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Abbreviations

ACC: 1-aminocyclopropoane-1-carboxylic acid

CFD: computational fluid dynamics

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1 Assessment of the tested approach

1.1 Overall requirements

A chamber setup with environmental factors controlled within fixed limits (based on a trade-off of plant growth requirements for optimal yield to be consolidated based on applicable literature and experimental evidence) is a first prerequisite.

In this study, due to constraints relating to available building space, a chamber with common gas loop was fitted with the sensor technology put forward in the PSDU conceptual design. To compare the effect of a stress factor with control growth conditions, two independent pH and EC controlled nutrient film technique setups were installed: independent liquid loops.

An even air flow distribution avoids wind stress and in extreme cases local heating through less efficient air mixing.

Light level is difficult to homogenize, but with addition of reflectors and side-illumination, a deviation in intensity at the edges, lower than the in this study measured 65% of maximum values (in a setup consisting of eight closely spaced fluorescent lamps for one shelf), can be achieved. An approach to reduce homogeneity should first be modeled using a ray-tracing approach based on a 3D chamber model.

The pH and EC control, and water addition strategies have to yield comparative kinetics in order to achieve a sound basis for comparative studies in 2 separate nutrient solution monitoring systems. Adequate mixing and calibration of dosing pumps are prerequisites here.

Flow control of the nutrient solution also needs to be comparable among gullies. The problems in this study encountered were largely caused by the organization of gullies on different levels (dictated by the need for efficient growing space usage), and employing of adjustable needle valves, that are first of all subject to clogging by microbial growth and/or debris accumulation, and are fixed in PVC distribution manifolds in which gas (air released from the nutrient solution) accumulates over several days and further obstructs flow. A procedure for flushing of the distribution valves at regular intervals is a solution to be further explored.

Ideally comparative growth tests would be carried out in completely independent systems, having comparative measurement capabilities for as well gas as liquid phases.

1.2 Ethylene measurement

Ethylene monitoring is, given the extensive body of former research on the topic, clearly a requirement for inclusion in a future stress monitoring strategy. In chambers with sealed atmosphere the here tested photo-acoustic system would provide on-line measurement capability.

To guarantee long term stability and accuracy of this measurement, a source of pure (hydrocarbon free) calibration air (gas bottle) should be accounted for.

The detector module (30x40x5cm) should be placed in an environment with stable temperature for optimal performance of the laser based detection system.

1.3 pH, EC and water level control logging

Parallel monitoring of added amounts of acid (or base), concentrated nutrient solution and pure (distilled or condensed) water has the potential of giving a first indication of changes in (root) growth response (see TN 89.53 and 89.54, response to ACC-treatment and Fe-deficiency).

1.4 On-line weight measurement

The use of load-cells positioned under NFT gullies permits to have an average measurement of the weight of several plants (in the setup of the current experiment 7 for each control and treatment gully). For stable measurement, the input nutrient solution flow rate of the hydroponics system should remain stable, since otherwise weight measurements will be confounded by the changed liquid contents in the gully.

This was exemplified by the shifts in the recorded weight signals (See TN 89.54) caused by a gradual decrease of flow followed by a manual adjustment and restoration of the original higher flow rate and associated higher liquid volume in the gully.

1.5 Plant monitoring

Visual assessment - primarily of the shoot, but also applicable to the root of gullies are employed fitted with removable lids - remains the most trustworthy approach, but implies the expert knowledge of plant nutrient deficiency and disease symptoms to be able to detect these changes at an early stage in order to mitigate losses in growth yield.

Fixed camera's, typically visible spectrum CMOS or CCD camera's positioned at several individual plant positions (to avoid monitoring only a single plant with a possible deviant response not representative of the whole crop) can provide detailed growth assessments of individual plant shoots and roots, indicative of overall performance. An overview camera can be used to identify plants with slow growth, however only so at a late stage and without detailed info, hence limiting its early warning merit.

Individual plant monitoring by imaging sensors, when fitted with dedicated illumination domes, can generate visual spectrum images of constant intensity, hue and saturation independent of the imaging position, and will be useful for detailed colour and projected leaf area assessment. A mobile camera avoids blocking of light when operating between light source and plants, and permits to achieve higher resolution. Mass, volume and energy penalties for implementing this additional system have to be minimised by choice of miniaturised systems with a positioning accuracy and imaging resolution able to detect and follow up features in time, at the mm to cm scale, to be further determined based on sensor and actuator characteristics.

To monitor plant water usage and plant assimilation imaging also provides a possibility of visualising tresses by a heterogeneous response at the leaf level. Given the higher cost of thermal cameras, and their inherent lower resolution as compared to CCD or CMOS cameras, implementing positioning technology for individual plant imaging is a strategy with obvious benefits, but several issues need to be tackled to deliver trustworthy data..

The choice of a miniature low-resolution thermal camera, as used in this project, proved to yield usable images from a point of view of whole plant visualisation and within-plant heterogeneity detection (dependent on leaf age or treatment-induced phenomena).

A prototype of software to convert the recorded digital signals (array of pixels) to a temperature image was realised in collaboration with UGent-Faculty of Engineering, department of electronics and information systems. The use of a mobile temperature reference (material that immediately adjust to the room temperature fluctuations) attached to the camera support and always present within the field of view of the camera allows to scale the pixel values of each captured image to a similar T-value for the reference surface present in each image. This approach will permit to obtain a platform that can effectively compare the T of all plants within a growing facility, without being confounded by the air temperature fluctuations induced by the chamber climatisation control system.

Stress factors can emerge as spots of higher or low T (or chlorophyll fluorescence). The semi-automatic detection thereof depends on the quality of the images. The miniature thermal imaging system employed in this study has an inherent noise with clear orientation among X and Y axis, which would impair the application of image processing algorithms aiming at automatic plant, leaf, or within-leaf spot (or other feature) detection. Therefore in collaboration with UGent - Faculty of Engineering, department of telecommunications and information processing, noise reduction was concluded to be feasible from a first pre-test, and needs to be further pursued in order to be able to tackle (semi-automatic user-assisted) shape recognition and quantification.

1.6 Plant performance characterisation

After the above-described observation step, consisting of expert assessment (human plus multi-sensor imaging coupled to a stress-classification expert-system), characterisation is needed for prompt selection of a corrective action.

When adhering to proper sanitation (in a space-context), pathogens are supposed to pose little threat; however in ground-based testing systems this will be hard to achieve unless draconic safety measures would be taken.

A Q-PCR based approach in which the most important pathogenic and beneficial micro-organisms in hydroponic culture (on a per plant species basis if needed) can be detected in parallel with a single assay [1] was identified as a promising tool for stress characterisation after targeted (observation-guided) destructive sampling.

Additional evidence can be gathered by devising a set of PCR primers indicative of suboptimal conditions, e.g. expression of a marker gene for anoxia (too deep or long submergence of roots) thigmotropic response (shoot movement by ventilation) or oxidative status (high light or

more general stress response). A lower growth speed can then be correlated to a higher induction of one or more of these genes. Indeed, stress is commonly a superposition of several additive and thus aggravating factors.

In addition to an analysis of the nutrient solution, possibly indicative of depletion of particular elements, the analysis of plant fluids gives an indication of actual transport and thus immediate availability of the different elements (in contracts to already fixed in plant material and possibly not anymore available). In addition ICP-OES samples could be taken, not necessary sacrificing the whole plant, and analysed according to proposed nutrient interaction models, to derive possible early indications of deficiencies [2].

Within this study, a pathogen problem emerged, but was confined to the germination/propagation stage, and easily identified by conventional means (microscopical observation followed by petridish culture evaluation – UGent –faculty of bioscience engineering, department of crop protection) and easily mitigated by seed sterilisation in a stronger bleach solution. The above-mentioned PCR approach required sending nutrient solution samples to the laboratory that devised the test, however the pathogen was specifically associated with the root/shoot junction.

For future microbial characterisation of the rhizosphere (and phyllosphere), such a PCR-based approach will be considered for local regular diagnosis of pathogen presence.

The ethylene inducing treatment employed in the test (ACC addition to the nutrient solution) lead as expected to an emanation of gaseous ethylene from the leaf part and a loss of geotropy the roots. However the leaf area of treated plants was larger and plant roots alkalinised the solution at a faster rate. Moreover the treated plants appeared greener (in contradiction to the senescence-prompting effect of ethylene), and had a significantly higher biomass yield. In spite of this, the treated leaves displayed marked necrosis three to four days of ACC addition to the medium. This proved an opportunity for assessing the detection ability of the thermal imaging system. The ACC in the nutrient solution could be microbially degraded (e.g. to CN) which is a known necrotic agent, but pursuing the identification of the cause of this necrosis was beyond the scope of this project. In conclusion, ethylene and control gassing in 2 separate chambers appears the better approach for further comparisons, but as described above was not realisable due to chamber and sensor availability.

Additional evidence for the presence of non-microbial but sub-mm size organisms also depends on microscopic observation. Mites were detected in Petri-dishes used for lettuce germination after indications based on visible trails left in the moisture-covered lids.

When observing sub-optimal growth in plant growth test runs, root or shoot histology could shed light on the tissue affected and hint at the underlying mechanism, if formerly established information on ‘normal’ morphology is available (e.g. morphology of root and leaf sections).

2 Consolidated requirements

The combination of monitoring

- atmosphere monitoring, CO_2 assimilation – NCER (net carbon exchange rate), Ethylene
- nutrient solution pH EC H_2O compensation volume input rates
- nutrient solution composition
- shoot and root observation
- weight

and characterisation

- expert system with database based on baseline and stress test runs
- Q-PCR for pathogen and stressfactors
- Microscopy for localisation or structural info

will lead to a setup and protocol for growth under optimal safeguard and adaptive control.

References

[1]www.dnamultiscan.com

[2] Baxter, I. R., Vitek, O., Lahner, B., Muthukumar, B., *et al.*, The leaf ionome as a multivariable system to detect a plant's physiological status. *Proceedings of the National Academy of Sciences* 2008, *105*, 12081-12086.