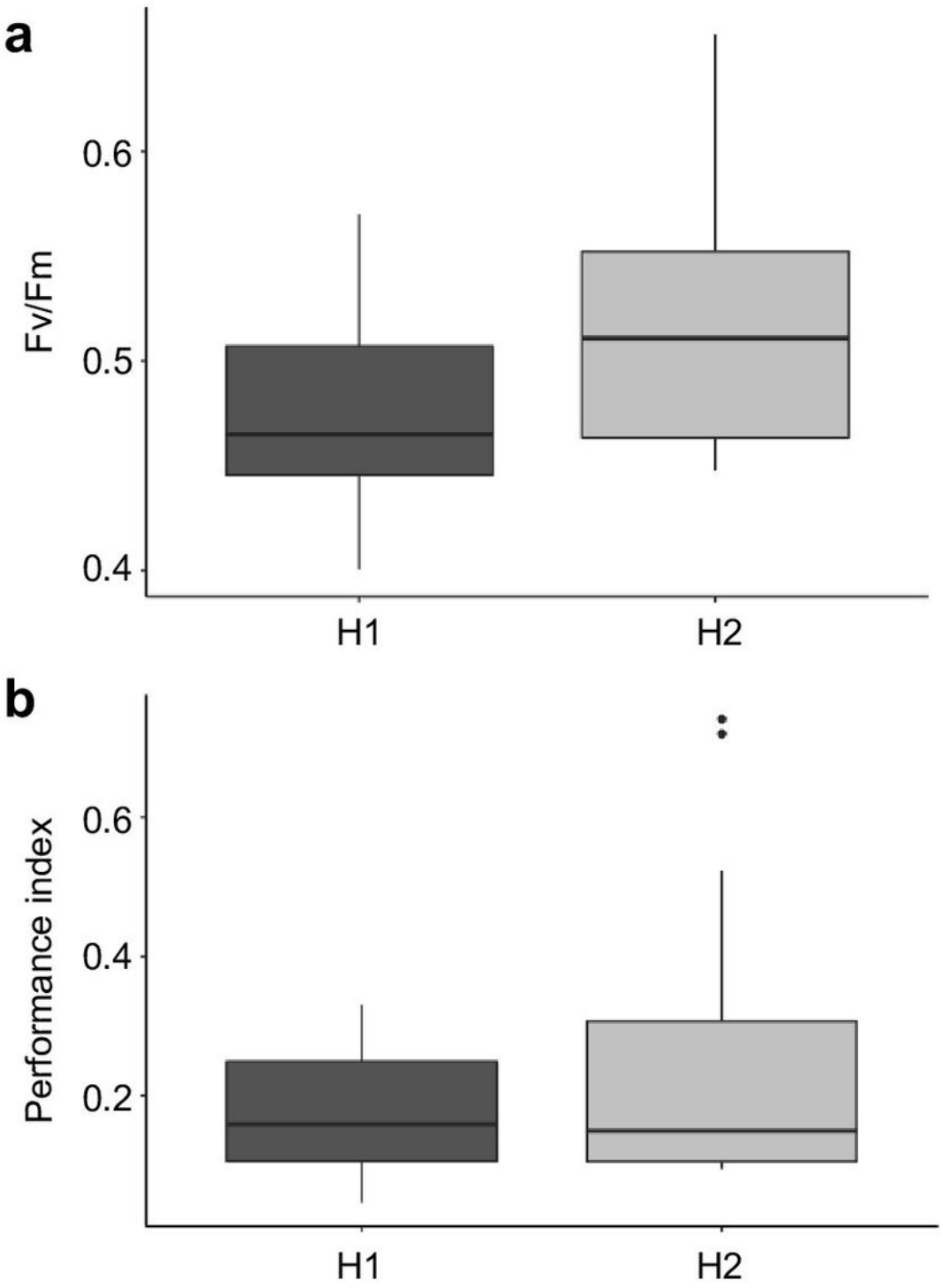

Figure 1

Fv/Fm and b P.I. for non-treated plants in different types of soil (H1, H2).

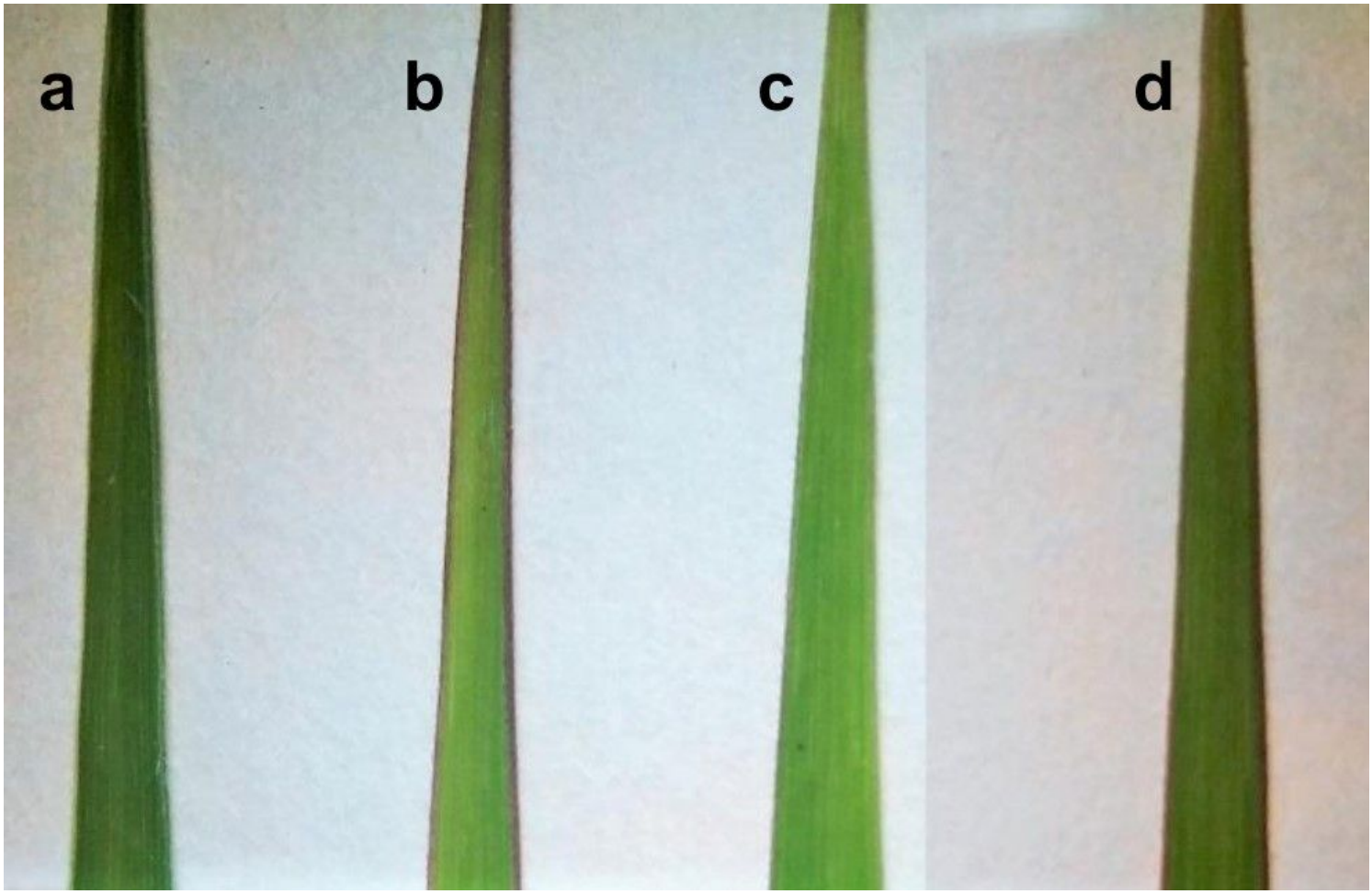

Figure 2

Mxg leaves used for microscopy- : a) C soil, b) H1 soil, c) H2 soil and d) H2_R250x250.

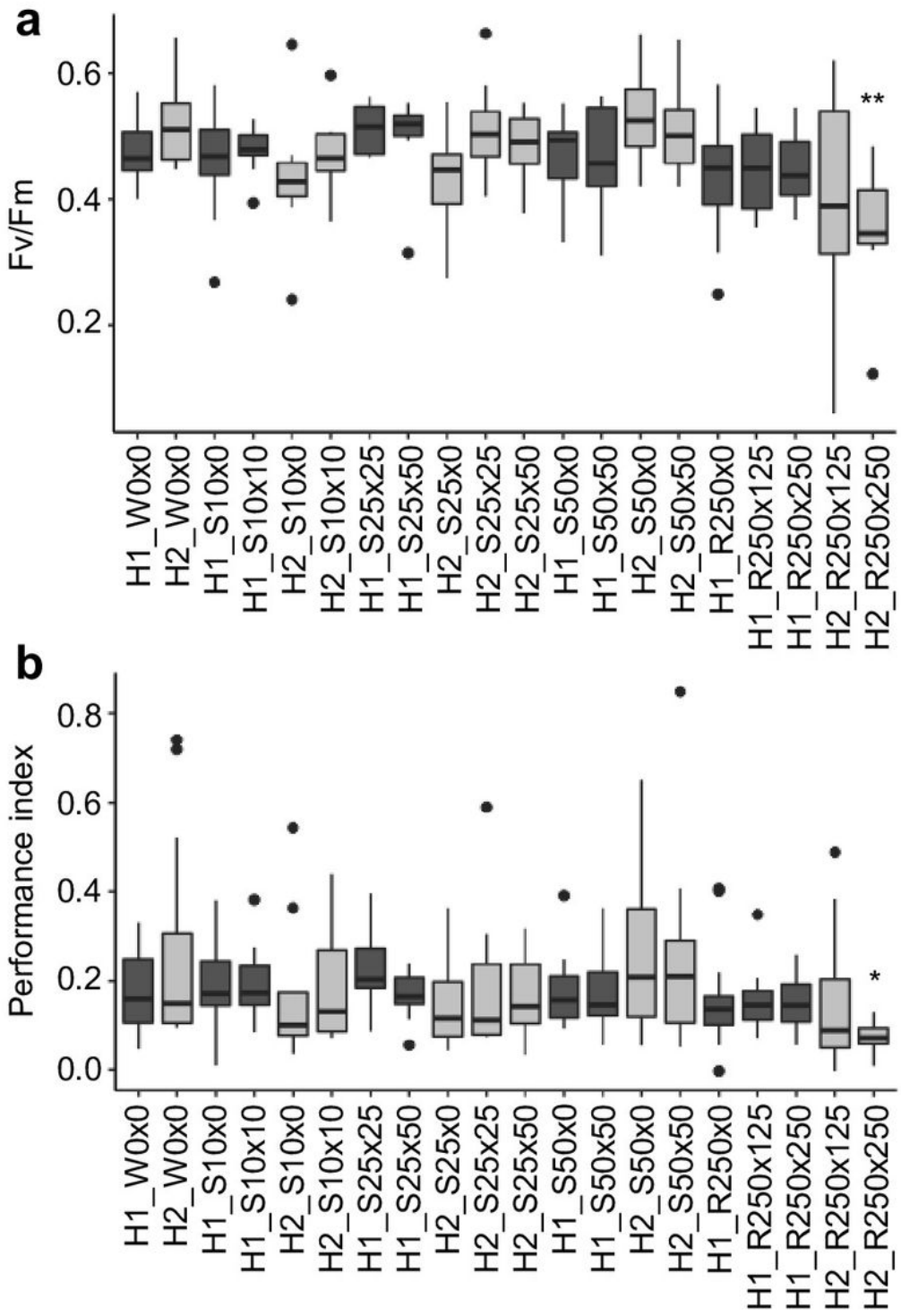

Figure 3

Comparison of Fv/Fm and b P.I. for plants with different treatment; results significantly different from untreated control for the same soil are marked * P<0.05 and ** P<0.01).

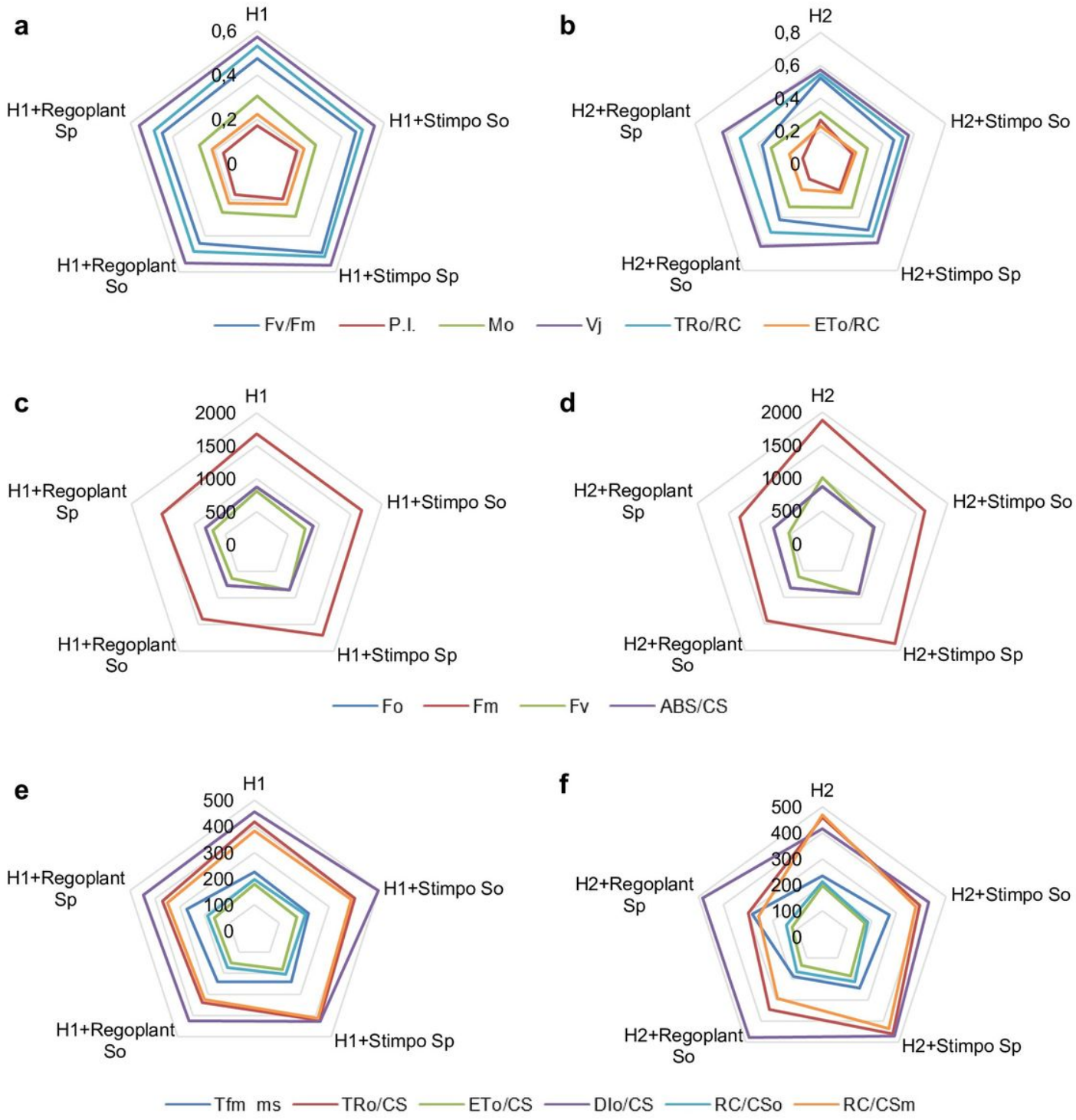

Figure 4

Physiological parameters of Mxg for two types of soil H1 and H2 and different type of PGRs application (non-treated plants are marked H1 and H2).

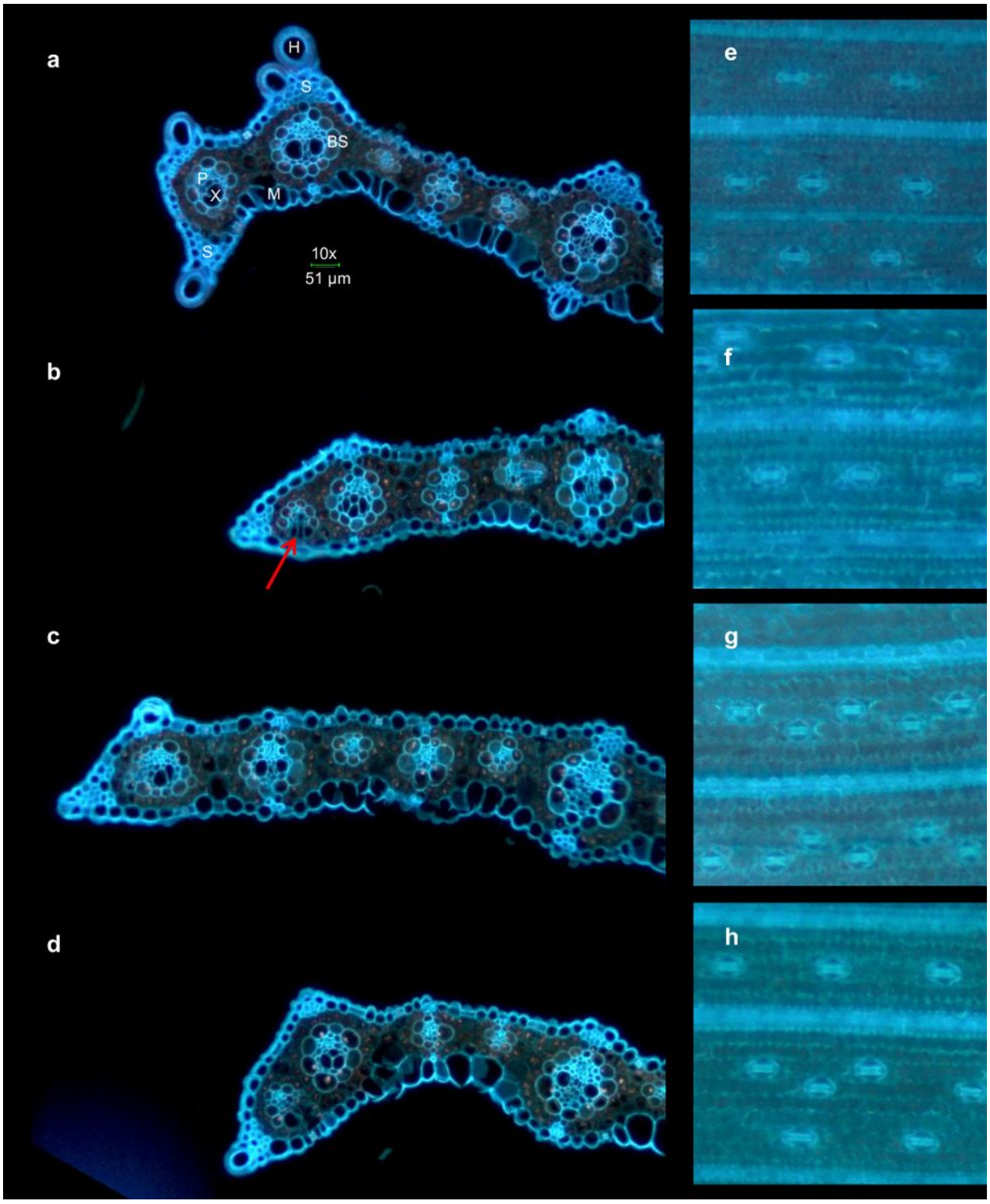

Figure 5

Mxg leaves used for microscopy; C soil (a, e), H1 soil (b, f), H2 soil (c, g) and H2-R250x250 (d, h); leaves used for microscopy using autofluorescence (a, b, c, d), stomata on the bottom side of the leaf, autofluorescence (e, f, g. h); X - xylem, P - phloem, M - motor cell, S - sclerenchyma cell, H - hook, BS - bundle sheath cell, red arrow points altered bundle sheath.

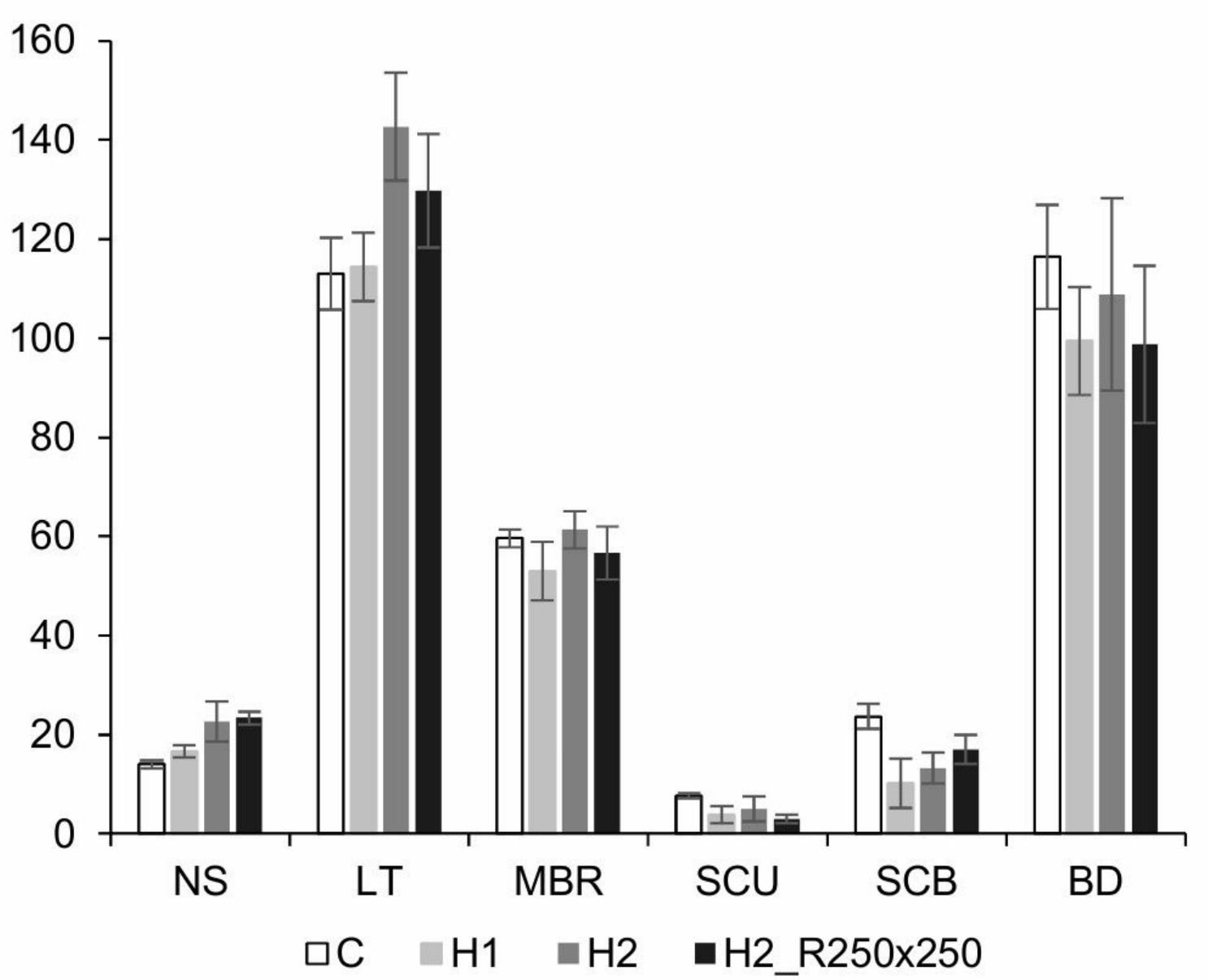

Figure 6

Morphology traits of plant grown in different soils (NS = number of stomata, LT = leaf thickness (µm), MBR = main bundle radius (µm), SCU = sclerenchyma cells – up, SCB = sclerenchyma cells – bottom, BD = bundles distance (µm)).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx
- Table1.docx
- Table3.docx

- Table4.docx