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Technical Note

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Preliminary trade-off of crop cultivars: Test results evaluation and selection methods consolidation

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List of Abbreviations

BT1 / BT2:	Bench Test 1 / Bench Test 2
CES:	Controlled Environment Systems
CESRF:	Controlled Environment Systems Research Facility
DM:	Dry Matter
DW:	Dry Weight
EC:	Electrical Conductivity
FW:	Fresh weight
HZPC:	Consultant for hydroponic potato growth
IPL:	Institut Paul Lambein
NCER:	Net Carbon Exchange Rate
NFT:	Nutrient Film Technique
RH:	Relative Humidity
SEC-1 /SEC-2:	Sealed Environment Chambers
T:	Temperature
TDF:	Total Dietary Fibre
TGA:	Total glycoalcaloids
TN:	Technical Note
UBern:	University of Bern
UCL:	Université Catholique de Louvain
UGent:	Ghent University
UNapoli:	University of Naples
UoGuelph:	University of Guelph
USDA:	United States Department of Agriculture
VOC:	Volatile Organic Compound
VPD:	Vapour Pressure Deficit

1 Introduction

This document evaluates the **performance of the cultivars** pre-selected in TN 98.3.1, according to the selection method established in TN 98.3.1 and developed into the measuring plan as described in TN 98.4.11.
First **experimental performance** as reported in TN 98.4.21 is evaluated and critical points discussed.
When possible the experimental layout as described in TN 98.4.11 will be adjusted, while limiting the impact on repeatability if the first test was successfully completed.

Ranking of the cultivar performance will be presented in a preliminary way, since only the input of the repeat experiment foreseen will enable to obtain a more trustworthy result, given the occurrence of unanticipated problems in some of the setups.
The selection method as presented in TN 98.3.1 will be assessed per crop in tabular form.

Bench test evaluation includes the following key parameters:
Benchtest setup performance. Measures needed to counteract culture-technical problems are discussed under 1.1, 2.1 3.1 4.1 5. 6.1

Nutrient solution composition evolution. This includes observations on element depletion or accumulation. (see 1.2, 2.2.7, 3.2.7, 4.2.7, 5.2.7, 6.2.7). Emphasis should be put on the fact that elemental nutrient composition is the key to optimal plant production.

Cultivar edible yield, and comparison with reference field crop data (as reported in TN 98.3.1) (See 1.4 2.4 3.4 4.4 5.4 and 6.4)
Cultivar harvest proximate composition, and comparison with data obtained from commercial agriculture. (See 1.4 2.4 3.4 4.4 5.4 and 6.4)

Additional Parameters that were proposed as of particular interest for the plant bench test evaluation were
Crop size
Harvest index or ratio edible/enible biomass
Harvest composition regarding antinutritional compounds
Water use efficiency
Stress resistance preliminary observations
Choice of the crop initiation procedure
These topics are discussed under the appropriate headings below, where relevant

Limitations of the used protocols (sensitivity and timing) are indicated.

This evaluation will lead to the proposal of a consolidated crop cultivar selection method.

1.1 Experimental Layout

UBern and UoGuelph carried out the wheat trials without nutrient solution cooling.
UNapoli also cultivated soybean without control of the solution T.
For potato this parameter is more critical, since T>20˚C inhibits tuber induction, hence nutrient solution cooling was foreseen in UGent and UCL.

Active humidification is used in the UGent and UNapoli setups, which have air exchange with the outside atmosphere (in addition to condensation in the cooling system).
The UoGuelph setup relies on extra dehumidification capacity to keep the RH setpoint in the sealed chambers.
In the UBern chamber, bread wheat culture necessitated the installation of extra dehumidification, given the high level of transpiration from the mature wheat crop, and the limited ventilation capacity with facility supplied conditioned outside air of the UBern setup.
The RH in the UCL setup remained stable by means of the condensation in the cooling system.
Dehumidification needs for further tests will depend on the level of crop development on the available cultivation surface, versus growth chamber volume.

1.2 Evaluation of growth environment follow-up

The foreseen loggers for each of the setups were adequate to follow-up on chamber T and RH (VPD).
Frequent manual adjustment of pH and EC are time-demanding and the setpoint is difficult to obtain with precision (mixing being critical during adjustment). The automatic pH adjustment setup at UGent proved to suffer from sensor stability problems.
Nutrient solution elemental analysis can give an indication on accumulation of elements. Depletion of elements can be a consequence of efficient uptake by plants (characteristic for N P and Mn), hence this parameter has to be considered per element in relation to uptake characteristics.
The key parameter to be considered in future experiments is plant tissue elemental composition, which should be compared with published reference values for MFC experimental crops. These analysis have not been done for BT1 as the results would have been unreliable due to either phyto-sanitary problems or suboptimal growing conditions.

1.3 Evaluation of monitoring of plant development

Access to the plants is for the closed, sealed UoGuelph setup logically limited, it was hence problematic to obtain time-lapse data when plants grow tall, largely obscuring viewing window and monitoring camera placed inside. Intermittent opening (e.g. on a bi-weekly basis), can allow for a suitable level of data collection, on a subset of plants and will be considered for BT2.
For potato tuberisation assessment, with the aim to develop a nutrient delivery strategy for optimal tuber development, a gully setup with easily removable lid proved workable. However

tuber greening remains a concern, hence the light-tightness needs improvement. This can be accomplished by:

- using improved gully covers
- better closing the gully lids
- avoiding to open the gullies
- reducing the time of opening of the lid during the observations.

1.4 Evaluation of crop harvest

1.4.1 Yield

Crop yield is based on cultivated surface, defined as illuminated surface: whole chamber area in the closed Guelph chambers, shelve or table areas covered by canopy and gullies in other setups.
The durum wheat trials gave an average edible yield of 0.5kg/m^2 yield for 2 cultivars tested so far (next 2 are grown in bench test2).
For bread wheat, 3 cultivars, yielded 0.4 kg/m^2, while the fourth performed worse with 0.3kg/m^2.
The potato yield obtained by UGent-consultant HZPC in hydroponic greenhouse culture attains a highest value of 3kg FW/m^2. Potato on average has 20% DW content, hence a value of 0.6kg/m^2 is also attained for DW/m^2.

Suboptimal start or growing conditions and subsequent phytosanitary problems severely limited growth in the bench tests at UGent and UCL (potato), and UNapoli (soybean).

1.4.1.1 UGent/UCL

For the UCL/UGent potato experiment, the problem was caused by a combination of factors:

1. Plants were produced at HZPC, initially under sterile in-vitro conditions, then transferred to non-sterile conditions and transported to UGent. Further growth at UGent was continued in non-sterile conditions. Plants were thus in non-sterile conditions at HZPC, during transport and in Belgium, which most likely increased the contamination hazard. This needs to be avoided for future experiments.
2. Suboptimal regulation of the nitrogen addition during the tuberisation phase. No nitrogen was added in the beginning of the tubersiation phase and later there was only one nitrogen addition per week. The plants were already weak when we started the N additions and addition of the weakly N amount in one time is not optimal for plants growth. In the future we will test whether better performance can be obtained upon addition of lower amounts on a more regular basis. Based on plant pathologist comments, the root contamination is possibly a secondary infection due to plant weakness (physiological problem most probably due to a bad nitrogen distribution). So resolving the physiological problem (nitrogen application) must solve the infection problem.

In order to avoid contamination in future experiments, we envisage the following countermeasures:

1. Between Bench Tests thorough cleaning of the growth chamber and cleaning of the gullies using bleach will be done.
2. The procedures in the above mentioned points 1-2 will be optimized.

1.4.1.2 UNapoli

In soybean hydroponics cultivation, according to pathology specialists, the main problems seemed to be determined by nutrient deficiencies (expecially Mn) due to pH fluctuations. We will aim to improve the nutrient solution management in BT2 (using a more concentrated nutrient solution, EC target from 1.2 to 2 dS/m), in order to prevent similar problems.

1.4.2 Nutritional analysis

In proximate analysis, carbohydrate content is obtained by difference (between starting sample weight and water, protein, lipid and ash determinations)
Fiber content determination: IPL and UNapoli according to the same AOAC 985.29 protocol
ETHZ determination
UoGuelph
Available carbohydrate is defined as the difference between total carbohydrate and fiber.

1.5 Cultivar selection method and ranking

Preliminary ranking of the tested cultivars of BT1 can (for all the crops) as a minimum be based on:
Edible harvest yield /m^2
Growth period (maturation)
Harvest index (ratio edible/total yield)
Attained plant height
Nutritional analysis
Total amount of water transpired

2 Bread Wheat (UBern)

2.1 Evaluation of experimental layout

2.1.1 Measuring plan

The setup with 2 different cultivars per shelf (0.6m^2 illuminated growth area) in a randomised layout generates access difficulties in following up on growth, and problems related to different maturation duration between cultivars.
Full production is advised only to be carried out with the same cultivar in the 2 independent gullies on each shelf.

2.1.2 Setup

A plant density of 200 plants / m^2 (2 x 60 plants / 0.6m^2) is considered adequate based on the bench test 1 results.
Seed germination, as carried out in closed boxes at 100% humidity (for 3 days) allowed to carry out a preselection of synchronously germinated seeds (seedlings of the same early developmental stage), and allowed a 100% plant survival till harvest.
The density was reduced to 100 plants/m^2 (one gully instead of two gullies per shelve) in BT2.
In BT2 the concentration of macro and micronutrients in the nutrient solution was step-wise decreased after flowering to reach an EC of 0.4 mS/cm and the pH compensating acid was changed (H_2SO_4 replaced HNO_3). This change in the nutrient medium diminished the number of extra side-stems (tillers) for CH Rubli and Greina (the two other cultivars are not yet mature).

2.2 Evaluation of growth environment follow-up

2.2.1 Settings

RH could be reduced to some extent in BT2 as compared to BT1 using the added dehumidification capacity.

2.2.2 Chamber T/RH evolution

Extra dehumidification was installed and functionality proven to reduce the RH to the 60% to 80% (depending on developmental stage of the wheat) regardless of time of day.

2.2.3 Chamber CO_2 level

Only a minor depletion of CO_2 concentration was observed throughout the day (values > 300 ppm).

2.2.4 Nutrient solution environment

Adjustment of the nutrient level needs frequent manual additions given the high transpiration rates of the crop.

2.2.5 pH and EC evolution

EC was used as the setpoint (1.2 mS/cm) when adjusting the gully reservoir nutrient medium level.
pH values were recorded, but not compensated by acid additions. The nutrient medium was exchanged every month (see the days of the exchange in the table below). In between the exchange, the gully reservoirs were refilled with water and replenish solution to adjust the EC to 1.2 mS/cm. Sometime after these exchanges, the EC and the pH evolved in opposite way for a short period of time, for example after the 23 of September (all gullies). To improve the maturation of the ears, the gully reservoirs were refilled with water only since the 15 of December. Refilling reservoirs with water only allowed a step wise decrease of the EC. Again after this step, the pH and the EC evolved sometimes in an opposite way, for example Aletsch and Greina.
For subsequent tests, the amounts of N and S from acids used to adjust pH have to be included in the nutrient level adjustment strategy as linked to development and maturation.

Tab. 1 Days of monthly exchange of nutrient medium for the 8 gullies

	Gullies							
	A1	A2	B1	B2	C1	C2	D1	D2
1st exchange of nutrient medium	15.09.2009	15.09.2009	16.09.2009	16.09.2009	16.09.2009	16.09.2009	16.09.2009	16.09.2009
2nd exchange of nutrient medium	21.10.2009	21.10.2009	21.10.2009	21.10.2009	21.10.2009	20.10.2009	21.10.2009	21.10.2009
3rd exchange of nutrient medium	24.11.2009	24.11.2009	24.11.2009	24.11.2009	24.11.2009	24.11.2009	24.11.2009	24.11.2009
refilling with water only since this day	15.12.2009	15.12.2009	15.12.2009	15.12.2009	15.12.2009	15.12.2009	15.12.2009	15.12.2009
exchange of nutrient medium with water only	19.01.2010		19.01.2010				19.01.2010	

2.2.6 Nutrient solution T

The solution T was higher than optimal. Plants yielded normal ears and kernels, hence this parameter is not the most critical.
It is logistically difficult to rapidly setup a cooling system for all separate gully reservoirs

2.2.7 Nutrient solution analysis

Phosphate was nearly completely depleted after 4 weeks. P is rapidly taken up by plants, hence this is expected.
The 4 week nutrient exchange cycle likely permitted to avoid P-limitation, final confirmation can only be obtained by the analysis of plant material.

2.3 Evaluation of monitoring of plant development

2.3.1 Photographic follow-up

Crop development and differences in maturation between the cultivars was described. CH Rubli and Greina matured first, Aletsch was intermediate, and Fiorina was the latest maturing cultivar. The developmental stages of the four cultivars are shown in the following figures.

The germination, stem elongation, ear emergence, flowering, grain filling, ear yellowing and harvest are shown on a timeline. Special events such as chlorosis, lodging and mould contamination are also shown on this timeline.

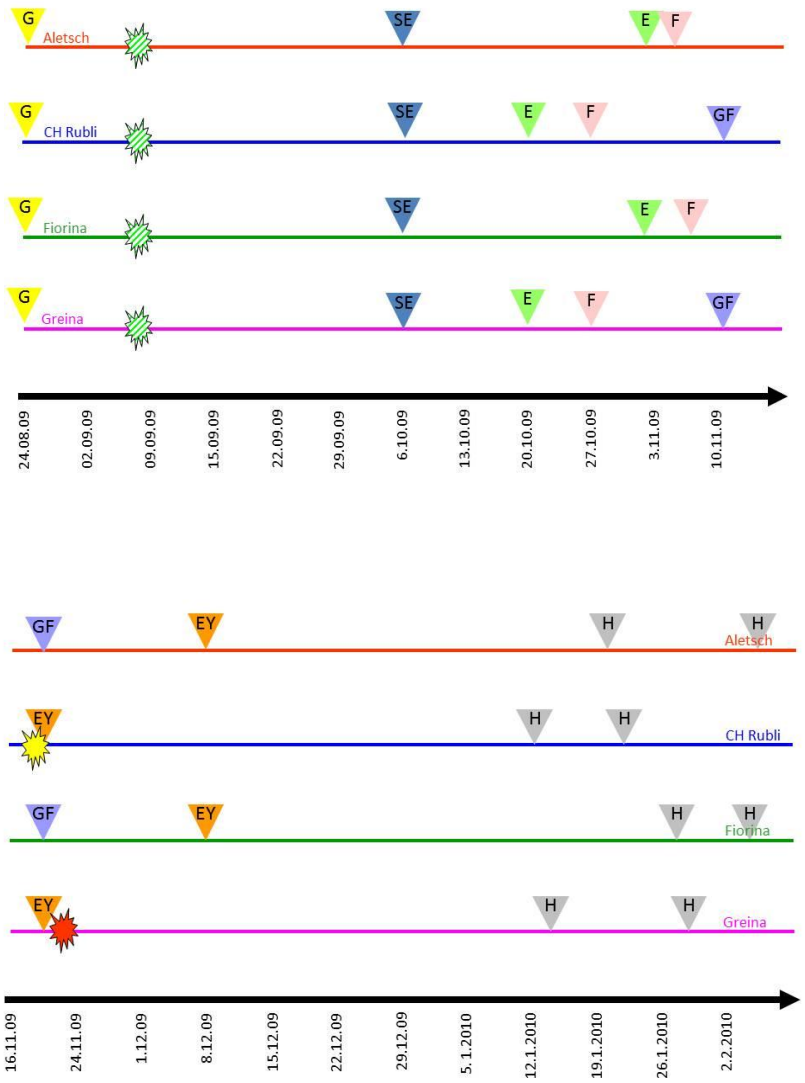
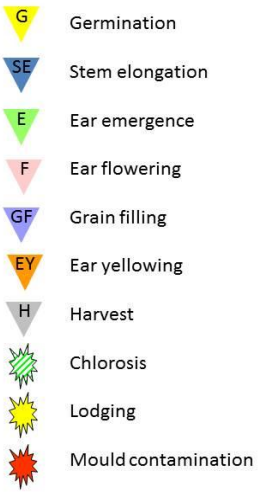


Fig. 1 Developmental stages of the 4 cultivars from the germination to the harvest



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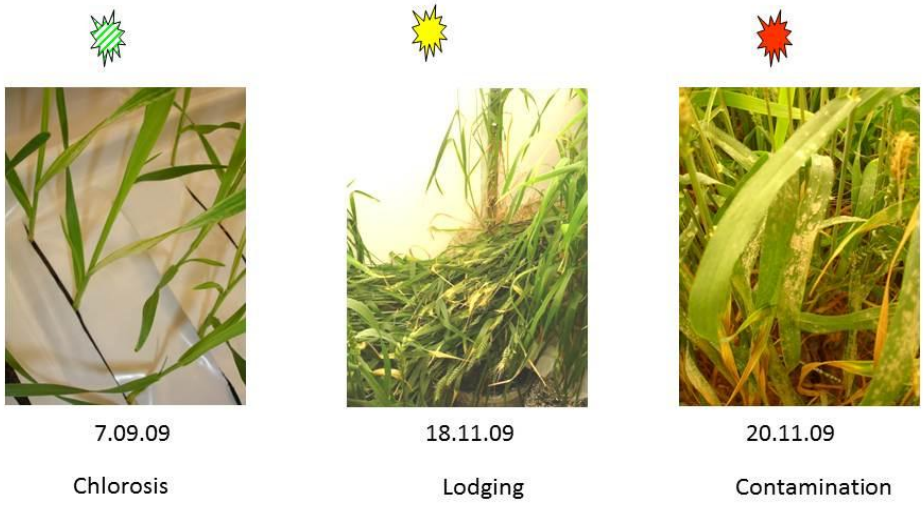


Fig. 3 Pictures of chlorosis, lodging and mould contamination

2.3.2 Detailed photographic observations

The ripening of the ears, on which harvest time was based, was documented.

2.3.3 Growth assessment

Double gully layout precludes access to the gully on the wall side of the shelf.

Resistance to lodging: plants of CH Rubli Gully C1 lied down after grain filling and the plant of Fiorina (both gullies) suffer for lodging already before stem elongation and some threads were placed around Gullies D1D2 to maintain the plants. Second gully of Fiorina suffer also of lodging but the plants were maintained by the wall.

Fig. 4 UBern - Lodging of CH Rubli Gully C1 (20.11.2009)

Fig. 5 UBern - Thread placed around Fiorina Gully D1 to maintain the plants

2.3.4 Gas exchange data

Not foreseen in the measuring plan for bread wheat.
Global plant water usage determined volumetrically from the nutrient solution usage was correlated with length of the growth period of the cultivars, the first maturing cultivars having the lowest water consumption.
The length of the growth period explains a part of the water usage. But the water usage is also related to the cultivar. Greina and CH Rubli have more or less the same length of growth period but CH Rubli used more water than Greina (Fig. 6). Transpiration during maturation was presumably also affected by late tillering caused by excess nutrient availability. BT2 may give some further indications.

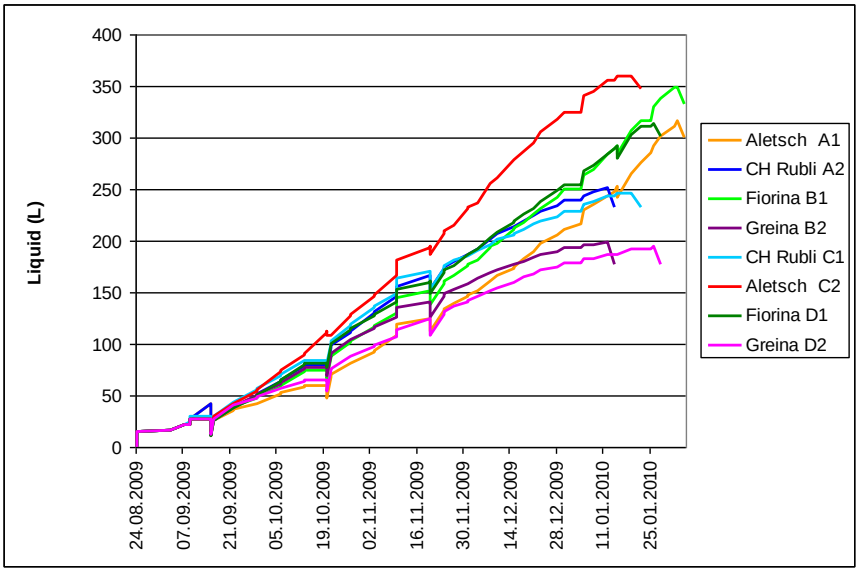


Fig. 6 UBern - Liquid (water, start-up solution and replenish solution) cumulative uptake by the plants

2.4 Evaluation of crop harvest

Separation of the kernels from the rachis (central 'stem' of the wheat ear) and the glumes was carried out by a dry separation method.
The Fiorina cultivar proved the most difficult to manually separate kernels from the ears.
Greina produced 248.05 g of kernel, CH Rubli 247.47 g, Fiorina 239.95 g and Aletsch 180.35 g. The 100 kernel weights were 3.67 g with Greina, 3.22 with Aletsch, 3.12 with Fiorina and 3.04 with CH Rubli. They were similar but not identical with market samples (depending on genotype). The harvest index [DW kernels/(DW kernels + DW straw + DW roots + DW treshing debris)] was 0.31 for Greina, 0.24 for CH Rubli, 0.16 for Fiorina and 0.14 for Aletsch. In literature, the harvest index is defined as the ratio of grain yield to aboveground biomass (Li et al., 2011), the roots are therefore not counted in the dry weight. When comparing the harvest

index shown in table 1 (which is calculated with the dry weight of roots) with the harvest index shown in table 2 (which is calculated without the roots), the value for the harvest index (without the roots) are higher: 0.36 for Greina, 0.28 for CH Rubli, 0.19 for Fiorina and 0.17 for Aletsch. The harvest index found in the literature for winter wheat was in between 0.15 and 0.44 (Li et al., 2011), around 0.45 (McIntyre et al., 2010) or around 0.5 (White and Wilson, 2006). The green tillers (which didn't produce grains) were an important part of the dry weight of shoot, and thus decreased the harvest index.

Tab. 2 UBern - Harvest index

	DW Kernels in g	DW straw in g	DW roots in g	DW treshing debris in g	Harvest index for dry matter
Aletsch	168.82	699.78	222.73	126.93	0.14
CH Rubli	231.65	491.45	119.96	109.26	0.24
Fiorina	224.15	733.83	194.52	207.72	0.16
Greina	232.49	337.34	91.76	78.57	0.31

Tab. 3 UBern – Harvest index (without roots)

	DW Kernels in g	DW straw in g	DW threshing debris in g	Harvest index for dry matter
Aletsch	168.82	699.78	126.93	0.17
CH Rubli	231.65	491.45	109.26	0.28
Fiorina	224.15	733.83	207.72	0.19
Greina	232.49	337.34	78.57	0.36

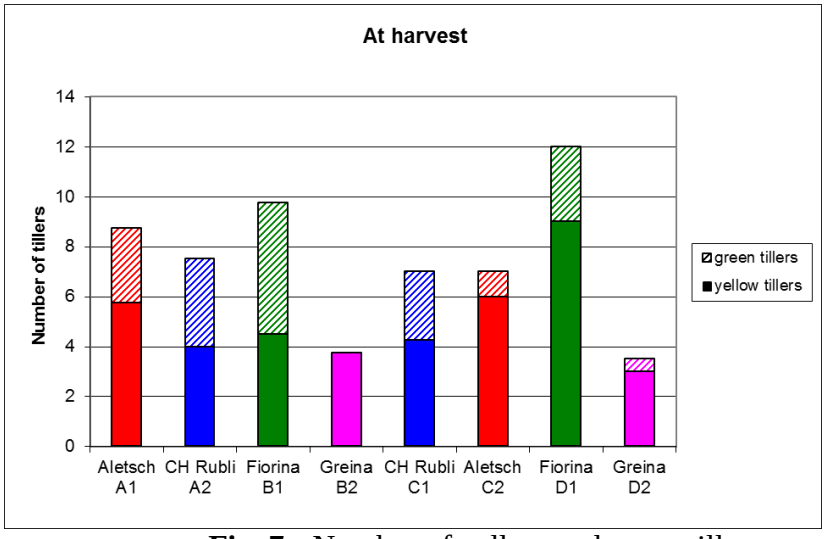


Fig. 7 Number of yellow and green tillers per plant at harvest

Tab. 4 UBern - BT1 harvest and ripening

Cultivars	Gully	Germination	Harvest	Number of days	Ripeness	Number of days for ripeness
Aletsch	A1	24.08.2009	04.02.2010	164	not completely mature at harvest	more than 164
CH Rubli	A2	24.08.2009	13.01.2010	142	13.01.2010	142
Fiorina	B1	24.08.2009	03.02.2010	163	not completely mature at harvest	more than 163
Greina	B2	24.08.2009	14.01.2010	143	13.01.2010	142
CH Rubli	C1	24.08.2009	22.01.2010	151	13.01.2010	142
Aletsch	C2	24.08.2009	20.01.2010	149	13.01.2010	142
Fiorina	D1	24.08.2009	27.01.2010	156	not completely mature at harvest	more than 156
Greina	D2	24.08.2009	28.01.2010	157	13.01.2010	142

Tab. 5 UBern - BT1 yield

	Total per cultivar (g)
Aletsch	180.350
CH Rubli	247.470
Fiorina	239.944
Greina	248.052

2.5 Cultivar selection method and ranking

As mentioned in TN 98.3.1 (section 2.2.2), the following selection criteria were considered for the bench test trials:

The first 2 criteria below are pre-test criteria, and not to be used for the bench test evaluation
- Availability of the cultivar 4 pre-selected cultivars
- Vernalization excluded the use of winter wheat

Tab. 6 UBern - Cultivar overview

	Greina	CH Rubli	Aletsch	Fiorina
Shoot length (short)*	1	3	3	2
Generation time (short)	1	1	2	3
Precocity of ear emergence	1	1	2	2
Resistance to lodging	1	2	1	3
High yield	1	1	3	2
Total rank	**5**	**8**	11	12

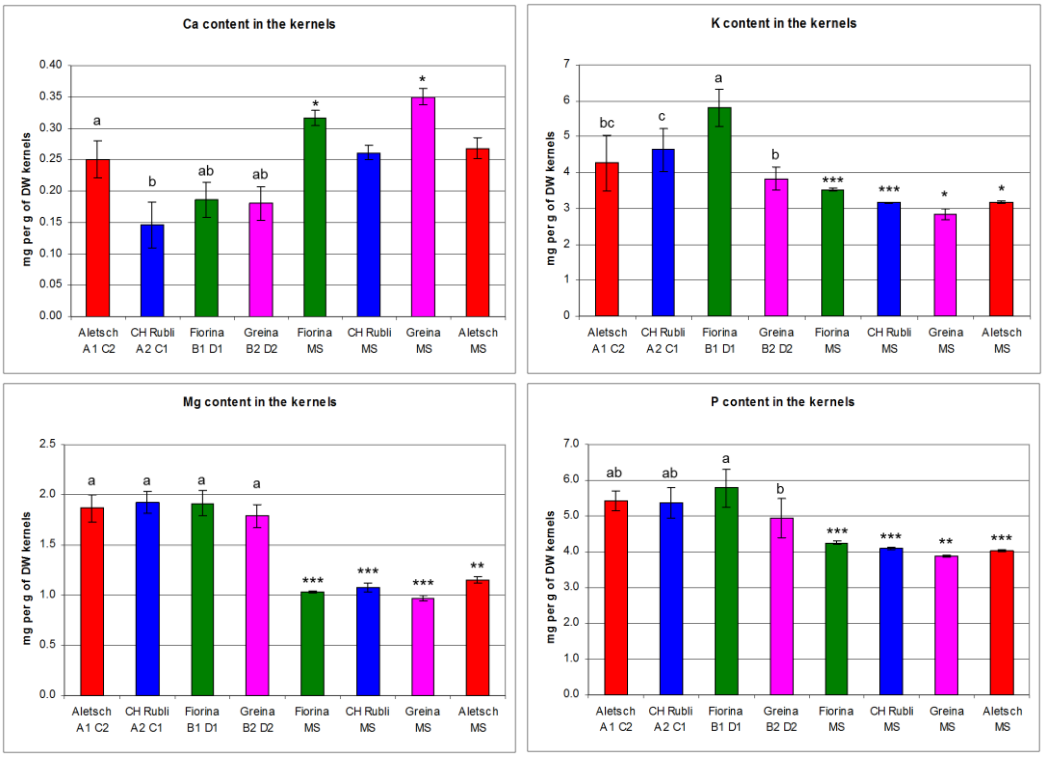
*shoot length Greina and Fiorina ears appear at the top of the stalks (see TN 98.4.12, 2.3)

- Disease resistance

The observed fungal infection first observed in Greina is most likely caused by the late maturation (and related to the high nutrient supply) and not indicative of a disease resistance during normal development, but to be confirmed.

- Levels of certain elements (e.g. K, Ca, Mg, P, Fe, Zn, Cu, Mn, Ni)

The plant macro and micronutrients (K, Ca, Mg, P, Fe, Zn, Cu, Mn and Ni) content in the kernels of BT1 and the market samples are shown in the figures below. In the kernels of BT1, the macro and micronutrient contents were higher (K, Mg, P, Zn, Cu, Mn and Ni) or lower (Ca and Fe) than the content in the market samples, with some exceptions: Ca in CH Rubli and Aletsch, Zn in Fiorina and CH Rubli and Mn in Fiorina and Aletsch. These higher contents of macro and micronutrients might be due to an inadequate supply. The difficulty of the wheat to get mature (high amount of green tillers at harvest time, flag leaves not senescent at the harvest) and the mould contamination on Greina were also a sign that the macro and micronutrient supply was inadequate. The EC was too high, especially after flowering. The pH of the nutrient medium might also be inadequate. For BT2, the pH will be adjusted to 5.6 – 6 with acids (HNO_3 replaced after flowering by H_2SO_4). In conclusion, the macro and micronutrient contents in kernels of BT1 were different but not too far away from the content in the market samples and may become closer to the content in the market samples by a better adjustment of the EC and pH of the nutrient medium.



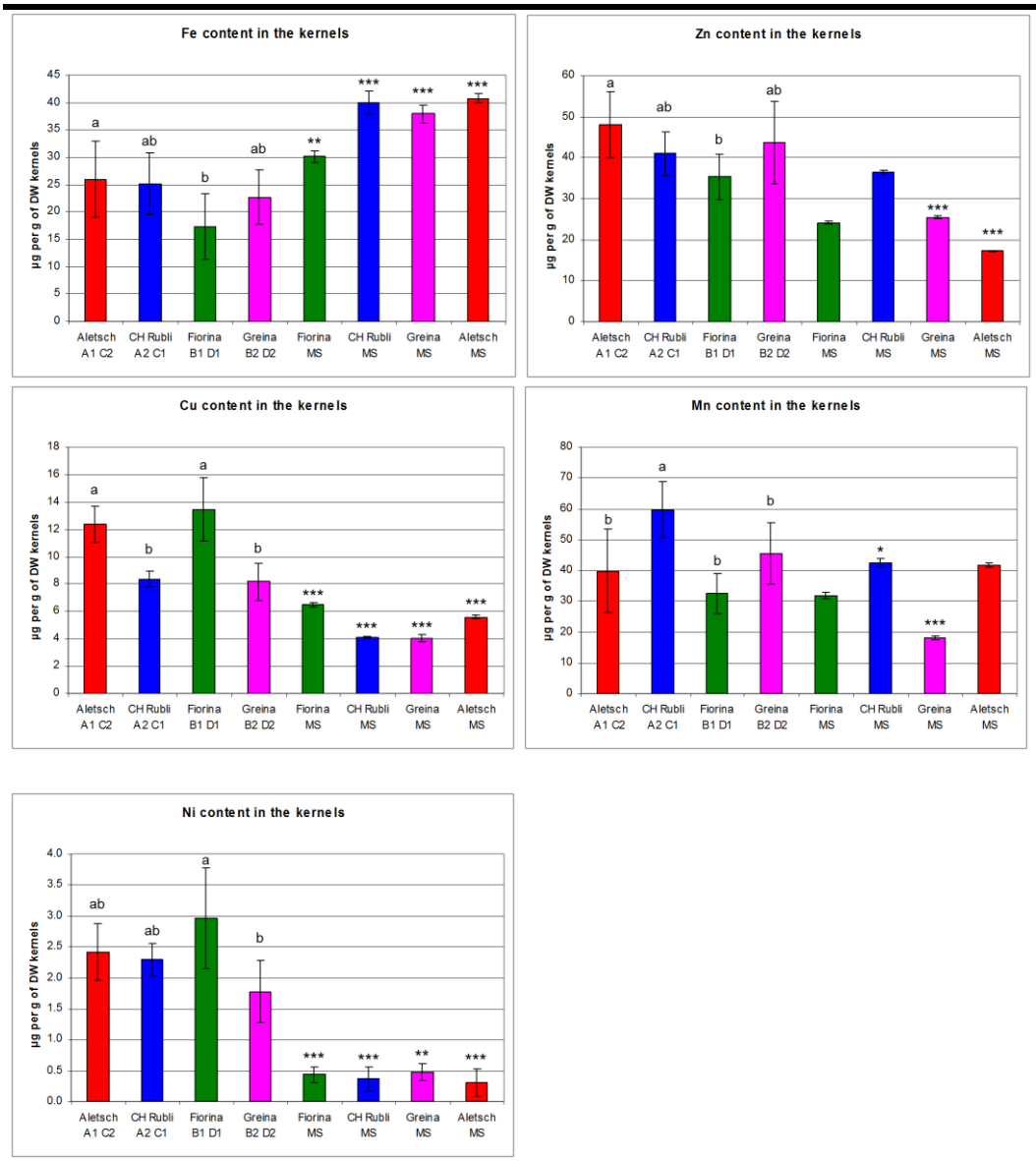


Fig. 8 K, Ca, Mg, P, Fe, Zn, Cu, Mn and Ni content in the kernels of BT1 and market samples (MS).

The content is in mg or µg per g of dry weight of the kernels. Samples of BT1 (the 2 gullies together) are on the left and the market samples are on the right. Values are means + SD (n = 4). The different letters indicate the statistically significant differences in between the four cultivars of the bench test. The asterisks represent the statistically significant differences for the same cultivar in between BT1 and MS (*: $P \leq 0.05$; ** : $P \leq 0.01$; *** : $P \leq 0.001$)

There were 2 post-harvest analysis criteria to be assessed:
- Protein content
- Processing properties

These have been assessed in TN 98.5.2 (section 3) and further evaluated in TN 98.5.3 (section 3).

Specific for controlled environment chamber plant growth additional parameters were mentioned under section 2.2.1.1 of TN 98.3.1:

- High yield of edible versus non-edible parts (see table harvest index above).
- Water use (integrated transpiration rate during the entire growth period,)
  This was positively correlated with the generation time, but was also related to cultivars (for instance CH Rubli and Greina had the same generation time but CH Rubli used more water).
- Senescence and maturation properties: linked to the abovementioned generation time. These properties will most likely be affected by modifications in the nutrient supply. Additional information can be expected from BT2.
- Photosynthetic rate and oxygen production (measurements not foreseen in the measurement plan for BT1 and 2).

2.6 References

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White EM and Wilson FEA (2006) Response of grain yield, biomass and harvest index in their rates of genetic progress to nitrogen availability in ten winter wheat varieties. Irish Journal of Agricultural and Food Research 45: 85-101

3 Durum Wheat (UoGuelph)

3.1 Evaluation of experimental layout

3.1.1 Measuring plan

Measuring plan was carried out as specified.
In former growth trial experiments in the sealed SEC-2 chambers at UoGuelph, a standard bi-weekly leaf level measuring schedule involved opening of the chambers, avoiding buildup of ethylene and oxygen (and possible other compounds).

3.1.2 Setup

The same layout of plants will be used for next test with the remaining 2 preselected cultivars.
The density corresponds to 135 plants per square meter growing area (corresponding to 1 gully and associated 1/5th of the illuminated chamber growth area of $5m^2$).
The plant survival rate of 69%, which equalled the in-situ (in the chamber, on the rockwool pads) germination rate, corresponds to a density of 93 plants / m^2.

3.2 Evaluation of growth environment follow-up

3.2.1 Settings

Setpoints confirmed for next 2 cultivar trial.

3.2.2 Chamber T/RH evolution

Apart from some minor interference by chamber opening T control was as expected.
Humidity control was not as effective as desired and the cause will be investigated.
T was raised from 23 to 26˚C after approximately 12 weeks in order to improve seed filling as recommended by the durum wheat expert Dr. Mark Jordan.
Humidity setting was reduced to a minimum to accelerate crop drying prior to harvest.

3.2.3 Chamber NCER, evapotranspiration, ethylene and oxygen production

The CO_2 control at 1200 ppm was achieved throughout the experiment, and allowed NCER calculation.

In Avonlea, oxygen levels exceeded ambient levels starting approximately 3 weeks after planting and were only reduced during chamber opening to repair flooding due to root growth. The high oxygen immediately prior to the observed decrease in NCER may have been a contributing factor as high oxygen reduces the efficiency of photosynthesis by competing with CO_2 for the acceptor Ribulose 1,5-bisphosphate (Warburg effect). Measurement of oxygen

production in the Strongfield trial failed due to a mechanical error. In order to reduce the effect of high oxygen on photosynthesis, biweekly chamber venting will be employed in the following trials.

Ethylene reached levels of 80 ppb in Avonlea, and 40 ppb in Strongfield. High ethylene levels may have contributed to the lower yield in Avonlea.
The difference in leak rate between the 2 chambers (0.51% for Avonlea versus 6.59% for Strongfield chamber) is a likely explanation of the different levels measured, although cultivar differences could also contribute. This can only be proven by replication of the data.
Ethylene should be mitigated in future trials by scheduled venting (on a biweekly basis).
Removal through adsorption or catalysis should not be performed at this time as indications of ethylene production are an important aspect of system sizing for future mitigation procedures.

3.2.4 Nutrient solution environment

Intermittent irrigation was used with a circulation pump on time of 2 to 3 minutes. The on times are part of a 10 minute cycle.
Intermittent irrigation is necessary in these hydroponic systems due to the draining requirements.
The approach is likely beneficial for oxygen provision to the roots, saves energy, and likely reduces nutrient solution T increases.

3.2.5 pH and EC evolution

pH and EC control was excellent with deviations from setpoint only during initial operation (Avonlea - injection pump failure) and during solution changes or flooding events.
pH and EC deviated from the setpoint at the end of the experiment in both chambers and was a direct result of the cessation of nutrient circulation to the plants in order to decrease maturation time.

3.2.6 Nutrient solution T

Suppression of nutrient solution T increases is seen as beneficial. Target setpoint would be about 1 degree below chamber atmosphere setpoint temperature.

3.2.7 Nutrient solution analysis

P, K and the micronutrient Mn were depleted at the time of nutrient solution exchange.
These elements are taken up rapidly by the plants, not necessary an indication of shortage.
Nutrient solution composition could be adjusted for the next 4 cultivar repeat test.

3.3 Evaluation of monitoring of plant development

3.3.1 Photographic follow-up

See 3.3.3

3.3.2 Detailed photographic observations

See 3.3.3

3.3.3 Growth assessment

Limited viewing ability by integrated webcam and small chamber window.
First plants in each gully can be used to assess key development stages (flowering, grain maturation) during scheduled venting.

Maturity assesment
There are several methods but for general comparisons we would use the number of days from seeding until 75% of the heads have no green colour (are yellow). This is obviously not precise as the heads do not turn instantly yellow on a single day but it is practical and often used.
For carefully controlled physiological studies the exact method is physiological maturity is when the head is at 20% moisture. For this you need to take samples of heads, weigh them then dry and weigh again. If the weight difference is 20% they are mature.

3.3.4 Gas exchange data

The chamber level assimilation and evapotranspiration data per cultivar allow for the comparison of integrated values over the whole growth period.

3.4 Evaluation of crop harvest

Results from both cultivars exceeded recorded field production yields (Clark et al., 2006) by 14 and 87 percent in Avonlea and Strongfield respectively, demonstrating that the sealed environments were suitable for durum wheat growth and development.
Avonlea produced over 2.1 kg of wheat kernels while Strongfield produced over 3.7 kg.
The 100 kernel weights were 4.17 with Avonlea and 4.57 with Strongfield, and are in the normal range for durum wheat. The harvest index was 17.7 and 27.9 for Avonlea and Strongfield respectively. No harvest index data is available from external sources for comparison with these values. Harvest index is not a common parameter used to evaluate durum wheat (Dr. Mark Jordan, Cereal Research Centre, Agriculture and Agri-Food Canada, personal communication).

Tab. 7 UoGuelph - Harvest overview

Cultivar	Total DW (g)	Height (cm)	Roots (g)	Straw (g)	Kernels (g)	Number of Plants	g/plant	Harvest Index
Avonlea	12 054	86	1 291	8 630	2 133	469	4.55	18
Strongfield	13 531	84	1 435	8 325	3 771	466	8.08	28

Ash content measurements were recorded for both cultivars and were 0.81 and 0.71 percent in Avonlea and Strongfield respectively. These values agree with previously published values for these cultivars (Hatcher et al, 2009).

At this preliminary results stage, the chemical CHNOP formula is not provided for the following reason: "C, N, and P together represent less than 50% of the durum wheat seed chemical composition. The major elements (i.e. oxygen and hydrogen) are necessary to provide a reliable biomass chemical formula, it is therefore recommended to provide this formula when complementary analyses are available".

3.4.1 Quality tests

A comparison was made with field data for the same cultivars at the Cereal Research Centre (AAFC/CRC).
The falling number measures starch degradation (due to alpha-amylase activity). The enzyme is activated by germination and premature activation in the grain that can occur under certain environmental conditions (known as pre-harvest sprouting). The conditions are generally wet weather around harvest. Low falling number can be associated with lower strength (the W and P strength alveograph parameters are lower in the chamber-derived samples).
Many premium customers want the falling number to be above 300: both cultivars did not reach that limit in sealed chamber culture. Below this level increased cooking loss (water gets cloudy as starch gets dissolved in it) and softer pasta can occur (pasta is mushier) as well as cracking when drying pasta.
The gluten index values are still good so the gluten itself is fine – the starch component is just altered which can affect the strength values in the alveograph (W,P).

The kernel colour was quite good so finished pasta, as one possible end product, should look fine.

As the low falling number is environmentally related, it should be fixable with alterations of conditions.
-A reduction in humidity gradually after flowering should help in this regard.

-Another method involves cutting off heads as they mature and drying them, however this is not possible as it requires extensive compromise of the sealed environment.
Other options include:
-harvesting before complete maturity, or
-reducing the watering interval gradually during flowering.
The basic problem seems to be that the usual imposition of dormancy that comes with gradually reduced water during maturation under field conditions is compromised in the sealed environment.
Mimicking field conditions at maturity should improve the falling number.
However, in practicality the issue may not matter – the wheat in this trial should make perfectly good pasta as the falling number is not low enough to completely compromise the quality. Astronauts will not need the same specifications as high throughput pasta manufactures and can play around with cooking times as needed.

The protein levels were quite good. Strongfield had a very high grain yield and there is usually an inverse correlation between yield and protein. In this case the protein level was not compromised by the high yield.

3.5 Cultivar selection method and ranking

Only 2 cultivars have been grown so far, and the selection criteria (yield and associated parameters) might be confounded by the different closure levels of the 2 chambers used, and the consequently different ethylene and oxygen level accumulation. Still yields were higher than for field harvests for both cultivars.
Appropriate venting will avoid accumulation to levels as experienced by the cultivar Avonlea for the next 2 cultivars to be grown in bench test 2.
Crop quality parameters (chosen to be diverse during pre-selection) correlated with data from field crop derived data.

Tab. 8 Durum wheat cultivars recommended for food characterization trials. Data is summarized from field trials where conditions are optimized for ideal growth and development.

Cultivar	**Habit**	**Gluten Index** (relative)	**Maturity** (days)	**Protein %** (field trial data)	**Yield tonnes/ha** (field trial data)
Avonlea	Tall	Low	101	14.3	3.6
Strongfield	Tall	Medium	102	14.5	3.6
Commander	Semi-dwarf	High	102	13.8	3.6
Eurostar	Tall	High	104	14.3	3.6

3.6 General Conclusions

Results from both cultivars exceeded recorded field production yields (Clark et al., 1999, 2005) by 14 and 87 percent in Avonlea and Strongfield respectively, demonstrating that the sealed environments were suitable for durum wheat growth and development Over the course of the trial, some chamber issues were identified including excessive root growth and elevated levels of oxygen and ethylene. To alleviate these problems, biweekly chamber venting and root trimming will be employed for the next cultivars to be tested.

3.7 References

Clarke et al. 1999. AC Avonlea durum wheat. Crop Sci. 39:880-881

Clarke et al. 2005. Commander durum wheat. Can. J. Plant Sci. 85:901-904

4 Potato (UGent)

4.1 Evaluation of experimental layout

4.1.1 Measuring plan

The measurements carried out during the first bench tests are feasible.
Given the high air mixing rate and through-canopy airflow in the bench test chamber, and its non-sealed nature, no localised chamber-level ethylene accumulation can be measured.
Measurements on plants or leaves enclosed in cuvettes with airflow comparable to the chamber growth environment did not allow to reveal increased ethylene levels.
On-line ethylene measurements within the current non-sealed chamber setup could still be tested with leaf level cuvettes, at a minimal flow rate that does not induce condensation in the cuvette. Otherwise sampling vials need to be used that enclose (part of) a leaf during a chosen timeframe.

4.1.2 Setup

No changes needed.
As an explanation to the extra step of 3 weeks in-vitro culture: in vitro plants are compared with tubers as starting material below:

Tuber seed:
For pre-test 1 and 2, chosen start up material were tubers obtained from the UGent consultant HZPC.
This has several inconveniences:
-tubers have to be stored during dormancy period, sprouting can take long depending on cultivar characteristics.
-tubers can possibly be contaminated by diseases.
-tuber buds and shoots grow vigorously and are produced in excess number (dominance of the distal bud depends on cultivar).so that many need to be removed to enable efficient growth in gully systems. This procedure requires a lot of working time.
-difference in weight and so, of nutrient reserve of mother tubers is a source of development heterogeneity.
In-vitro plants:
In-vitro plants are produced in 3 to 4 weeks' time, in large amounts on a limited surface:
-plant material is sterile
-plants are very homogenous with in theory the same genetic background.
With the current bench test setup, *in-vitro* plants must have a stem of minimum 5cm long to be placed through the plant-insertion openings at the side of the gully.

Below this length, it is hardly possible to have both roots in the nutrients solution layer, and youngest leaves exposed to light.
Acclimatization is a critical period that needs particular attention, but step-wise hardening and avoiding exposure to drying airflow is efficient.
After acclimatization a 4 or 5 days elongation phase is required to obtain plant about 10cm long. This step takes place in open gullies with NFT.

For these reasons, *in-vitro* plants are easier to use and more reliable than seed tubers.

4.2 Evaluation of growth environment follow-up

4.2.1 Settings

Confirmed.

4.2.2 Chamber T/RH evolution

Apart from the unavoidable short (stabilisation) day/night transients (see TN 98.4.11, section 4.1.3.2 and Table 37), no aberrations recorded.

4.2.3 Chamber CO2 level, ethylene measurement

4.2.3.1 CO2

Ambient concentrations of CO2 were applied. No decrease was measured, only increases during crop manipulations by operator presence.

4.2.3.2 Ethylene

Photo-acoustic measurements indicate accumulation of ethylene levels up to between 58-126 ppb. These are levels known to inhibit plant growth.

4.2.3.3 Oxygen

Maximum-minimum variation were +/- 0,3%. Moreover the average value was not higher then 18,5%.

4.2.4 Nutrient solution environment

Gully nutrient solution layer thickness is regulated by gully inclination and pump flow rate. Same values for the 4 gullies are adjusted.

4.2.5 pH and EC evolution

The observed instability of the pH sensor was presumed to be caused by the accumulation of gas bubbles at the sensor-liquid interface. To avoid the triggering of accidental addition of

phosphoric acid as during bench test 1, the sensors were placed in an aspirated tube connected to the circulation pump intake (similar to the setup for the manual dissolved O_2 measurement). Depletion of Nitrate induces acidification of the nutrient solution.

We cannot yet explain the alcalinisation of the nutrient solution observed during the first 2 weeks of culture nor the sudden change to acidification. These phenomenon's will need further research.

4.2.6 Nutrient solution T

Temperatures of all gully system solutions were stable at 20 degrees.

4.2.7 Nutrient solution analysis

Nitrate amount availability per plant is the control factor for plant height.

Tab. 9 UGent - Quantity of Nitrogen available per plant for each potato growth test

	mmol N /plant	
	Starting solution	Total amount
HZPC	3.1	3.1
UCL	2.8	9.5
UGent	0.78	1.15

UGent added a low amount of N, UCL dosed above the recommendations; both labs obtained a similar low yield due to initial stress and progressive development of opportunistic pathogens (see TN 98.4.21 and section 4.3 below).

N content in the plant samples needs to be determined in the future; UCL has carried out a preliminary analysis (Fig. 9).

Phosphate should be high (in comparison to N: high P/N ratio) during tuberisation (lack of potassium can then be a problem) and lower before tuber initiation, Potassium phosphate is not used for EC compensation during vegetative plant growth (Potassium sulphate only).

High Zn levels will be remediated by limiting the extra micronutrient additions as specified in the HZPC protocol.

4.3 Evaluation of monitoring of plant development

4.3.1 Photographic follow-up

Phytosanitary problems were identified on stolons and later on stems, showing black dots (indicative of Colletotrichum coccodes).

Microscopical observation confirmed Colletotrichum coccodes, however this pathogen was not the major cause of plant die-off, as it is considered mostly a disease of tuber appearance (HZPC info).

DNA-multiscan PCR analysis of the nutrient solution at the moment of plant die-off indicated major presence of the oomycete Pythium, and also confirmed Colletotrichum and Fusarium. According to UGent consultant HZPC these pathogens can only develop when the plants are weakened by stress.

The list of pathogens identified by the DNA-multiscan PCR analysis (Sciencia Terrae Diagnosecentrum) is reproduced. No beneficial organisms were identified.
-Colletotrichum accutatum, medium inf.
-Colletotrichum coccodes, medium infec.
-Fusarium oxysporum, medium infection
-Pythium dissotocum, strong infection

In order to avoid contamination in future experiments, we envisage the countermeasures explained in section 1.4.1.1 of this document.

4.3.2 Detailed observations

Leaf and tuber size were small as well as plant's stature. Tuber formation and growth will be followed-up in more detail.

4.3.3 Growth assessment

Fixed camera hourly picture logging and weekly manual photographing guarantee a sufficient level of documentation of crop development.

4.3.4 Gas exchange data

Preliminary assimilation data measurements over the time course of 1 to 2 days on attached small leaf cuvettes did not reveal significant differences at the leaf level for the 4 cultivars. The trial proved the measurement possible, but it presents little added value.
Transpiration data obtained during the same time course neither showed significant differences.

It is clear that gas exchange data should be obtained on a more continuous basis. This will be the case in the future PCU (Plant Characterisation Unit) environment. In the current set-ups, priority should rather be given to optimization of growth (e.g. nutrient solution composition) rather than further gas exchange experiments.

4.3.5 Extra plant physiological measurements

On-line plant weight determination needs further validation to guarantee more stable data.

4.4 Evaluation of crop harvest

4.4.1 Yield

Due to weak plant development induced by suboptimal starting quality in the bench test room (non-optimal transport, acclimation and propagation), and insufficient nitrogen and calcium provision during the tuberisation phase, plants were susceptible to opportunistic plant pathogens likely present in the environment.
The amount of harvest at UGent allowed nutritional analysis to be carried out at IPL. The protein level was unexpectedly high in comparison with field grown and database values.
The ratio edible/total plant dry weight (Tab. 1) is only trustworthy from the HZPC greenhouse test, since prior to harvest the UGent bench test plants displayed severe foliage loss and disintegration.

During BT1, Annabelle and Bintje provided the highest yield with respectively 660 and 583g/m^2. These results are still very low and due to inappropriate starting conditions and nutrient solution. Improving these two step would lead to a yield increase.

A major difference in yield is reported between the pre-tests and the bench test. The yield increased due to nutrient optimization. Furthermore the pre-test was performed in a simplified hydroponic set-up and not in a nutrient film system.

Tab. 10 Potato - Harvest results

		Annabelle	Bintje	Desiree	Innovator
Tuber harvest (kg)	HZPC 2008	1.872		1.141	0.676
	HZPC 2009	4.420	1.984	3.998	0.663
	UGent pretest 1, 2009		0.4	0.283	
	UGent pretest 2, 2009		0.249	0.418	
	UGent bench test 1, 2009	0.511	0.466	0.274	0.415
	UCL bench test 1 2009	0.662	0.546	0.299	0.283
Tuber harvest (kg/ m²)	HZPC 2008	2.5		1.52	0.9
	HZPC 2009	4.91	2.2	4.442	0.74
	UGent pretest 1, 2009		2.67	1.89	
	UGent pretest 2, 2009		1.66	2.79	
	UGent bench test 1, 2009	0.660	0.583	0.343	0.501
	UCL bench test 1 2009	0.829	0.683	0.374	0.355
Tuber harvest (g/ plant)	HZPC 2008	93.6		57.1	33.8
	HZPC 2009	184.2	82.7	166.6	27.6
	UGent pretest 1, 2009		133.6	94.5	
	UGent pretest 2, 2009		83.1	139.5	
	UGent bench test 1, 2009	34.1	29.1	17.2	27.2
	UCL bench test 1 2009	41.4	34.1	18.7	17.7
Number of (tubers/plant)	HZPC 2008				
	HZPC 2009	20.4	12.9	10.5	3.7
	UGent pretest 1, 2009		7	4	
	UGent pretest 2, 2009		6.3	3.3	
	UGent bench test 1, 2009	9.2	6.5	3.2	2.1
	UCL bench test 1 2009	4.6	4.6	3.6	1.4

Tab. 11 Harvest index for potato BT1

Measure-ment / Cultivar	Tuber FW (g/plant)	Shoot FW (g/plant)	Root FW (g/plant)	Stolon FW (g/plant)	Total FW (g/plant)	Tuber DW (g/plant)	Shoot DW (g/plant)	Root DW (g/plant)	Stolon DW (g/plant)	Total DW (g/plant)	% DW	Harvest index (based on DW)
HZPC 2008												
Annabelle	-	54,81	17,04		71,85	-	4,18	1,06		5,24	7,29	-
Bintje	-	-	-		-	-	-	-		-	-	-
Desiree	-	39,52	19,27		58,79	-	2,53	1,35		3,88	6,60	-
Innovator	-	28,91	8,38		37,29	-	2,13	0,50		2,63	7,04	-
HZPC 2009												
Annabelle	-	140,00	20,29		160,29	-	-	-		9,75	6,09	-
Bintje	-	79,00	8,21		87,21	-	-	-		5,75	6,59	-
Desiree	-	169,25	32,38		201,63	-	-	-		10,75	5,33	-
Innovator	-	37,50	2,96		40,46	-	-	-		3,50	8,65	-
UGent BT1												
Annabelle	34,10	-	-	-	-	6,00	1,99	0,21		8,20	-	**73,18**
Bintje	29,10	-	-	-	-	6,66	3,65	0,23		10,54	-	**63,20**
Desiree	17,20	-	-	-	-	2,84	4,03	0,49		7,36	-	**38,57**
Innovator	27,20	-	-	-	-	6,39	3,15	0,21		9,75	-	**65,55**
UCL BT1												
Annabelle	41,40	-	-	-	-	9,04	2,64	0,38	0,06	10,53	-	**85,85**
Bintje	34,10	27,80	6,80	4,80	73,50	6,82	2,68	0,35	0,34	8,07	-	**84,51**
Desiree	18,70	30,01	11,3	3,37	63,38	3,33	4,63	0,57	0,35	8,70	-	**38,28**
Innovator	17,70	-	-	-	-	2,57	1,92	0,19	0,11	3,52	-	**73,01**

4.4.2 Anti-nutritional compounds - alkaloid levels

Alkaloids were selected as the most important anti-nutritional factor to be monitored in the potato bench tests. Solanine is a mixture of two compounds, α-chaconine and α-solanine which are the major compounds to be considered. Therefor the total solanine level can be considered as the total glycoalcaloid content. Solanine levels (= α-chaconine + α-solanine) are set at a maximum of 0,2 mg/g (200 ppm or mg/kg). Total glycoalcaloid content (TGA) is similarly set at maximum 0,2 mg/g (fresh weight) potatoes for commercial tuber varieties (Friedman, 2006; Bushway 1981).
Light exposed potatoes can reach alkaloid level values over 1mg/g, which represents a health hazard. Greening is an indication of solanine buildup, although solanine can be produced without being linked to greening (e.g. by mechanical damage).
From the pre-selected HZPC cultivars, Annabelle is most and Innovator least resistant to greening under the influence of (low levels) of light.
Levels for Annabelle were between 34 and 60 mg/kg solanine, and between 54 and 71 mg/kg chaconine.
Preliminary results indicate that gradients in light level within a single gully setup (from 100 to 200micromoles/m^2.s) are not correlated with increasing glycoalcaloid levels in cultivar Annabelle, and rather showed an inverse correlation.
Innovator contained as expected the highest levels, with 97 mg/kg solanine and 123 mg/kg chaconine in one sample surpassing the officially agreed limit for safe consumption.

The level of TGA is thé criterion for safe human consumption; therefore further thorough research will need to be done on the influence of growth conditions on TGA levels in potato.

4.5 Cultivar selection method and ranking

The data obtained by UGent in bench test 1 are not sufficient, given the cultivation problems encountered, to enable a comparison with the table that summarises the diversity of the 4 pre-selected cultivars (see TN 98.3.1).
The HZPC greenhouse NFT hydroponic results from a comparative test indicate Innovator to be the slowest developing, which is corroborated by the initial data from UGent and UCL.
Annabelle and Desiree were the best performing as expected from the selection data as obtained from HZPC for TN 98.3.1 (See Tab. 4).
The 4.9 kg FW/m^2 maximal estimation (cultivated surface in HZPC greenhouse) for Annabelle compares to a maximum of 7 kg FW/m^2 as obtained in NASA studies (Mackowiak Adv. Space Res. 2007). UGent got, in a growth chamber hydroponics pre-test a yield of 2.8 kg FW/m^2.

Tab. 12 UGent - Potato cultivar comparison table

Cultivar	Tuber FW yield (HZPC hydroponics)	Tuber DW yield – (field)	Tuber size	Plant height	Maturity
Annabelle	Very high	Low 18,4%	Small	Medium-High	Very early
Bintje	Low	Medium to high	Medium to large	Medium	Early to intermediate
Desiree	Very high	21,40%	Large	Medium	Intermediate to late
Innovator	Medium	High 21,30%	Large	Medium to low	Early to intermediate

This technical note describes a single experiment. Therefore it is impossible to draw scientifically sound comparative conclusions from a chemical and nutritional point of view. Further experiments are needed.

4.6 References

Mackowiak, C. L., G. W. Stutte, et al. (1997). "Hydroponic potato production on nutrients derived from anaerobically-processed potato plant residues." Life Sciences: Life Support Systems Studies-I 20(10): 2017-2022.

Friedman, M., Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. J Agr Food Chem 2006, 54, 8655-8681.

5 Potato (UCL)

See section 4, hydroponic system basic components identical to UGent, starting plant material also identical, as provided by HZPC.

5.1 Evaluation of experimental layout

5.1.1 Measuring plan

Gas exchange and chlorophyll-fluorescence data scheduled every month.

5.1.2 Setup

No changes intended.

5.2 Evaluation of growth environment follow-up

5.2.1 Settings

Location of sensors confirmed.

5.2.2 Chamber T/RH evolution

No significant deviations recorded.

5.2.3 Chamber CO2 level

Measurements not foreseen.

5.2.4 Nutrient solution environment

NFT thickness follow up OK.

5.2.5 pH and EC evolution

pH was always kept below pH 6.5 and EC evolved between 1.5 and 2.1.

5.2.6 Nutrient solution T

Control confirmed as sufficient.

5.2.7 Nutrient solution analysis

A preliminary assessment of a mass balance of nitrogen of the nutrient solution was estimated. The nitrogen present in the plant was underestimated since the fallen leaves were not taken into account (exact DW not known).

Tab. 13 Estimation of the nitrogen (mg) available in the solution and the nitrogen (mg) present in the plant at the end of the experiment

cultivar	solution	plant
desiree	4418.83392	2387.04482
annabelle	4266.48862	2993.40562
bintje	4280.46984	1532.71438
innovator	4059.36297	1061.58413

All the nitrogen present in the solution was not present in the plants at the end of the experiment suggesting that a part of the available nitrogen was used by microorganisms present in the solution.

5.3 Evaluation of monitoring of plant development

5.3.1 Photographic follow-up

See 5.3.3

Fungal symptoms were observed, and microscopical observation confirmed *Colletotrichum coccodes*

5.3.2 Detailed photographic observations

See 5.3.3

5.3.3 Growth assessment

Due to the phytosanitary problems, the cultivar comparison by the used parameters might not be reproducible for the later timepoints.
Overall the Innovator cultivar develops slowest. Bintje has the lowest number of branches and leaves, but produces a lot of stolons.

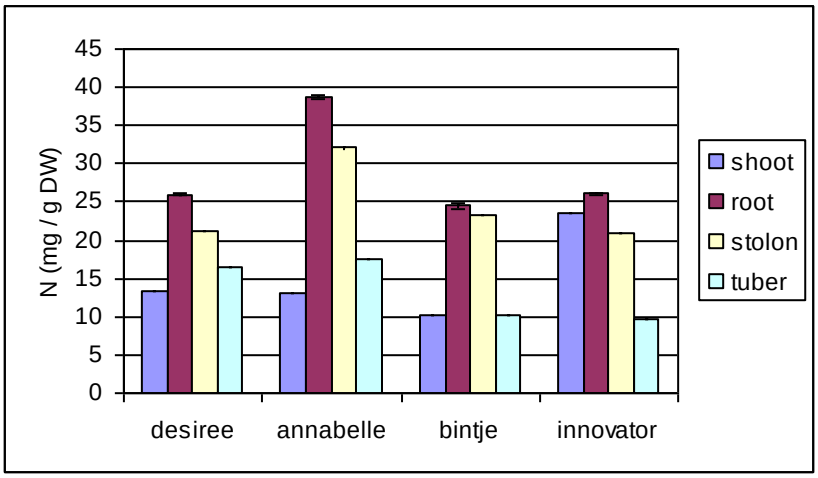


Fig. 9 UCL - Nitrogen content in BT1's plants

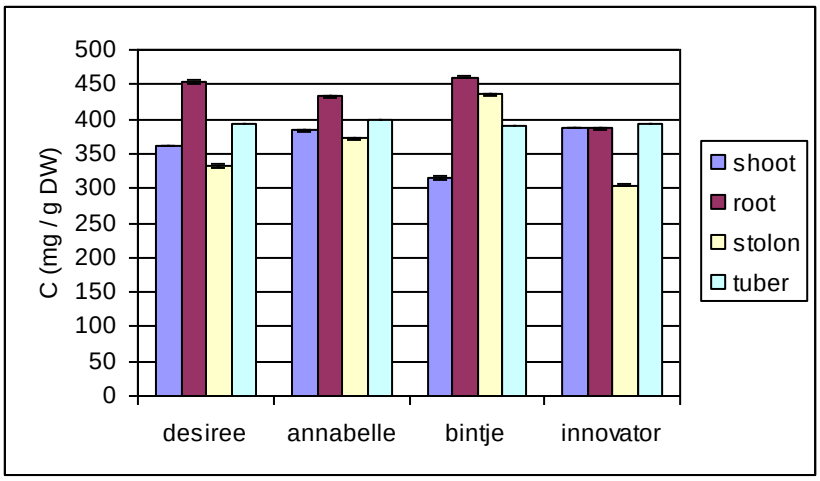


Fig. 10 UCL - Carbon content in BT1's plants

5.3.4 Gas exchange data, water usage

Water use efficiency, expressed as amount of biomass produced per litter of water transpired, is a critical parameter in agriculture when irrigation is limited.
Amount of water transpired can also be of interest in a CELSS context for production of potable water.

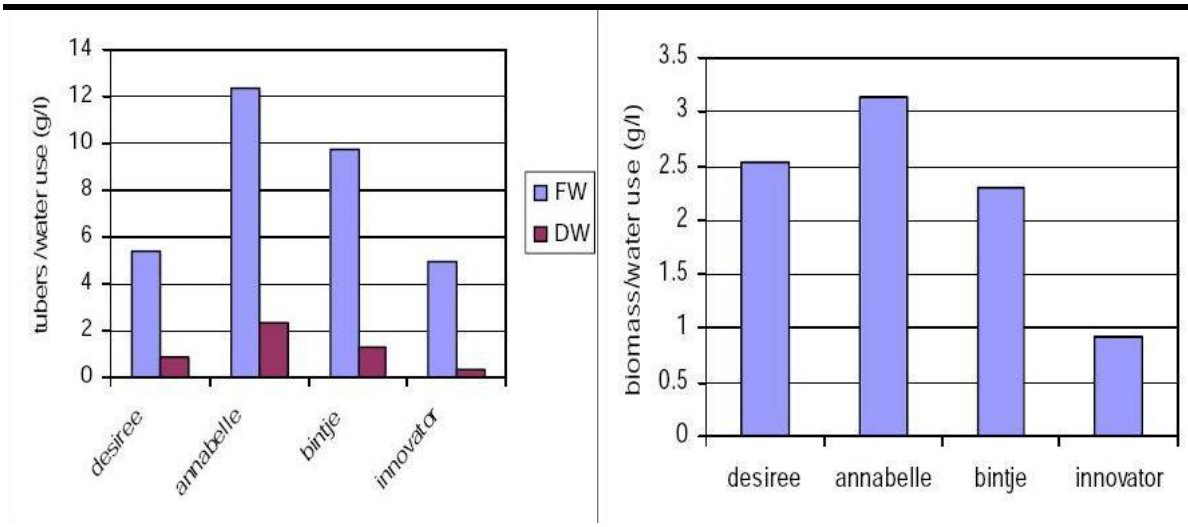


Fig. 11 UCL - Potato water use efficiency

5.4 Evaluation of crop harvest

See also section 4.4

From the UCL bench test, the Annabelle cultivar produced the largest amount of tubers compared to the inedible plant part (on a DW basis).

5.5 Cultivar selection method and ranking

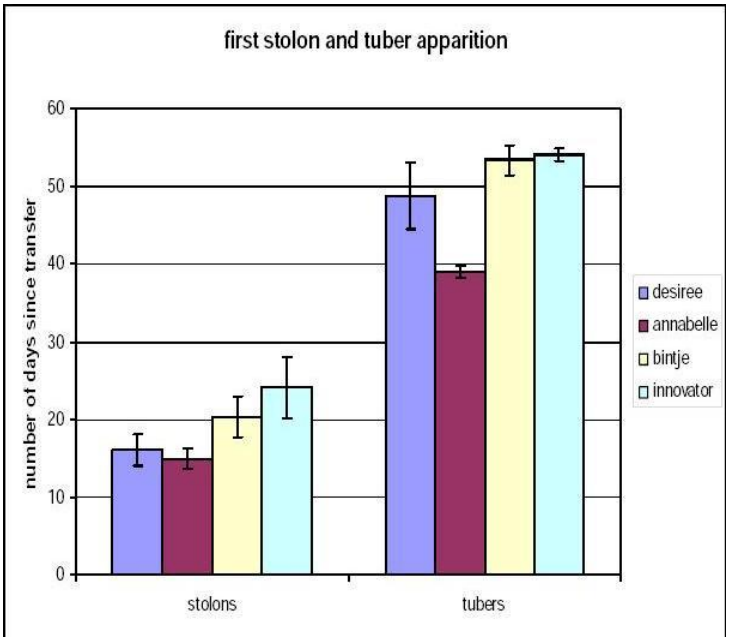
See also section 4.5

Given the cultivation problems encountered, the data obtained by UCL in bench test 1 are not sufficient to enable a comparison with the table (table 9) that summarises the diversity of the 4 pre-selected cultivars.

Our observations confirmed that Annabelle is the most precocious cultivar and Innovator the latest.
Annabelle is also the highest yielding cultivar (Tab. 10), but DW is lower than in other cultivars.

The other selection criteria are based on diversity, including processability. There are no strong differences between cultivars according to nutritional analysis (Table 29 in TN 98.4.21). Annabelle and Bintje produced nevertheless less solanine and chaconine (antinutritional compounds) than Desiree and Innovator.

Fig. 12 UCL - Potato stolon and tuber initiation times – precocity parameter



5.6 Conclusions

The NFT-systems function properly and the set-up system of BT1 will be maintained in BT2. Concerning the solution composition, the amount of nitrate available per plant must be better controlled. For BT2, the amount of nitrate added during the tuberisation phase must be reduced and it must be added on a more regular basis. The extra addition of micronutrient will be limited in BT2 to remediate to the high Zn levels.
Development of pathogens will be followed up and in case of pathogen development, the plants will be treated to avoid plant death as in BT1.
The gas exchange and chlorophyll fluorescence analysis will be planned once per month in BT2 to reduce the damages to the leaves due to these measurements.
Due to the cultivation problems encountered, the data obtained by UCL in BT1 are not sufficient to enable clear conclusions. Annabelle and Bintje were the best performing cultivars in terms of tuber production and are the most promising ones.

6 Soybean (UNapoli)

6.1 Evaluation of experimental layout

6.1.1 Measuring plan

6.1.2 Setup

The hydroponics Nutrient Film Technique (NFT) system consisted of 12 independent double gullies. In BT1, 3 soybean cultivar were tested (Atlantic, Regir, PR91M10).
3 double gullies (corresponding to 42 plants) were used for each cultivar, in a randomized experimental layout.

6.2 Evaluation of growth environment follow-up

6.2.1 Settings

Tab. 14 UNapoli - Settings

Photoperiod	12-h Long Day
Light intensity	600 µmol m^{-2} s^{-1}
Room temperature Humidity	20/26 °C (Night/Day) 65-75% (set point 70%)

6.2.2 Chamber T/RH evolution

Temperature and Relative Humidity values were always within the target intervals.

6.2.3 Chamber CO2 level

CO_2 air concentration was at the ambient level and it was monitored in the daytime, during the gas exchange measurements.
UNapoli growth chamber was not sealed. On the contrary, periodic changes of the internal air were performed by two small fans (for suction and extraction), placed at the top and at the bottom of one wall.
This implies that no correlation can be done between air CO_2 concentration and assimilation rate.

6.2.4 Nutrient solution environment

Gully inclination and flow rate will be kept for BT2. The nutrient solution will have to be improved in order to discard nutrient deficiency like observed in BT1. This will also lead to an increase of the EC.

The nutrient solution composition showed alternation of accumulation and depletion of nutrients, especially for NO_3 and K. This time course was probably due to the problems in plant growth: plants were not able to constantly adsorb nutrients from the solution.

6.2.5 pH and EC evolution

The strategy for nutrient solution management was very efficient in keeping the EC values within the target interval.
As expected, solution pH tended to rise to alkaline level, requiring acid for pH control during the whole cycle.

6.2.6 Nutrient solution T

Not critical for soybean.

6.2.7 Nutrient solution analysis

Correlate with possible nutrient deficiency signatures.
A phyto-sanitary problem occurred during the cultivation. Particularly, some brown spots appeared on the leaves, with higher frequency in younger leaves, and, within a few days, the surrounding leaf area turned yellow. In the most serious cases, the affected leaves died prematurely because of the broad necrosis. This problem occurred in the most sensitive developmental stage: the soybean pod formation. According to pathology specialists, the main problems seem to be determined by nutrient deficiency (probably Mn) due to pH fluctuations, even though possible subsequent infections occurred.

6.2.8 Photographic follow-up

Overview of plant development for 1 gully per cultivar.

6.2.9 Detailed photographic observations

Flowering and pod-set time points for the 4 cultivars.
Phyto-sanitary problems: necrosis was observed.
Cultivars showed similar time for flowering and pods formation. Flowering started 56 Days After Sowing (DAS) and the beginning of pods formation started 65 DAS.

6.2.10 Growth assessment

Cultivar differences to be specified.
Plant height was different among the cultivar, particularly Pr91m10 formed the highest plants. Moreover, this cultivar showed the highest number of leaves and the highest leaf area, while Atlantic had leaves with smallest size, with a consequent lower plant leaf surface.

Due to the nutrient deficiency, measurements of plant growth were not reliable.

6.2.11 Gas exchange data

The rate of net photosynthesis and transpiration at the leaf level was lower compared to the values reported in literature for soybean. As explained, this result is related to the plant health problems.

6.3 Evaluation of monitoring of plant development

Data about yield and harvest index (HI, calculated as DM of seeds/DM total) are not reliable because of the nutrient deficiency problems and the reduced plant growth. More useful information can be obtained by the performances of the selected cultivars in the BT2.

	HI
Atlantic	0.16±0.052
Pr91m10	0.07±0.039
Regir	0.02±0.002

Tab. 15 Nutritional composition of soybean seeds obtained in BT1 and data for soybean traditionally grown in open field.

Soybean Cultivar	**Atlantic**		**PR91M10**		**Regir**	
Value	BT1	field	BT1	field	BT1	field
Sum proximate FW Harvest	100	100	100	100		100
Water (%)	19.08	10.19	15.15	6.93	18.18	6.53
Protein (%)	29.80	29.17	35.61	32.83	-	30.40
Fat (%)	13.62	14.82	10.83	15.54	-	15.88
TDF (%)	22.40	19.50	25.14	20.56	-	22.13
Carbohydrates (%) [by difference]	14.79	25.71	13.01	23.10	-	24.53
Minerals (%) [ash]	0.31	0.61	0.25	1.04	-	0.53
N (%) [Protein = N * 5.7]	5.23	5.12	6.25	5.76	-	5.33
Crop specific compounds						
Phytic acid (%)	1.27	1.26	1.16	1.13	-	0.84
Total isoflavones (%)	0.17	0.11	0.07	0.10	0.09	0.17

Seeds obtained in BT1 in hydroponics shows higher protein and total dietary fiber contents but lower fat and mineral contents, respect to the field control. Phytic acid content does not vary greatly and total isoflavones content shows different trends in different cultivars.

In Atlantic, total isoflavones content increases in BT1 but in PR91M10 and in Regir seeds total isoflavones content is lower in BT1 then in field control. In BT1, Atlantic presents the higher fat and mineral content.
In conclusion, PR91M10 can be considered the best cultivar, for the high protein content and the lower dietary fiber and phytic acid content.

6.4 Cultivar selection method and ranking

Parameters not considered in BT1 and BT2:
- Volatile organic compound (VOC) production and Root exudates production
- Resistance to stress (plants during bench test should be cultivated under optimal conditions)

From Table 23 from TN 98.3.1, page 68 the selection criteria applicable to the bench test data are shown below.

Tab. 16 UNapoli - Soybean cultivar bench test selection criteria

Criteria	Major parameter(s)	Associated parameter
Crop cultivar stature	Growth space	Handling (harvest)
	Growth period length	Crop senescence
Cultivar harvest index	Waste production	Waste degradability
	Influence of plant growth system	
Cultivar nutritional composition	Absence of anti-nutritional compounds	Pro-nutritional compounds
Cultivar edible part composition	Processability	Possible conflict with levels of pro-nutritionals
	Storage stability	Storage time
Water use efficiency	Maximum water use Increases growth efficiency	High water turnover rate – regeneration rate

Tab. 17 UNapoli: Soybean cultivar comparison table

This table is based on the final score obtained by each cultivar after the application of the theoretical procedure reported in TN 98.3.1 and four main characteristics basic for cultivation and nutritional value.

Cultivars	Final Score	Yield (t/ha)	Height (cm)	Protein content (%)	Earliness (days)
Pr91m10	**30.75**	3.3	81	high	105
Regir	**23.42**	4.5	90	38.6	120
Atlantic	**23.05**	4.3	90	37.6	120
Cresir	**22.37**	3.9	100	data not available	105

7 Conclusion

A summary of preliminary conclusions from the first bench test including the most important 'lessons learned' can be found below are:

- Newly built NFT-systems function properly, nutrient control management needs to be optimised for future experiments.
- Sealed chamber tests at UoGuelph deliver continuous assimilation and evapotranspiration data, but ethylene and oxygen accumulation need to be limited. With the current hardware available, the approach consists of scheduled venting (opening of the access door) of the chamber.
- The conventional walk-in room setups can obtain a total evapotranspiration amount per cultivar based on water usage, as recorded from the hydroponic tank (manual) refill adjustments.
- Maturation of wheat crops is a critical issue, and implies modulation of the nutrient solution composition. Some bread wheat cultivars seem relatively insensitive to the high N-levels. This issue needs further thorough investigation.
- The need for nutrient solution composition changes upon crop development precludes the approach of staggered growth with a common hydroponic feeding system, where all gullies regardless of the developmental stage of the plants growing in it, would receive the same nutrient mix.
- Harmonised psychopathological and pest management protocols would enable efficient and reproducible control of the most common stresses in future test trials.
- Wheat culture start-up benefits from 3 days of germination in optimal conditions, to allow a start with germinated seeds that would all develop to mature plants.
- Nitrogen level in the starting nutrient solution of UGent and UCL was inappropriate. Furthermore, nitrogen should have also been regularly added after tuber set to favour bulking and keep plants vigorous.
- From the available data up to now a yield of 0.5kg DW / m2 is indicative (to be confirmed for soybean and potato in the FC1 bench test setups). Given 20% DW in harvested potato, a yield of 2.5 kg at harvest would be needed.

As this technical note describes a single experiment, it is impossible to draw scientifically sound comparative conclusions from a chemical and nutritional point of view. Further experiments are needed.