TECHNICAL NOTE 96.3

Test Protocols and procedures for lettuce cultivation

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ACRONYMS

CESRF	Controlled Environment Systems Research Facility
HPC	Higher Plant Chamber
MSDS	Material Safety Data Sheet
PAR	Photosynthetically Active Radiation
PP	Polypropylene
PPF	Photosynthetic Photon Flux
PAR	Photosynthetically Active Radiation
UAB	Universitat Autònoma de Barcelona
UoG	University of Guelph

1.Introduction

This TN outlines the general operational requirements for growth of lettuce (*Lactuca sativa* L.) in batch culture within the UAB HPC, from seeding up to harvesting. As cultivation requirements vary with lettuce variety, this method will focus on the cultivar (Grand Rapids) used in previous HPC test procedures. Any lettuce cultivar may be used, however the number of days to maturity will vary for each.

2. Objectives

There are a number of experimental objectives associated with HPC prototype operation. The procedures described below are primarily for chamber operation in autonomous (stand-alone) mode, however many of the methods will be common with operation in integrated mode when connected to other MELiSSA compartments.

Common objectives of lettuce study are as follows:

- Monitor CO_2 assimilation/evolution
- Monitor O_2 evolution/respiration
- Monitor evapotranspiration
- Monitor hydroponic nutrient system parameters (pH, EC, selected ions – requires HPLC analysis or alternative methods)
- Evaluation of growth parameters (fresh/dry weight, leaf area, leaf size, etc)
- Evaluation of lettuce mineral composition
- Evaluation of C and N balances in the system

Although a number of objectives are considered, only those required by the separately documented experimental design are to be performed.

3. Personal protective equipment

There are a number of chemicals and products required in this protocol which require protective equipment. You MUST consult your laboratory MSDS records and laboratory safety protocols, and have proper training prior to their use.

4. Chemicals and consumables

4.1.1. Required chemicals

The following analytical grade (or better) chemicals are required for lettuce growth experiments.

- $Ca(NO_3)_2 \cdot 4H_2O$
- $FeCl_3 \cdot 6H_2O$
- Na-EDTA
- $MgSO_4 \cdot 7H_2O$
- KNO_3
- $NH_4H_2PO_4$
- $(NH_4)_2SO_4$
- H_3BO_3
- $MnSO_4 \cdot H_2O$
- $ZnSO_4 \cdot 7H_2O$
- $CuSO_4 \cdot 5H_2O$
- H_2MoO_4 (85%MoO3)
- HNO_3
- KOH

4.1.2. Consumables

1. Rockwool - small cubes - Grodan AO 36/40 6/15W (2940 in carton)
2. Rockwool - large cubes – Grodan Delta 4G 42/40(383 in carton)
3. Seed germination trays and covers
4. Lettuce seeds – cv. Grand Rapids

5. Equipment

1. Balance for micro-nutrient and salt measurement (500 g ± 0.01g)
2. Solution stock storage tanks (2 x 20 L tanks with spigot, PP)
3. Seedling nutrient storage tank (1 x 10 L with spigot, PP)
4. Solution transfer tank (1 x 200 L tank, PP)
5. Submersible pump (5 L min^{-1} or greater) and connection tubing
6. Growth cabinet for seedling establishment (300 µmol s^{-1} m^{-2} PAR minimum). HPC can be substituted, if available, with all lamps on and appropriate temperature/RH setpoints
7. Higher plant chamber (1 or more)
8. Magnetic stirring plate, stirring bars
9. Tweezers

6. Procedures – full maturity crop growth

6.1 Scope

This procedure outlines the steps required to prepare nutrient solutions and start lettuce seedlings (cv. Grand Rapids) prior to growth and development in the UAB HPC. This procedure is for growth to full crop maturity as a single batch only.

6.2 Study period

The time to maturity for the lettuce cultivar 'Grand Rapids' is 45 days, resulting in a total HPC growth period of 40 days. Five days are required for seedling establishment and initial development before transfer to the HPC. For alternative lettuce cultivars, subtract 5 days from the number of days to maturity to obtain the HPC full growth period.

6.3 Chamber Start-Up and Functional Verification

Chamber start-up begins with a functional verification and subsystem calibration. Ensure that all sensors are functioning and calibration/maintenance procedures are followed as per 'TN 85.91 Operation Manual' and 'TN 85.92 Prototype Maintenance Plan' prior to the start of any experiment.

6.4 Leak Testing

Following equipment verification, a 48 hour leak test should be completed using CO_2 as a marker gas. The chamber should be operated at the temperature and humidity conditions of the pending experiment with a CO_2 demand of 1500 ppm. The CO_2 injection system should remain on during equilibration, and once demand levels are reached, it should be deactivated. The leakage rate is determined from the slope of the decay profile in CO_2 over time, bracketing the intended CO_2 concentration for the experiment. The leakage rate is used as a correction term in the calculation of net carbon exchange rate and should be determined prior to and following a plant growth experiment in the chamber.

6.5 Solution Preparation

The feed strength (nutrient solution reservoir final concentration) is provided by adding concentrated stock solutions (A and B) to deionized water, and adjusting the pH. Calcium nitrate and chelated iron (Stock A) are separated from the remaining components in Stock B to prevent precipitation. During the HPC1 automated operation, the solution EC is maintained by the control system with timed injections from chamber stock A and B reservoirs.

Component	Mol. Wt. (g)	Feed Strength		Stock (g) grams per 20L
		mM	g/L	
Stock A				
$Ca(NO_3)_2 \cdot 4H_2O$	236.16	434.4	102.6	2052
$FeCl_3 \cdot 6H_2O$	270.30	9.6	2.6	51.9
Na- EDTA	372.24	12	4.5	89.3
Stock B				
$MgSO_4 \cdot 7H_2O$	246.48	120	29.6	591.6
KNO_3	101.10	600	60.7	1213
$NH_4H_2PO_4$	115.08	180	20.7	414.3
$(NH_4)_2SO_4$	132.00	120	15.8	316.8
Micronutrients – include with Stock B				
H_3BO_3	61.83	2.4	0.15	2.97
$MnSO_4 \cdot H_2O$	169.01	0.6	0.10	2.03
$ZnSO_4 \cdot 7H_2O$	287.54	0.42	0.12	2.42
$CuSO_4 \cdot 5H_2O$	249.68	0.096	0.02	0.48
H_2MoO_4 (85% MoO_3)	161.97	0.06	0.01	0.2
Acid				
HNO_3	63.01	0.5 M	31.5	
Base				
KOH	56.11	0.5 M	28.0	

Table 6.1: Hydroponics stock nutrient solution components

Component	Mol. Wt. (g)	Feed Strength	
		mM	g/L
$Ca(NO_3)_2•4H_2O$	236.16	3.62	0.85
$FeCl_3•6H_2O$	270.30	0.08	0.02
Na- EDTA	372.24	0.10	0.04
$MgSO_4•7H_2O$	246.48	1.00	0.25
KNO_3	101.10	5.00	0.51
$NH_4H_2PO_4$	115.08	1.50	0.17
$(NH_4)_2SO_4$	132.00	1.00	0.13
H_3BO_3	61.83	0.02	0.0012
$MnSO_4•H_2O$	169.01	0.0050	0.0008
$ZnSO_4•7H_2O$	287.54	0.0035	0.0010
$CuSO_4•5H_2O$	249.68	0.0008	0.0002
H_2MoO_4 (85% MoO_3)	161.97	0.0005	0.0001

Table 6.2: Hydroponics final nutrient solution components

P	S	Ca	Mg	K	N -NO_3^-	N-NH_4^+
0.05	0.06	0.14	0.02	0.20	0.17	0.05

Table 6.3: Concentration of minerals in the final nutrient solution, g/L

Cu	Mn	B	Zn	Mo	Fe	Na	Cl
0.05	0.3	0.2	0.2	0.06	4.5	4.9	8.5

Table 6.4: Concentration of trace elements in the final nutrient solution, mg/L

a. Stock Solution Preparation:

1. add 20L of deionized water to stock tank A
2. add analytical grade fertilizer salts as listed (Table 6.1 – Stock A)
3. mix thoroughly until all salts have dissolved

4. add 20L of deionized water to stock tank B
5. add reagent grade fertilizer salts as listed (Table 6.1 – Stock B and micronutrients)
6. mix thoroughly until all salts have dissolved
7. keep stock tanks in the dark when not in use

b. Acid and Base preparation:

Acid and base preparation should only be performed by staff trained in the use of, and safety protocols associated with, strong acids and strong bases. Care must be taken when using both the concentrated solutions and the diluted solutions prepared for HPC use. For each litre of acid or base required, add the amount of acid or base indicated in Table 6.1. ***Never add water to concentrated acid or base***, fill the container with water first, then slowly add the concentrated acid or base.

WARNING: Nitric acid MUST be dispensed in a fume hood suitable for use with concentrated acids. Appropriate safety equipment must be worn. Read and understand the product label and MSDS and follow laboratory safety procedures at all times.

Acid:

1. Add ~900 mL deionized water to a 1.0 litre volumetric flask
2. Add 31.5 g HNO_3 to the water (**fume hood and protective equipment!**)
3. mix well with a magnetic stirring bar on a stirring plate
4. remove magnetic stirring bar
5. Top up to the 1.0 liter mark with deionized water

Base:

1. Add ~900 mL deionized water to a 1.0 litre volumetric flask
2. Add 28.0 g KOH to the water (**protective equipment!**)
3. mix well with a magnetic stirring bar on a stirring plate
4. remove magnetic stirring bar
5. Top up to the 1.0 liter mark with deionized water

HPC Nutrient Solution Preparation:

1. add 160 litres of deionized water to the 200L solution transfer tank
2. add 1.3 litres of stock A and mix well

3. add 1.3 litres of stock B and mix well
4. continue mixing and adjust pH to 5.8 using 0.5M nitric acid or 0.5M potassium hydroxide
5. ensure the HPC nutrient solution reservoir is empty
6. connect the outlet of the submersible pump to the HPC nutrient tank drain valve using suitable tubing

7. place the submersible pump in the 200 L solution transfer tank
8. open the HPC nutrient tank drain valve
9. turn on the pump and pump the solution from the solution transfer tank to the HPC nutrient tank until the transfer tank is empty
10. close the drain valve and remove the pump and tubing
11. clean the pump, tubing and nutrient storage tank with deionized water

HPC Stock Tank Solutions:

1. Add 2.5 litres of Stock A to the Stock A tank on the HPC
2. Add 2.5 litres of Stock B to the Stock B tank on the HPC
3. Add 2.5 litres of Acid to the Acid tank on the HPC
4. Add 2.5 litres of Base to the Base tank on the HPC

DO NOT mix up the tanks and the solutions in them.

Seedling Solution preparation:

After planting, and prior to transfer to the HPC, seedlings should be watered regularly with ½ strength nutrient solution. To prepare 10L of solution:

1. Fill the 10 litre seedling nutrient storage tank with 10 litres of deionized water
2. add 40mL of stock A
3. add 40mL of stock B
4. adjust pH to 5.8 with nitric acid or potassium hydroxide
5. water seedlings as needed
6. check pH prior to each watering and adjust if required

Procedure for the Rockwool safe manipulation.

a. Manipulation with Rockwool - small cubes - Grodan AO 36/40 6/15W

1. Use Personal Protective Equipment: particle mask, gloves, goggles, lab coat.
2. Place the two flats of Rockwool cubes into a big plastic bag.
3. Fill this bag with deionized water until it will cover all the cubes.
4. Having gloves on, wash the hands.
5. Tie up the bag and let the cubes to dump.
6. Wash the hands with the gloves on.
7. Take the Rockwool cubes out of the bags and place them wet into thermostable vessels, cover with aluminium foil and fix well with sticky paper tape.
8. Sterilise these Rockwool cubes by autoclaving.
9. Wash again the hands with the gloves on.
10. Take off the gloves and put them into a garbage bag.

b. Manipulation with Rockwool - large cubes – Grodan Delta 4G 42/40

1. Use Personal Protective Equipment: particle mask, gloves, goggles, lab coat.
2. Place 100 Rockwool large cubes into a big plastic bag.
3. Fill this bag with deionized water until it will cover all the cubes.
4. Having gloves on wash the hands.
5. Tie up the bag and let the cubes to dump.
6. Wash again the hands with the gloves on.
7. Take off the gloves and put them into a garbage bag.

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6.6 Planting

1. Thoroughly rinse the two flats of Rockwool cubes that have been placed in seed germination trays with deionized water.
2. Allow to drain thoroughly.
3. Sterilise by autoclaving 1 L of deionised water
4. Soak seeds in 5% bleach for 15 min
5. Rinse seeds 3 times with sterilised deionised water
6. Using tweezers, place a single lettuce seed in each hole. Expand holes slightly if required (Figure 6.1).
7. Add a light transparent plastic tray cover (Figure 6.2) and place in HPC1 with a temperature of 20°C and lighting period of 16 hours. Light intensity should be not less than 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (1HPS+1MH lamps per module are enabled), blower should be enabled, set point for relative humidity are: 50% at day time and 70% at night

8. Water the seeds as needed (daily) with about 900-1200 mL of the seedling solution. Do not over water (no liquid water present beneath the Rockwool) - drain trays if required.

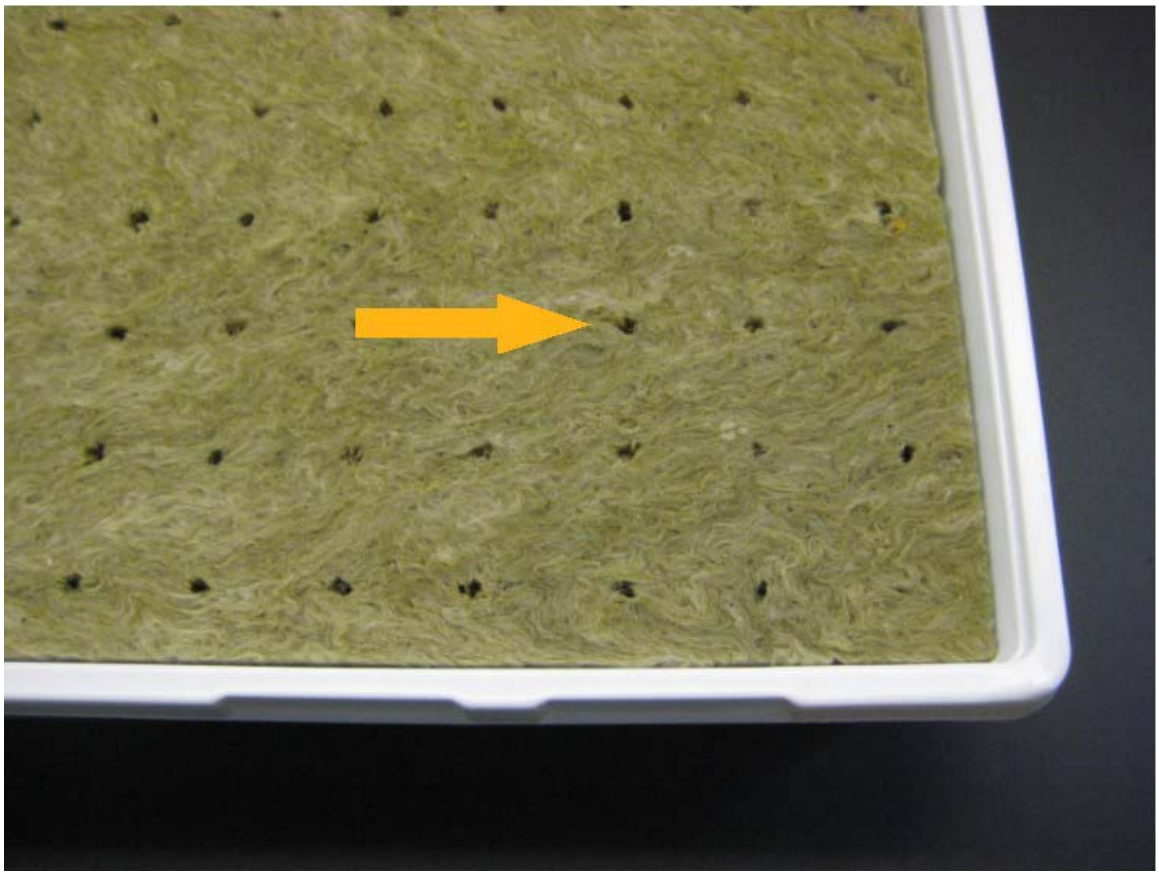

Figure 6.1: Rockwool cubes with arrow showing hole placement for seed

9. After > 60 percent emergence, remove the plastic cover.
10. After 5 days, break off individual cubes containing viable seedlings and set them into the larger (pre-rinsed with deionized water) Rockwool cubes (Figure 6.3).
11. If the HPC is empty (no troughs) skip to step 17.
12. Turn off the irrigation pump.

Figure 6.2: Rockwool cubes in seedling tray with clear plastic cover

13. Remove one trough from the harvest end and evenly space 5 large Rockwool cubes with plants inside within it, being sure not to block the drain hole and leaving space for the irrigation spout.
14. Cover the trough with 5 mil black/white blackout film which has 40mm holes centred over each plant. Include a 60mm hole directly beneath the irrigation spout. Ensure that the white side faces up and affix with binder clips to the sides of the trough if required.
15. Place the trough in the HPC planting end and attach to the trough already in the chamber. Repeat at step 13 and continue until the entire chamber has been filled with seedlings.

Figure 6.3: Placement of seedling in the larger rockwool cube

16. Skip to step 20.
17. Take a single trough and evenly space 5 plants within it, being sure not to block the drain hole and leaving space for the irrigation spout.
18. Cover the trough with 5 mil black/white blackout film which has 40mm holes centred over each plant. Include a 60mm hole directly beneath the irrigation spout. Ensure that the white side faces up and affix with binder clips to the sides of the trough if required.
19. Place the trough in the HPC planting end and attach to the trough already in the chamber. If there is no existing trough, simply place the planted trough on the interior rollers. Repeat at step 17 and continue until the entire chamber has been filled with seedlings.
20. Turn on the irrigation pump and adjust pump speed until all troughs receive a flow of water.
21. Close the inner airlock doors.
22. Close and latch the outer doors.

Additional instructions for chamber operation, if required, are found in TN 85.91 HPC Operation Manual.

6.7 HPC Environment control requirements

Environment control conditions should be set in the control system as follows:

- CO_2 concentration set point– 1000 ppm
- Temperature – 26/20 º C (day/night)
- Humidity – 50-75% day, 70-75% night
- EC – 2 mS/cm

- pH setpoint– 5.9 [5.8-6.0]
- day/night of 16/8 hours
- Light Intensity – All lamps on
- VPD – 9.0 day, 6.0 night

6.8 Harvest requirements

Specific harvest procedures are presented in TN 96.4 'Sampling and Analysis Protocols'. For full maturity crop growth experiments, the following procedures and calculations should be performed after harvest:

- fresh weight
- dry weight
- percent carbon and percent nitrogen
- plant mineral analysis
- crop nutrient solution analysis

Additional analysis could be as well performed if needed:

- leaf area
- proximate analysis
- plant nitrate analysis
- plant Acid Detergent Fiber (ADF)-Neural Detergent Fiber (NDF)

6.9 Transfer of the troughs outside of the HPC1

1. Turn off the irrigation pump
2. Turn off the lighting system
3. Open the exterior harvest air lock door
4. Open the inner harvest air lock door
5. Remove troughs one at a time and harvest each separately (detailed harvesting procedure is given in TN 96.4)
6. Clean trough and return to the HPC for storage

7.Procedures – short term crop growth

7.1 Scope

This procedure outlines the steps required to prepare nutrient solutions and start lettuce seedlings (Grand Rapids) prior to short term lettuce

growth and development in the UAB HPC. This intended use of short term experiments is to test overall HPC system functioning in a shorter period of time than that required for a full crop growth experiment. In this procedure, plants are transferred to the chamber at a later stage to ensure proper system control and sensor response during transpiration, photosynthesis, and respiration.

7.2 Study period

Although the time to maturity for the lettuce cultivar 'Grand Rapids' is 45 days, plants will only be grown for a seven day period within the HPC. For better performance of all control loops the plants should be in the phase of active growth and fourteen days are required for seedling establishment and initial development before transfer to the HPC, resulting in a total growing time of 21 days.

7.3 Chamber Start-Up and Functional Verification

Chamber start-up begins with a functional verification and subsystem calibration. Ensure that all sensors are functioning and calibration/maintenance procedures are followed as per 'TN 85.91.

Operation Manual' and 'TN 85.92 Prototype Maintenance Plan' prior to the start of any experiment.

7.4 Leak Testing

Leak testing is not required for short term plant growth experiments.

7.5 Solution Preparation

The feed strength (nutrient solution reservoir concentration) is provided by adding concentrated stock solutions (A and B) to deionized water, and adjusting the pH. Calcium nitrate and chelated iron (Stock A) are separated from the remaining components in Stock B to prevent precipitation. During the HPC1 automated operation, the solution EC is maintained by the control system with metered injections from chamber stock A and B reservoirs.

Stock Solution Preparation:

1. add 20L of deionized water to stock tank A
2. add analytical grade fertilizer salts as listed (Table 6.1 – Stock A)

3. mix thoroughly until all salts have dissolved
4. add 20L of deionized water to stock tank B
5. add reagent grade fertilizer salts as listed (Table 6.1 – Stock B and micronutrients)
6. mix thoroughly until all salts have dissolved
7. keep full stock tanks in the dark when not in use

WARNING: Nitric acid MUST be dispensed in a fume hood suitable for use with concentrated acids. Appropriate safety equipment must be worn. Read and understand the product label and MSDS and follow laboratory safety procedures at all times.

Acid and Base preparation: Acid and base preparation should only be performed by staff trained in the use of, and safety protocols associated with, strong acids and strong bases. Care must be taken when using both the concentrated solutions and the diluted solutions prepared for HPC use. For each litre of acid or base required, add the amount of acid or base indicated in Table 6.1. ***Never add water to concentrated acid or base***, fill the container with water first, then slowly add the concentrated acid or base.

Acid:

1. Add ~900 mL deionized water to a 1.0 litre volumetric flask
2. Add 31.6 g HNO_3 to the water (**fume hood and protective equipment!**)
3. mix well with a magnetic stirring bar on a stirring plate
4. remove magnetic stirring bar
5. Top up to the 1.0 liter mark with deionized water

Base:

1. Add ~900 mL deionized water to a 1.0 litre volumetric flask
2. Add 28.0 g KOH to the water (**protective equipment!**)
3. mix well with a magnetic stirring bar on a stirring plate
4. remove magnetic stirring bar
5. Top up to the 1.0 liter mark with deionized water

HPC Nutrient Solution Preparation:

1. add 160 litres of deionized water to the 200L nutrient transfer tank

2. add 1.3 litres of stock A and mix well
3. add 1.3 litres of stock B and mix well
4. continue mixing and adjust pH to 5.8 using 0.5M nitric acid or 0.5M potassium hydroxide
5. ensure the HPC nutrient tank is empty
6. connect the outflow of the submersible pump to the HPC nutrient tank drain valve using suitable tubing
7. place the submersible pump in the 200 L nutrient transfer tank
8. open the HPC nutrient tank drain valve
9. turn on the pump and pump the solution from the nutrient transfer tank to the HPC nutrient tank until the reservoir is empty
10. close the drain valve and remove the pump and tubing
11. clean the pump, tubing and nutrient storage tank with deionized water

HPC Stock Tank Solutions:

1. Add 2.5 litres of Stock A to the Stock A tank on the HPC
2. Add 2.5 litres of Stock B to the Stock B tank on the HPC
3. Add 2.5 litres of Acid to the Acid tank on the HPC
4. Add 2.5 litres of Base to the Base tank on the HPC

DO NOT mix up the tanks and the solutions in them.

Seedling Solution preparation:

After planting, and prior to transfer to the HPC, seedlings should be watered regularly with ½ strength nutrient solution. To prepare 10L of solution:

1. Fill the 10 litre seedling nutrient storage tank with 10 litres of deionized water
2. add 40mL of stock A
3. add 40mL of stock B
4. adjust pH to 5.8 with nitric acid or potassium hydroxide
5. water seedlings as needed
6. check pH prior to each watering and adjust if required

Procedure for the Rockwool safe manipulation.

a. Manipulation with Rockwool - small cubes - Grodan AO 36/40 6/15W

1. Use Personal Protective Equipment: particle mask, gloves, goggles, lab coat.
2. Place the two flats of Rockwool cubes into a big plastic bag.
3. Fill this bag with deionized water until it will cover all the cubes.
4. Having gloves on wash the hands.
5. Tie up the bag and let the cubes to dump.
6. Wash the hands with the gloves on.
7. Place wet Rockwool cubes into thermostable vessels, cover with aluminium foil and fix well with sticky paper tape.
8. Sterilise these Rockwool cubes by autoclaving.
9. Wash again the hands with the gloves on.
10. Take off the gloves and put them into a garbage bag.

b. Manipulation with Rockwool - large cubes – Grodan Delta 4G 42/40

1. Use Personal Protective Equipment: particle mask, gloves, goggles, lab coat.
2. Place 100 Rockwool large cubes into a big plastic bag.
3. Fill this bag with deionized water until it will cover all the cubes.
4. Having gloves on wash the hands.
5. Tie up the bag and let the cubes to dump.
6. Wash again the hands with the gloves on.
7. Take off the gloves and put them into a garbage bag.

7.6 Planting

1. Thoroughly rinse the two flats of Rockwool cubes that have been placed in seed germination trays with deionized water.
2. Allow to drain thoroughly.
3. Sterilise by autoclaving 1 L of deionised water.
4. Soak seeds in 5% bleach for 15 min.
5. Rinse seeds 3 times with sterilised deionised water.
6. Using tweezers, place a single lettuce seed in each hole. Expand holes slightly if required (Figure 6.1).
7. Add a light transparent plastic tray cover (Figure 6.2) and place in HPC1 with a temperature of 20°C and lighting period of 16 hours. Light intensity should be not less than 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (1HPS+1MH lamps per module

are enabled), blower should be enabled, set point for relative humidity are: 50% at day time and 70% at night.

8. Water the seeds as needed (daily) with about 900-1200 mL of the seedling solution. Do not over water (no liquid water present beneath the Rockwool) - drain trays if required.
9. After > 60 percent emergence, remove the plastic cover.
10. After 5 days, break off individual cubes containing viable seedlings and set them into the larger (pre-rinsed with deionized water) Rockwool cubes (Figure 6.3).
11. If the HPC is empty (no troughs) skip to step 17.
12. Turn off the irrigation pump.
13. Remove one trough from the harvest end and evenly space 5 large Rockwool cubes with plants inside within it, being sure not to block the drain hole and leaving space for the irrigation spout.
14. Cover the trough with 5 mil black/white blackout film which has 40mm holes centred over each plant. Include a 60mm hole directly beneath the irrigation spout. Ensure that the white side faces up and affix with binder clips to the sides of the trough if required.
15. Place the trough in the HPC planting end and attach to the trough already in the chamber. Repeat at step 13 and continue until the entire chamber has been filled with seedlings.
16. Skip to step 20.
17. Take a single trough and evenly space 5 plants within it, being sure not to block the drain hole and leaving space for the irrigation spout.
18. Cover the trough with 5 mil black/white blackout film which has 40mm holes centred over each plant. Include a 60mm hole directly beneath the irrigation spout. Ensure that the white side faces up and affix with binder clips to the sides of the trough if required.
19. Place the trough in the HPC planting end and attach to the trough already in the chamber. If there is no existing trough, simply place the planted trough on the interior rollers. Repeat at step 17 and continue until the entire chamber has been filled with seedlings.
20. Turn on the irrigation pump and adjust pump speed until all troughs receive a flow of water.
21. Close the inner airlock doors.
22. Close and latch the outer doors

Additional instructions for chamber operation, if required, are found in TN 85.91 HPC Operation Manual.

7.7 HPC Environment control requirements

Environment control conditions should be set in the control system as follows:

- CO_2 concentration set point – 1000 ppm
- Temperature – 26/20º C (day/night)
- Humidity – 50-75% day, 70-75% night
- EC – 2 mS
- pH - 5.8
- day/night of 16/8 hours
- Light Intensity – All lamps operational

7.8 Harvest requirements

There are no specific harvest requirements for short term growth experiments as they are intended to test system operation only.

7.9 Transfer of the troughs outside of the HPC1

1. Turn off the irrigation pump
2. Turn off the lighting system
3. Open the exterior harvest air lock door
4. Open the inner harvest air lock door
5. Remove troughs one at a time and harvest each separately (detailed harvesting procedure is given in TN 96.4)
6. Clean trough and return to the HPC planting end for storage

8. Comments

TN 96.3
Test Protocols and procedures for lettuce cultivation

Comments

General comments

From Minutes of Meeting of the Progress meeting on HPC1 and TRR functional tests with SCHNEIDER **(**MPP-MOM-09-4105(0)-EP-20090716), page 7, third paragraph:

The presence of algae in the seedling incubation was discussed, and it was agreed that it should be prevented for the future crop tests. The treatment of the rockwool in order to reduce the contamination in it before the use could be of help. CP will send a protocol for that, to be included in the protocol for lettuce cultivation.

TN amended accordingly.

Detailed comments

Page/paragraph	Comment
2/Section 2	*"Common objectives of lettuce study"* Tissue composition is missing , C balance, N balance as well Required information is added: *"- Evaluation of lettuce mineral composition* *- Evaluation of C and N balances in the system"*
11/Section 6.5	*"HPC Nutrient Solution Preparation* *4. Continue mixing and adjust pH to 5.8"* Set-point is 5.8 in TN 96.2 In TN 96.2 there is a range of pH from 5.7 to 6.3. However during crop test with Argus controller set point was 5.8. For crop tests with Schneider controller set point was 5.9 but dead zone was ±0.1 and pH actually was maintained at 5.8. So pH value here is changed to 5.8
12/Section 6.6	*"Planting:* *7. Add a plastic tray cover (Figure 6.2) and place in a growth cabinet with a temperature of 20°C and lighting period of 16 hours."* Please specify: clear? Light transparent? Specified: *"light transparent plastic tray cover"*
12/ Section 6.6	*Planting:* *"7. Add a plastic tray cover (Figure 6.2) and place in a growth*

	cabinet with a temperature of 20°C and lighting period of 16 hours." What about RH? Ventilation requirements? Rephrased: *"Add a light transparent plastic tray cover and place in HPC1 with a temperature of 20°C and lighting period of 16 hours. Light intensity should be not less than 300 µmol·m^{-2}·s^{-1} (1HPS+1MH lamps per module are enabled), blower should be enabled, set point for relative humidity are: 50% at day time and 70% at night"*
13/Section 6.6	*"Planting:* *10. After 5 days, break off individual cubes containing viable seedlings and set them into the larger (pre-rinsed with deionized water) Rockwool cubes."* Is this step really necessary? Could we manage without doing it? This step is necessary as the small Rockwool cubes are designed for the seedlings and there is no space in a small cube for a root system of an adult plant. Moreover, they are not stable and in the beginning of the crop test when the plants are still rather small, the cubes might fall aside.
13/Section 6.6	*"Planting:* *10. Remove one trough from the harvest end and evenly space 5 plants within it, being sure not to block the drain hole and leaving space for the irrigation spout."* In 5 large Rockwool cubes? Rephrased: *"Remove one trough from the harvest end and evenly space 5 large Rockwool cubes with plants inside within it"*
13/Section 6.6	*"Planting:* *10. Remove one trough from the harvest end and evenly space 5 plants within it, being sure not to block the drain hole and leaving space for the irrigation spout."* Is it the same planting density than previously used in UoG? please specify Planting density, used in UoG and reported in TN 85.83, was 6 large Rockwool cubes per trough. But it was not the only change in comparison with the test in UoG. Those changes were proposed by UoG.
15/Section 6.6	*"Planting:* 16. Skip to step 17. Modified: *"Skip to step 20".*

15/Section 6.7	*"- CO2 Demand – 1000 ppm"* Demand or concentration in the HPC? CO2 conc. set-point. Rephrased: *" CO2 concentration set point– 1000 ppm"*
15/Section 6.7	*"Humidity – 50-75% day, 70-75% night"* What about the set-point? There were different set points for different tests. With Argus controller Rh day/night set point was 75%/75% (but the actual value was about 60% for a day time). For the next 1-week crop test with Schneider controller Rh set point for day was 60% and for night -70% as it was not possible to reach higher values. For 45-days test Rh day set point was 50% as the previous crop test demonstrated that in the beginning of a crop test when the plants were small and transpiration was very low, it's not possible to reach higher values of Rh. Night set point was 70 %. For above mentioned reasons we provided the range of humidity set points.
15/Section 6.7	*"EC – 2 mS"* 2 mS/cm? Amended: *"EC- 2 mS/cm*
15/Section 6.7	*"pH high/low 6.0/5.6"* What about the exact set-point 5.8? With the Schneider controller the set point was 5.9 but dead zone was ±0.1. Amended: "*pH setpoint– 5.9 (5.8-6.0)"*
16/Section 6.7	Please mention VPD, although not measured yet, for information Included: *"-VPD – 9.0 day, 6.0 night"*
16/Section 6.9	*"5. Remove troughs one at a time and harvest each separately"* What are you doing with the plants? The procedure is added to TN 96.4. Added: *"(detailed harvesting procedure is given in TN 96.4)"*
17/Section 7.2	*"Fourteen days are required for seedling establishment and initial development before transfer to the HPC"* Clarify why, when compared to the 5 days mentioned in the full maturity crop growth Rephrased: *"For better performance of all control loops the plants should be in the phase of active growth and fourteen days are required for seedling establishment and initial development before transfer to the HPC"*
19/Section 7.5	*"HPC Nutrient Solution Preparation:*

	4. continue mixing and adjust pH to 5.9 using 0.5M nitric acid or 0.5M potassium hydroxide" pH to 5.8? Yes, amended: *"pH to 5.8"*
20/Section 7.6	See comments on paragraph 6.6 Amended in the same way as 6.6.
22/Section 7.7	See comments on paragraph 6.7 Amended in the same way as 6.7.
22/Section 7.9	*"5. Remove troughs one at a time and harvest each separately"* What do yo do with the plants? Store them for analysis? The procedure is added to TN 96.4. Added: *"(detailed harvesting procedure is given in TN 96.4)"*