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APPLICATION NOTE 30449

Online $^{13}C^{18}O_2$ analysis with IRIS for nondestructive plant phenotyping for water use efficiency in sugar beet

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Keywords

Carbon, Infrared spectrometry, Plant phenotyping, Sugar beet, Water

Goal

To present a new phenotyping strategy in which whole plant physiology and plant physiology processes are considered using Isotope Ratio Infrared Spectrometry.

Introduction

It is estimated that the global demand for feed crops will almost double by 2050 due to growing world population (Foley et al., 2011). Considering the increase in global temperature and water scarcity, crops in the future needs to be more water and nutrient use efficient to sustain food security. Photosynthesis or net canopy CO_2 exchange is one of the driving forces of crop yield formation. Since most commercially available equipments have been designed for single leaf measurements, photosynthesis at a leaf level has been studied more intensively than canopy photosynthesis. Leaf photosynthesis measurements are often poorly correlated with crop yield, whereas whole plant (canopy) photosynthesis measurements correlate well with crop yield (Kim et al., 2006). Whole canopy measurements bypass the problem of finding a representative leaf and give information about the whole plant physiology and other plant physiological processes. In addition to canopy photosynthesis measurements, non-destructive approaches such as stable isotope measurements via online lasers are excellent tools to study the efficiency in transpiration and photosynthesis in crop plants (Senbayram et al., 2015).

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Effective plant phenotyping platforms are from great importance for breeding industry to develop new cultivars that are less susceptible to the biotic and abiotic stress factors. Here, custom-built phenotyping system attached with the Thermo Scientific™ Delta Ray™ Isotope Ratio Infrared Spectrometer (IRIS) was established to impose accurate determination of whole plant photosynthesis, transpiration and water use efficiency (**WUE**) in a continuous flow whole plant chamber. In this study, we examined sugar beet genotypes during the wetting-drying cycle and studied their efficiency in water use.

Analytical instrumentation

Laser-based isotope ratio infrared spectroscopy allows scientists to continuously monitor CO_2 isotope ratios. Sampling and measurements are instantaneous allowing feature rich data to be immediately generated. Robust and simple by design, isotope ratio infrared spectrometers are readily also field deployable. The Thermo Scientific Delta Ray IRIS (Figure 1) represents a new solution for the continuous measurement of isotope ratio and concentration of CO_2 in air both in laboratory and field studies.

Figure 1. Thermo Scientific Delta Ray IRIS.

In the mid-infrared range absorption lines are strong and simple direct absorption approach requires laser path of only 5 m in length. Specifically, the fundamental bands of CO_2 at 4.3 microns are accessed to simultaneously determine $\delta^{18}O$ and $\delta^{13}C$ of carbon dioxide by measuring both of the major isotopologues at an atmospheric concentration. The gas is continuously flowing through the analyzer and has a gas exchange time of about 30 seconds. Thus, while the isotope ratios are calculated once per second, they are not truly independent due to the time it takes to completely replace the sample in the cell. Universal Reference Interface (URI) Calibration is key to accurate and precise measurements isotope ratio measurements. The Universal Reference Interface (URI) is an integral part of the Delta Ray IRIS, which includes linearity and scale contraction calibration into your measurement workflow.

Method

Whole plant gas exchange chambers to measure photosynthesis and transpiration of the shoots were designed and built in house (Figure 2). Chamber was continuously flushed with ambient air (380 – 420 ppm CO_2) with a flow rate of 30 liters per minute. The air at the input of the chamber (“chamber in”) and outlet (“chamber out”) was continuously sampled by the Delta Ray IRIS for CO_2 concentration and isotopology isotope analysis. Experiments were performed within a greenhouse set to an air temperature of 20 - 25 °C and a light intensity of 200 - 400 µmol quanta m^{-2} s^{-1}.

For the determination of the area based photosynthetic carbon assimilation rate and the transpiration rate, whole plant leaf area was determined via taking a digital picture of each plant. The pictures were converted to black-and-white images and the leaf area was calculated with dedicated software. Sugar beet plants (at six leaf stage) were irrigated (75% WHC) and placed into the cuvette. During one week of drying period all analyses were performed.

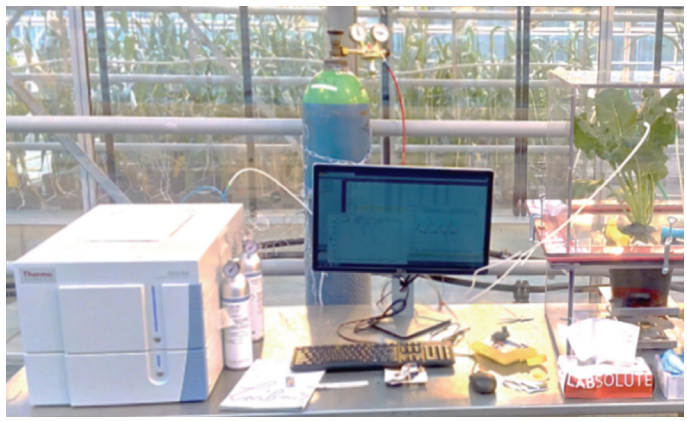

Figure 2. Whole plant chamber equipped with IRIS.

Results

Net photosynthesis (A_N) of sugar beet plants varied between -1.5 to 9 µmol CO_2 sec^{-1} m^{-2} leaf throughout the experiment. During the night, there were net respiration up to -1.5 µmol CO_2 sec^{-1} m^{-2} leaf. Although there was a light source in the greenhouse, A_N varied significantly during the day mainly reflecting variation in sunlight intensity. During the 10 days of drying period, A_N remained unchanged until day 4, however, then decreased gradually from 9 to 5 µmol CO_2 sec^{-1} m^{-2} leaf indicating severe drought at the end of the experiment. The $\delta^{13}C$ values of CO_2 in the plant cuvette also varied significantly over time. During the 10 days of drying period, minimum daily $\delta^{13}C$ values increased gradually over time, however response to drying was already clear in day 1 whereas no change in A_N was observed. The increase in $\delta^{13}C$ values reflects the CO_2 partial pressure of the carboxylation site (cc, that can be calculated by keeling plot) and therefore can be used to estimate time-integrated cc/ca ratios and intrinsic-WUE (A_N/transpiration). Overall there were significant negative correlation between transpiration per unit leaf area and $\delta^{13}C$ values at mid-day (R^2=0.55) indicating ^{13}C enrichment of CO_2 at the site of carboxylation due to stomatal closure.

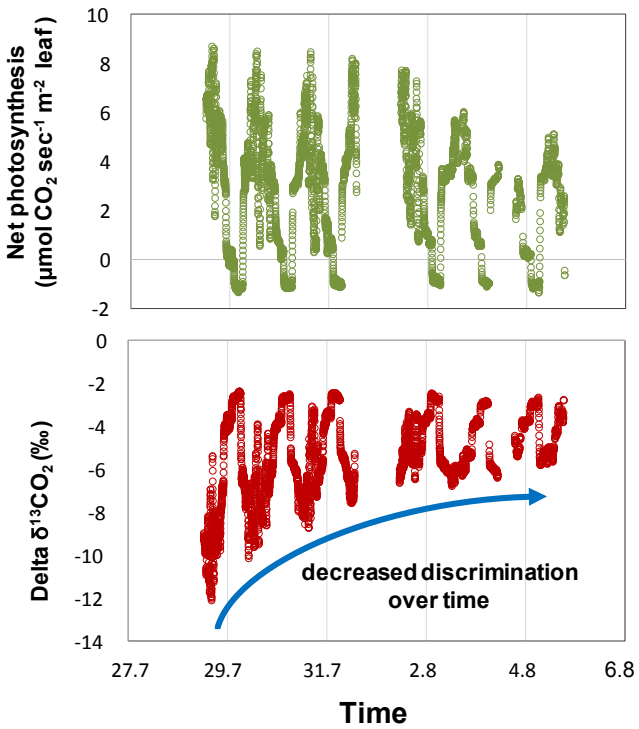

Figure 3. Net photosynthesis of sugar beet line and $\delta^{13}C$ values of CO_2 over 10 days of drying period.

The Keeling plot intercepts $\delta^{13}C$ during the day time shown for an example sugar beet line in Figure 3 vary between –26 and –19‰. In a plant cuvette, soil and roots were isolated and only above ground biomass were analyzed. Therefore, $\delta^{13}C$ values calculated via Keeling plot during daytime is associated to photosynthetic discrimination of the heavier isotope (resulting in a heavier composition of the CO_2 remaining in the atmosphere). As seen clearly in Figure 4, $\delta^{13}C$ values calculated via Keeling plot increased over time indicating clearly less discrimination and increase in intrinsic-WUE over time throughout the experiment. The latter can be used to study the behavior of various plant lines to stress factors, e.g. change in intrinsic-WUE, mesophyll conductance and level of the stress. As indicated above, ^{13}C values responded much faster compared to A_N. Thus, measuring online ^{13}C values of canopy or leaves seems to be an excellent way of studying real time response of plants to environmental stresses. This set up can easily be converted to multi-cuvette system via multi-positional valves that allow comparing response of number of different plant lines simultaneously thanks to IRIS.

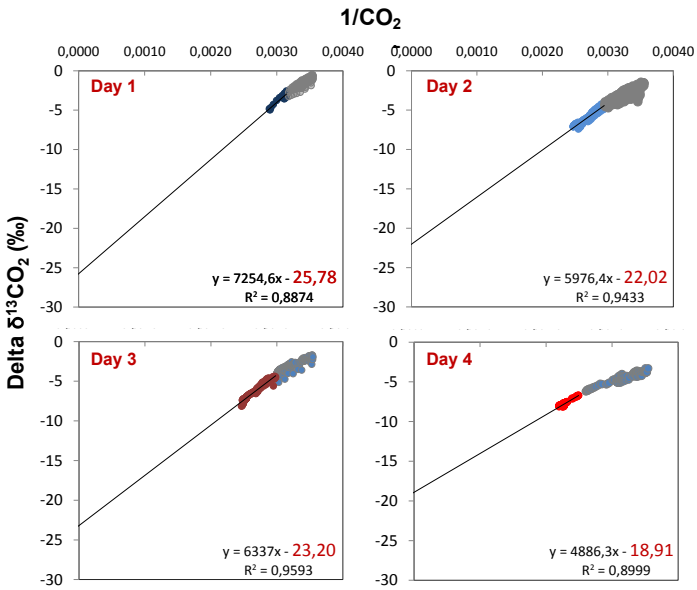

Figure 4. Example for Keeling plots and their intercept δKP, different colors are different day time.

Conclusions

When attached to a whole plant cuvette system, IRIS can determine hourly or daily ^{13}C photosynthetic discrimination of crop plants ($\delta^{13}C$ values calculated via Keeling plot) very precisely. Compared to the photosynthesis, $\delta^{13}C$ values of the CO_2 is a much faster indication of water limitation and plant response to drought. The latter indicates that measuring online ^{13}C values of canopy or plant leaves seems to be an excellent way of studying real time response of plants to the environmental stresses.

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