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1. PhD project description

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This PhD is part of the ClimaFruit project (European Regional Development Funded project). It will provide underpinning knowledge for growers to be able to sustain both productivity and quality when climatic conditions become limiting. The project will evaluate the ability of elite North region (Scandinavia and United Kingdom) blackcurrant cultivars to cope with water stress during flower induction or flowering and to recover after a period of drought stress by looking at plant water relations, flowering and root growth, and will also look at gene expression in stress conditions.

Introduction

Changing climatic conditions are becoming a limiting factor in the sustainable production of blackcurrants from existing commercial cultivars in Denmark. The overall aim of this project is to identify elite cultivars that have adaptive and phenotypic plasticity and are therefore more likely to remain productive during unfavourable weather conditions. This will be achieved by identifying specific traits such as an ability to maintain productivity and quality, and increase water use efficiency during and after drought stress.

Background – state of the art

In Denmark blackcurrants are commercially produced in the field. During the last 5 growing seasons more extreme weather conditions have had a negative impact on the productivity and sustainability of the crop. Weather conditions that currently threaten the production of blackcurrants in Denmark are extended periods of droughts, or heavy rain during the production season and/or lack of winter chilling (1).

There is a large diversity in the genetic material available in blackcurrants; many different cultivars are grown in different countries to fit the global production (2). Traditionally selections are chosen based on productivity and disease resistance (3). However, with changing climatic conditions and reduced productivity, breeding material needs to be re-evaluated for increased adaptive and phenotypic plasticity (4, 5). The term plasticity reflects the ability of a plant to alter its phenotype under changing conditions, plasticity in plant growth also reflects the genotype by environment interaction (6). Therefore, as climatic conditions become unpredictable it is

important to grow plant material that has plastic or adaptive traits so that they can cope with extreme drought and retain and maintain a commercial crop. These traits will become important given the increased frequency of extreme weather events.

Exploitation of germplasm to identify the genetic diversity in response to extreme climatic conditions (drought stress) has not been investigated in blackcurrants. There is significant potential to select cultivars with an increased ability to respond to and recover from stressful conditions (7). Cultivars with an ability to modify root systems as visualised by root distribution and root density are therefore likely to adjust to water and nutrient uptake (8, 9).

Short project description with hypothesis and aim

The overall aim of this project is to identify elite cultivars that have adaptive and phenotypic plasticity and therefore are more likely to remain productive during unfavourable weather conditions. This will be achieved by identifying specific traits such as an ability to maintain productivity and quality during and after drought stress.

The project will more specifically investigate root and shoot growth as well as flowering in different elite selections of blackcurrants, grown under limited/irregular water availability under protected culture. The response of plant material will be evaluated from a physiological and genetic perspective.

Drought conditions may result in differences between cultivars in evapotranspiration, stomatal conductance and stomata limitation, reduction in leaf water potential and changes in osmotic potential and turgor. Water deficit may result in reduced biomass production and therefore a reduced leaf area, fresh and dry weight, water use efficiency and finally, a reduction in production. Differences in root growth under and during recovery from reduced water availability can be measured using root window observations for quantification of root growth, WinRHIZO for estimation of total root length and root diameter classes and ¹⁵N application for estimation of root N uptake activity. The difference in gene expression between the stressed and unstressed samples will be evaluated using Agilent microarrays, and should enable the identification of some of the key genes involved in water stress tolerance.

More specific hypotheses are:

- Water use efficiency is expected to increase under drought stress but to different degrees dependent on blackcurrant cultivar.
- Blackcurrant genotypes respond differently to reduced water availability with regard to plant water status and gene expression.
- Root density, regrowth ability and root uptake activity of elite blackcurrant cultivars are phenotypic traits that are significantly correlated to growth plasticity during conditions of drought or water stress.
- Plant water relations are related to phenotypic and genotypic growth plasticity during water stress.

Industrial interests in the project

- The industry will gain basic knowledge on the potential to breed new cultivars more suited to climate extremes (drought stress) thereby ensuring a sustainable production of blackcurrants in future.
- Increased knowledge about the effects of drought stress on blackcurrant production and how to maintain a high productivity, uniformity and quality.
- Knowledge from the project will increase our overall understanding of how plants respond to and recover from drought conditions. More specifically the impact of restricted water availability during flowering and after the harvest period during flower induction on productivity will be determined.

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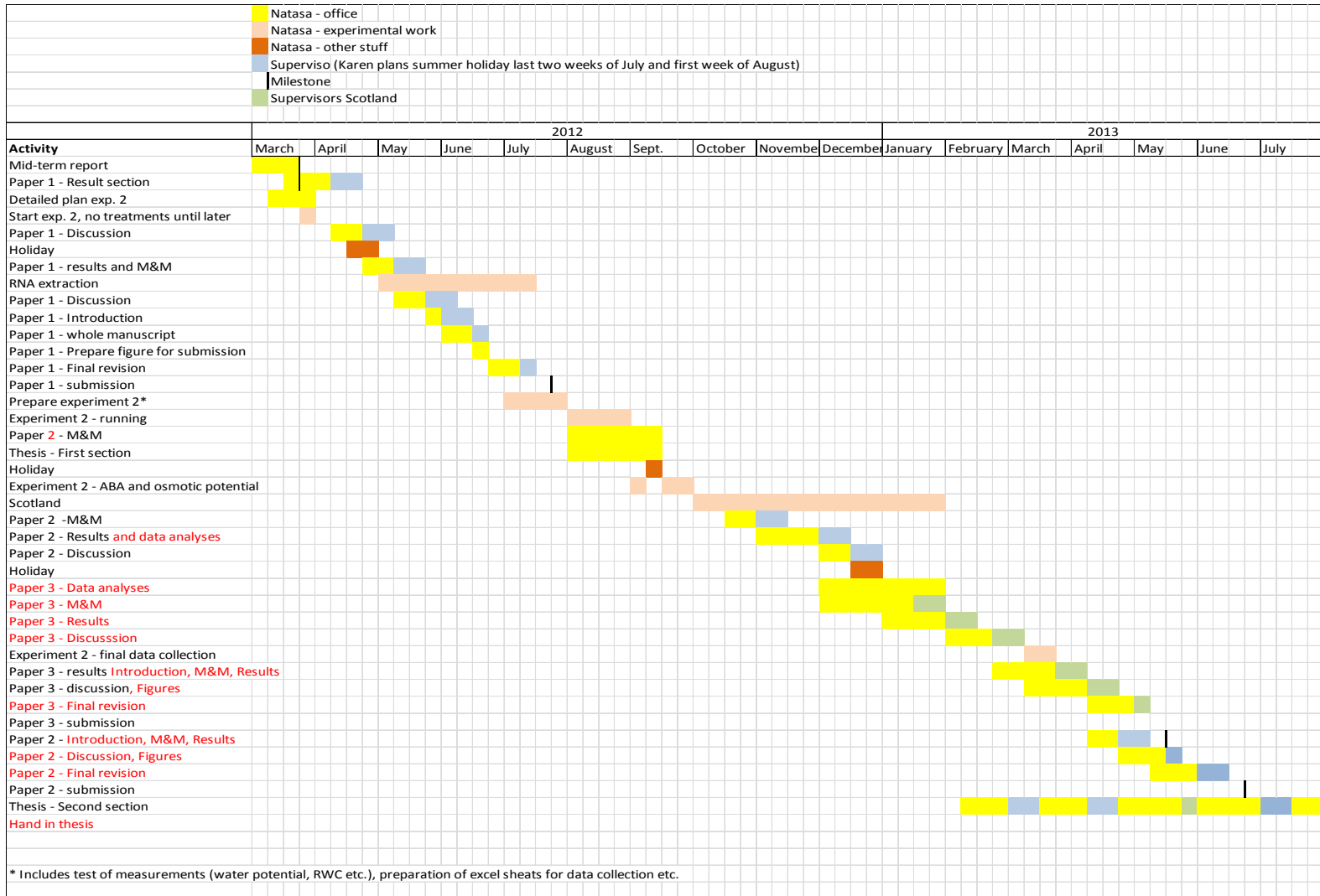
2. Progress so far and plan for remaining PhD period

TIME	EXPERIMENTAL WORK	OFFICE
Aug-Dec 2010		Literature study, PhD courses
Jan-Feb 2011	Pre-trial to estimate flowering time, evapotranspiration (ET_c), stomata conductance (g_s) data analyses from pre-trial experiment	Literature study, PhD courses
Mar-Apr 2011	Experiment I to determine drought stress during flowering, water status, growth, flower abortion and root and top regrowth ET_c , g_s , ^{15}N uptake, basal and stem shoot growth	Data analyses of g_s from experiment I
May-Aug 2011	Preparing minirhizotron tubes for field trial, washing roots and preparing for WinRHIZO from experiment I	PhD courses Data analyses
Sep-Oct 2011	Preparing samples C and ^{15}N	Data analyses
Nov-Dec 2011	osmotic potential WinRHIZO	PhD course Data analyses
Jan-Mar 2012	osmotic potential	Data analyses

Deviations from original PhD plan

In the original PhD plan three experiments were planned: one in greenhouse, one outdoors in pots to follow the plants from the greenhouse experiment and one field experiment. A greenhouse experiment (exp. 1), modified compared to the original plan to be manageable for one PhD-student, has been performed during the spring 2011 and one lot of plants were further evaluated after transfer to outdoor conditions. A field experiment was established (2011) with minirhizotron tubes and tubes for measuring soil moisture content. But, due to heavy precipitation during most of the 2011 growing season, no differences in growth can be expected so far. Therefore a second greenhouse experiment is planned to run during the autumn 2012, in order to have enough data to publish within the timeframe of the PhD period.

Milestones for remaining 1^{1/2} year PhD plan is presented in a table on the page 6.



3. Preliminary results

Effects of water stress during flowering of two pot grown blackcurrant (*Ribes nigrum* L) cultivars

Abstract

Two cultivars of blackcurrant (*Ribes nigrum* L.), ‘Narve Viking’ and ‘Ben Gairn’, were studied to evaluate growth responses to drought stress during flowering and during a recovery phase to identify if there is any significant difference in physiological responses between the cultivars.

Plants were selected based on growth vigor and differences in genetic background and grown in 3 l pots sandy loam soil mixture until the beginning of flowering where irrigation treatments were started. Plants were either hand irrigated with a volume replacing the previous day’s evapotranspiration (FI) or not irrigated (NI) for 12 days. Hereafter irrigation was continued of all plants to allow for 17 days of recovery.

Water limitation severely decreased the evapotranspiration and leaf anatomy. Stomatal conductance was highly influenced with drought stress, but also with daily temperature and VPD, time of measurements and cultivar difference. Plant water status was highly influenced by the day of measurement. Leaf water potential and osmotic potential significantly decreased during a twelve days of withholding water. Turgor remains positive, except the day 12, when leaves for both cultivar lost turgor and wilt.

Aboveground plant growth was evaluated by leaf area and fresh and dry weight of flowers, leaves and stems at the end of the drought treatment and after one and two weeks of recovery. Basal and stem shoot growth were recorder during drought stress period.

Roots were quantified at the vertical soil surface, feeding a stable isotope ^{15}N to the roots showed root nitrogen uptake activity, then extracted from the soil to estimate total root length and distribution in diameter classes by WinRHIZO analysis.

The results indicate that ‘Ben Gairn’ was more tolerant to drought stress than ‘Narve Viking’ due to a faster recovery of root growth and less reduction in growth of aerial parts during the drought treatment.

Keywords: *Ribes nigrum*; Water stress; Evapotranspiration; Plant water status; Root recovery

Introduction

Drought is one of the most important abiotic stresses that cause significant reduction in crop yield. The response of plants to water deficit varies with species. Plants have three main defences against drought stress: the first defence is a leaf area reduction, which results in reduced transpiration. The second is deeper root growth; which can allow water uptake from deeper soil zones that still have moisture. Stomata closure is third defence which serves to reduce

evaporation from the already existing leaf area (Taiz and Zeiger, 2002). Levitt in 1972 classified such mechanisms of plant response to drought stress as drought tolerance and drought avoidance which have long been considered to be important strategies in drought adaptation (Chaves et al., 2003, Blum 2011). In the concept of drought avoidance, plants use mechanisms like leaf movement, stomata closure and large root system in order to maximize water acquisition and reduce transpiration to be able to maintain higher water potential under water deficit condition. Drought tolerance use mechanisms such as osmotic adjustment and cell membrane stability that allow metabolism to function under low water potential (Blum 2011).

Drought conditions may results in difference between cultivars in evapotranspiration, stomata conductance and stomata limitation, reduction in leaf water potential and changes in osmotic potential and turgor. Water deficit results in foliage area biomass resulting reduce in leaf area, fresh and dry weight, water use efficiency and finally, a reduction in production (Asraf and O Leary, 1996; Chartzoulakis et al., 2002; Liu et al., 2006; Guerfel et al., 2009; Wang et al., 2009; Álvarez et al., 2011; Mahdid et al., 2011)

Weather conditions that currently threaten the production of blackcurrants in Denmark are extended periods of droughts, or heavy rain during the production season and/or lack of winter chilling (1) have had a negative impact on the productivity and sustainability of the crop.

Therefore, as climatic conditions become unpredictable it is important to grow plant material that has plastic or adaptive traits so that they can cope with extreme weather conditions and maintain a commercial crop. Specific traits for blackcurrants will become increasingly important given the increased frequency of extreme weather events (Heide and Sonsteby, 2010; Sonsteby et al., 2011).

In future, episodes of drought stress may be more frequent during the flowering period of blackcurrants due to climate change, but little is known about how different blackcurrant cultivars recover during growth after drought (Wilson and Jones, 1980; Cosmulescu et al., 2008). Therefore, the aim of this study is to investigate the effect of stress response in a twelve days and recovery phases of two weeks in a several anatomical and physiological parameters of the two important Scandinavian and UK cultivar, 'Ben Gairn' and 'Narve Viking'. Specifically, we study these two cultivars to identify adaptive and phenotypic adaptations that reduce water loss and are therefore more likely to remain productive during unfavourable weather conditions.

Material and methods

Plant material and growing conditions

A pot experiment was conducted in a greenhouse conditions at The Department of Food Science, Faculty of Science and Technology, Aarslev, Aarhus University, Denmark in April 2011 under optimal temperature, light, wind and nutrient conditions. Two blackcurrant (*Ribes nigrum L.*) cultivars 'Ben Gairn' and 'Narve Viking' were selected based on growth vigor and differences in genetic background. One year old cold stored plants propagated from cuttings were grown in 3 l pots in a sandy loam soil mixture from 13th of January until the beginning of flowering where irrigation treatments were started (6th of April).

Evaluations of blackcurrant cultivars focussed on phases of drought response of the plants during a 12 day period and seventeen days of recovery phase.

Experimental design

A split plot design was used with drought levels as the main treatments, cultivars as sub treatments, and four replications. On the beginning of the experiment, each cultivar was placed on separately table and mixed them randomize inside each of this table. According to the days of drought stress, we have inside of each table 6 blocks randomized. In a one block at day 0 we had 4 plants, while in other 5 blocks we have 8 plants, 4 fully irrigated and 4 non irrigated. Every time after finishing a day of drought treatment, we placed the pots on the free table completely randomized and irrigated in order to recovery.

Evapotranspiration and water treatment

Irrigation for plants in recovery phases was managed once per day. From the day of potting all plants were irrigated on a daily basis in order to maintain 100% water holding capacity. During the period of treatment (flowering time) two different water availabilities were established: 1. full irrigated (FI) and 2. non irrigated (NI) for 0, 2, 5, 7, 9 and 12 days followed by recovery at full irrigation.

Evapotranspiration, as well irrigation for control plants, and drying out was recorded on a daily basis with an electronic weighting scale, for each pot individually. Full irrigated plants were given water individually every day in the late afternoon according to the weight loss that we maintained at pot water holding capacity. For the plants that were exposed to drought (non irrigated treatment), we recorded the water loss every day.

Plant water relations

Stomata conductance of four plant per treatment was measured on the days of drought treatment (0, 2, 5, 7, 9 and 12) with a portable porometer (steady state porometer PMR-2, PP-Systems, UK) in the morning between 9-10 am (m) and afternoon - midday between 12:30-13:30 pm (a) and a second fully expanded upper canopy leaf were used per pot recording a three times. Midday leaf water potential (Ψ_l) and osmotic potential (Ψ_π) were measured on the same leaf using a pressure chamber and dew point microvoltmeter, respectively. After stomatal conductance and leaf water potential measurements

each leaf was immediately wrapped in aluminum foil, frozen in liquid nitrogen, and transferred to a -80°C freezer for subsequent leaf osmotic potential determination. Turgor potential (Ψ_T) was determined by the difference $\Psi_T = \Psi_\pi - \Psi_l$.

“Root window” method for quantification of roots at the vertical soil surface

Counting is performed at the end of drought treatment, one week of recovery and end of experiment (two weeks of recovery). A window is cut at a random place of the pot wall with minimal damage of roots by cutting the upper vertical edge and tearing the window downwards. The grid (4 x 4 cm cross) on transparent plastic is attached, 4 cm from the pot bottom. Roots touching the horizontal and vertical lines are counted. At the opposite side of the pot a similar

window is cut, grid attached and roots counted. Grids are removed and windows closed by tape until next counting.

¹⁵N experiment at the end of experiment for estimation of root N uptake activity

1 day before destructive sampling of plant shoots, 10 ml of solution of $\text{Ca}(\text{}^{15}\text{NO}_3)_2$ enriched 98% with ¹⁵N is injected into 10 cm depth in each pot in 4 holes. After e.g. 48 hours (exactly same period after injection for each pot), the shoot is harvested and dried at maximally 80°C for 24 hours and weighed for dry matter. Then store in plastic bags. One non enriched background sample per each cultivar was used. Later the samples are grinded, packed in tin capsules making a replicate samples and sent for ¹⁵N analysis at the US Davis facility.

Estimation of total root length and diameter classes by root extraction and WinRHIZO analysis

After harvesting shoot samples in the ¹⁵N experiment, the pots are stored cold. Within a few days, the roots from each pot are extracted by washing and collecting all roots on a sieve with 250 µm mesh size in the setup in the vegetable working room. The root samples are stored cold in water, coloured (solution) and then analyzed for total root length and diameter classes by use of the WinRHIZO software. The root samples are then dried and weighed for total root biomass. The root countings at the end of the experiment from the “root window method” are compared with the total root length and biomass measurements from the WinRHIZO.

Measurement of plants biomass after drought stress and recovery phases

The plants were harvested on 18th April and 5th of May after drought stress period and after ¹⁵N experiment, respectively. During harvest each plant organ was divided and flower, leaf, stem and root (only from recovery phases) and drying in an oven at 70°C for 48h to constant weight. The leaf area (LA) was measured after the twelve days of drought stress using leaf area meter.

Data analyses and statistic

A general linear model test was used to examine influence of the cultivar and irrigation treatments on two blackcurrant cultivars (SAS 8.02, Cary, NC, USA). In the case of stomatal conductance logarithm of g_s data ($\log g_s$) was used to examine cultivar, time of measurements and day of measurements and water availability treatment effects on stomatal conductance. Standard errors of the means (SE) were calculated. Tukey’s Studentised Range (HSD) Test was applied to separate measured parameters of the plants exposed to different irrigation regimes.

Results

Evapotranspiration

Water limitation for a period of 12 days during flowering and unfolding of leaves severely decreased the evapotranspiration and leaf area in blackcurrants (Fig.1, Table 1). After 3 to 5 days, the evapotranspiration for non-irrigated plants decreased as plants attempted to conserve water and closed stomata (Fig.2).

When grown at full irrigation (FI) the evapotranspiration from 'Narve Viking' was for the experimental period of twelve days an average of $2237.80 \text{ g plant}^{-1}$, but for 'Ben Gairn' only $1864.85 \text{ g plant}^{-1}$ (Fig.1) partly due to a smaller leaf area of 1385.02 cm^2 for 'Ben Gairn' compared with 1733.81 cm^2 for 'Narve Viking'. If irrigation was withheld for twelve days (NI) the evapotranspiration was almost the same in the two cultivars $683.70 \text{ g plant}^{-1}$ and $696.84 \text{ g plant}^{-1}$ for 'Narve Viking' and 'Ben Gairn' respectively. This indicates that almost no plant available water was left in the soil after 12 days of NI.

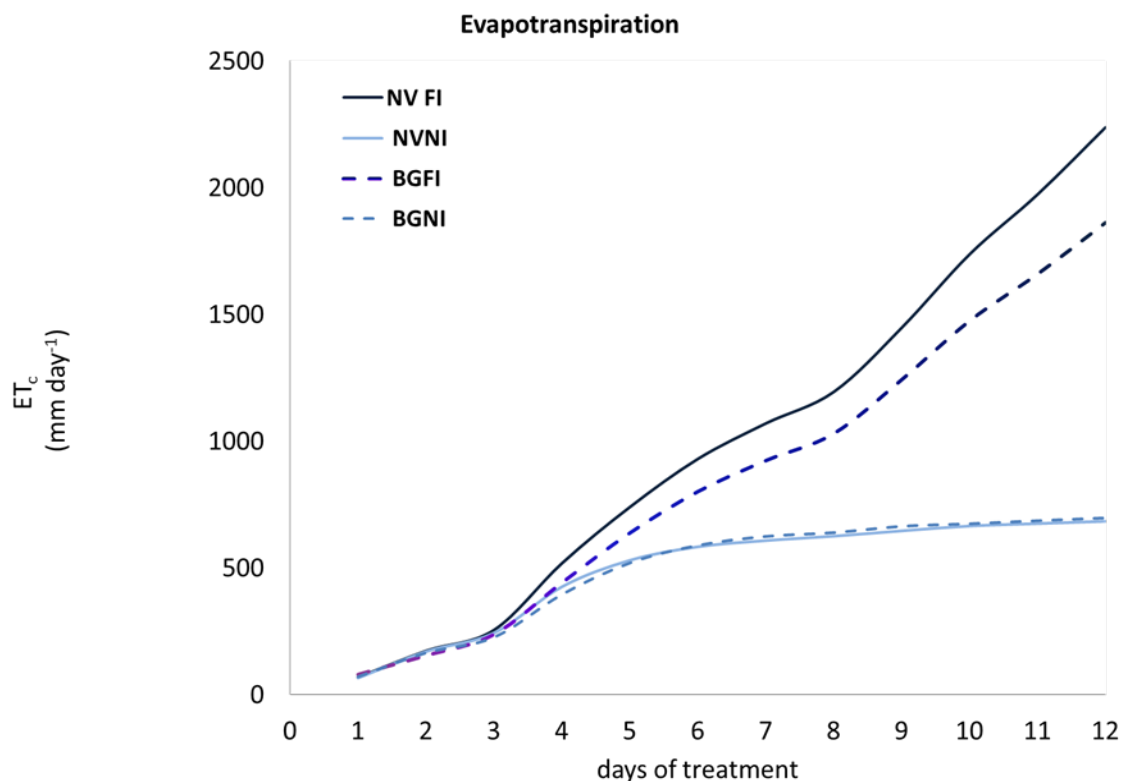


Figure 1: Accumulated evapotranspiration (ET_c) of fully irrigated (FI) and non-irrigated (NI) plants, of 'Narve Viking' (NI) and 'Ben Gairn' (BG) over a 12 day period

The leaf area, leaf dry weight and water content determined at the end of the drought stress period of 12 days were significantly lower for NI plants compared with FI plants of both cultivars whereas differences between cultivars were not significant (Table 1 and 2). In none irrigated 'Narve Viking' the specific leaf area showed a significant decrease but the highest water use efficiency (Table 1). The WUE in 'Ben Gairn' also tended to increase for NI plants.

Table 1. Effects of full irrigation (FI) or no irrigation (NO) on total leaf area, leaf water content, specific leaf area and water use efficiency (WUE) in two blackcurrant cultivars 'Ben Gairn' (BG) and 'Narve Viking' (NV) after 12 days of treatment. Values with the same letters within a column are not significantly different at $P < 0.05$ according to Tukey's test (n=4).

Cultivar/ treatment	Total leaf area (cm ²)	Leaf water content (g g ⁻¹ DW)	Specific leaf area (cm ² g ⁻¹ DW)	WUE (g g ⁻¹ DW)
BGFI	1385.02 ^a	3.06 ^a	225.18 ^{ab}	0.77 ^b
BGNI	623.87 ^b	2.41 ^b	217.20 ^{ab}	1.31 ^b
NVFI	1733.81 ^a	3.45 ^a	256.90 ^a	0.94 ^b
NVNI	576.43 ^b	2.04 ^b	195.62 ^b	2.33 ^a

Drought stress significantly decreased dry mass production after twelve days of withholding water and 'Ben Gairn' showed better leaf, stem and root recovery after seventeen days of recovery, when plants were fully irrigated, while flowers for both cultivars were severely damaged. The root dry weight, as well as the root length, after recovery were significantly higher for FI plants of 'Narve Viking' compared with NI plants (Table 2 and 6). In 'Ben Gairn' root dry weight and root length were not significantly affected in NI plants. Drought stress significantly reduced and influenced a total plant dry weight for 'Narve Viking' (Table 2 and 6).

Table 2. Dry mass distribution between different organs of blackcurrant cultivars 'Ben Gairn' (BG) and 'Narve Viking' (NV) (flower, leaf and stem) after 12 days of no (NI) or full irrigation (FI) and after seventeen days of recovery at FI (flower, leaf and stem, root). Different letters within the same column indicate significant differences according to Tukey's Test at $P < 0.05$ (n=4).

Cultivar/treatment	12 days of drought stress		Dry mass (g plant ⁻¹)			
			Flower	Leaf	Stem	Shoot
BGFI			0.33 ^{ab}	6.14 ^a	9.63 ^{ab}	16.10 ^{ab}
BGNI			0.09 ^b	2.88 ^b	6.25 ^b	9.22 ^b
NVFI			0.56 ^a	6.76 ^a	15.27 ^a	22.59 ^a
NVNI			0.14 ^b	3.01 ^b	12.06 ^{ab}	15.21 ^a
Cultivar/treatment	17 days of recovery - full irrigation		Dry mass (g plant ⁻¹)			
	Root	Total	Flower	Leaf	Stem	Shoot
BGFI	11.94 ^b	34.30 ^b	1.35 ^a	9.93 ^{ab}	11.03 ^b	22.36 ^b
BGNI	9.64 ^b	26.37 ^b	0.10 ^b	6.85 ^{bc}	9.78 ^b	16.73 ^b
NVFI	19.33 ^a	57.98 ^a	2.23 ^a	14.46 ^a	21.96 ^a	38.65 ^a
NVNI	11.90 ^b	26.59 ^b	0.05 ^b	3.63 ^c	11.01 ^b	14.69 ^b

Stomatal conductance

For stomatal conductance (g_s) four way interaction between cultivar, time of measurement, irrigation treatment and day of measurement was seen (Table 3).

Table 3. F values and significance level of general linear model test using logarithmic transformed data ($\log g_s$) for stomatal conductance data in two blackcurrant cultivars grown in the greenhouse

Main factors					
Cultivar	Day		Time		Treatment
34.0711 ***	210.8431 ***		18.1364 ***		2047.5480 ***
Two way interaction					
Cultivar:Day	Cultivar:Time	Day:Time	Cultivar:Treatment	Day:Treatment	Time:Treatment
7.0822 ***	5.4956 *	2.5038 *	52.8349 ***	256.1789 ***	11.2089 ***
Three way interaction					
Cultivar:Day:Time	Cultivar:Day:Treatment	Cultivar:Time:Treatment	Day:Time:Treatment		
5.4849 ***	6.8980 ***	13.7054 ***	17.4757 ***		
Four way interaction					
Cultivar:Day:Time:Treatment					
5.5809 ***					

In fully irrigated plants differences in g_s between days of measurement were seen due to differences in temperature and VPD (Table 3; Fig.2).

Drought stress highly influenced stomatal conductance and for 'Narve Viking' g_s was significantly reduced from day 5, both in the morning and afternoon measurements, while 'Ben Gairn' showed reduced g_s from day 7 in the morning and day 5 in the afternoon measurements.

In NI plants differences between the two cultivars were only visible on day 7 for the morning measurements, and day 5 for the afternoon measurements. Stomatal conductance did not differ between cultivars for FI plants. 'Narve Viking' FI morning measurement were significantly higher on the day 10 and 12, while 'Narve Viking' NI only at day 12. 'Ben Gairn' FI were significantly higher on the day 12, while 'Ben Gairn' NI was significantly higher at day 7.

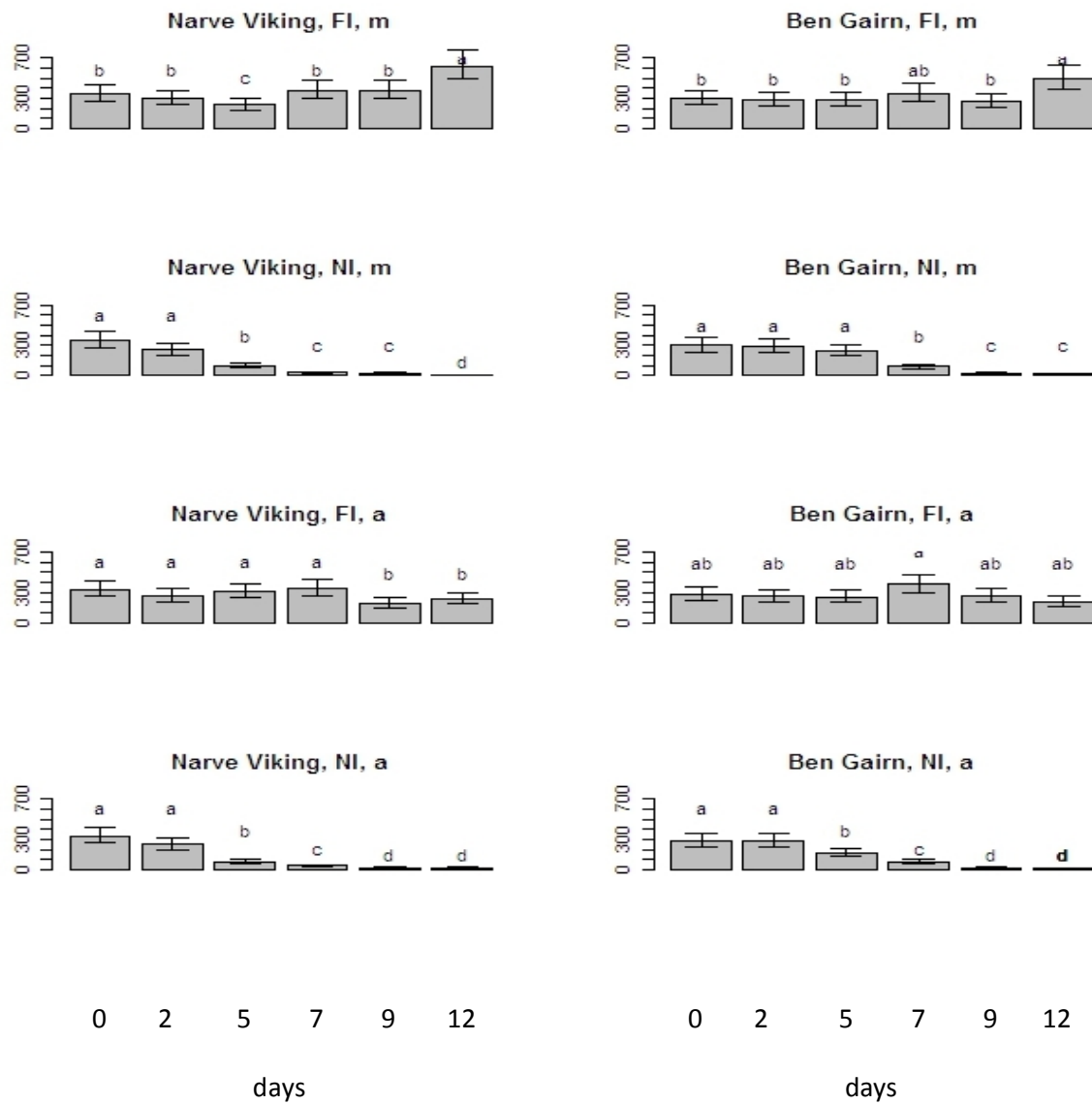


Figure 2. Stomatal conductance measured between 9-10 am (m) and between 12:30-13:30 pm (a) for blackcurrant cultivars 'Narve Viking' and 'Ben Gairn' grown at full irrigation (FI) or no irrigation (NI) for 0,2,5,7,9 and 12 days. Letters indicate differences between days of treatment, within each cultivar, irrigation treatment and time of measurement.

Plant water status (leaf water potential, osmotic potential and turgor)

Transpiration and changing in daily temperature (increasing of mean temperature) associated with drought stress reduce leaf water potential (Ψ_l) which become more negative for both cultivars, while turgor become more positive (Figure 3).

Water potential and turgor (Ψ_T) of fully irrigated plants for both cultivars ranked from -0.54 MPa and 0.86 MPa on days where the set point temperature was kept on 17.61 °C (day 7) and -1.51 MPa and 0.02 MPa on days with high temperature of (23.46 °C) on day 5, respectively. Osmotic adjustment (Ψ_n) as a mechanism of drought adaptation wasn't influenced by daily temperature and ranked -1.33 MPa on day 7 and -1.58 MPa on the day 5.

For fully irrigated plants no difference was seen in Ψ_l , Ψ_n and Ψ_T between the cultivars, whereas Ψ_l and Ψ_T for non-irrigated 'Narve Viking' was lower than for 'Ben Gairn' on day 7. When irrigation was withheld Ψ_l was significantly decreased in both cultivars from day 5 for 'Narve Viking' and day 7 for 'Ben Gairn'. The results measured on different days showing a highly influence by daily temperature and VPD.

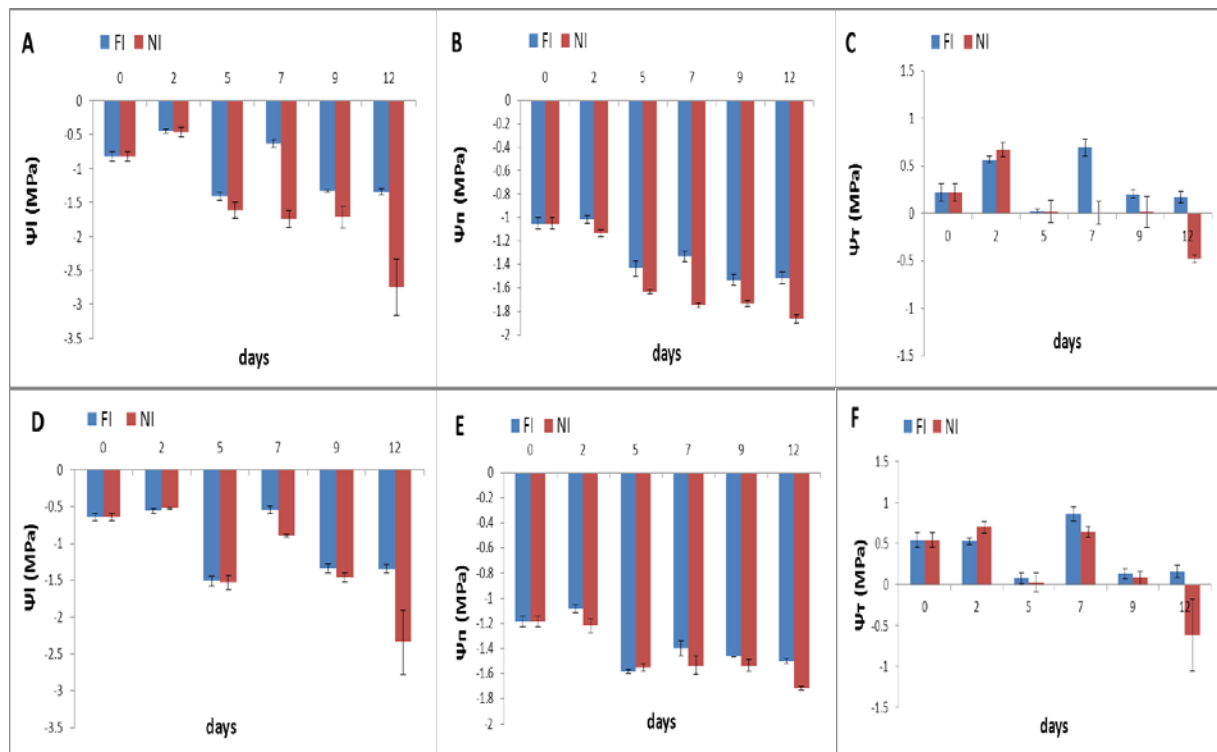


Figure 3. Water potential, osmotic potential and turgor of fully irrigated (FI) and non-irrigated (NI) plants, of 'Narve Viking' (A, B, C) and 'Ben Gairn' (D,E,F) over a 12 day period where data are presented as averages $n = 4$ with standard error bars

It is possible that due to smaller plant and through osmotic adjustment 'Ben Gairn' maintains cell water contents by increasing the osmotic force and thus increasing water uptake. With greater Ψ_l

more water is held in leaf cells resulting in higher Ψ_n comparing with Narve Viking. On day 12 NI plants of both cultivars lost turgor and wilted. On day 12 measurement of osmotic potential was really difficult which resulted in negative Ψ_n .

Table 4. F values and significance of general linear model for water potential (Ψ_I), osmotic potential (Ψ_n) and turgor (Ψ_T) in two blackcurrant cultivars grown in the greenhouse.

	Cul	Day	Irri	Cul*Day	Cul*Irr	Day*Irr	Cul*Day*Irr
Ψ_I (MPa)	14.53***	184.56***	63.11***	8.51***	8.97**	18.96***	1.55ns
Ψ_n (MPa)	0.10ns	115.19***	67.06***	5.65***	12.92***	6.20***	2.08ns
Ψ_T (MPa)	16.54**	41.11***	18.60***	4.20**	4.51**	8.56***	1.32ns

Root recovery measurements

In future, episodes of drought stress may be more frequent during the flowering period of blackcurrants due to climate change, but little is known about how different blackcurrant cultivars recover during growth after drought.

For root recovery measurements two way interactions between cultivars and irrigation, day and irrigation was seen (Table 5). The recovery of blackcurrant cultivars was significantly dependent with the time of recovery, increasing with the time of full irrigation. 'Ben Gairn' indicating a faster recovery and thereby a better drought tolerance.

Table 5. F values and significance of general linear model for root recovery data

Main factors		Cultivar 0.55ns	Day 20.72 ***	Irrigation 94.81***
Two ways interaction			Three ways interactions	
Cultivar:Day	Cultivar:Irrigation	Day:Irrigation	Cultivar:Day:Irrigation	
0.77ns	5.64*	3.26*	1.24ns	

Root window method for quantification of roots at the vertical soil surface during recovery phases in 'Narve Viking' and 'Ben Gairn' has been done during the two weeks after the treatment finished. The ability of root recovery was determined by evaluating the number of roots day 0, 7 and 14 after end of drought treatment. 'Ben Gairn' had a 52 % regrowth of roots in the non-irrigated relative to the full irrigated treatment at the 3. observation compared to only 10 % in 'Narve Viking'. (Fig. 4).

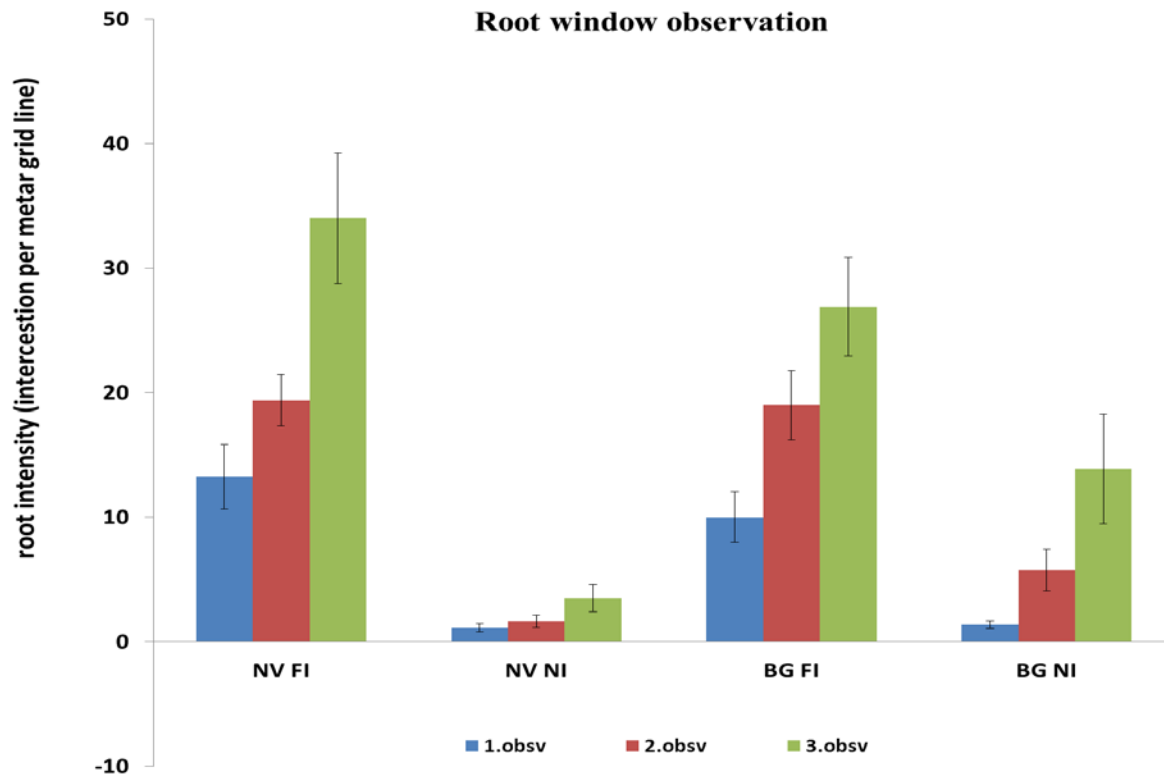


Figure 4. Ability of root recovery after drought treatment as determined by the number of roots counted in a given area for ‘Narve Viking’ (NV) and ‘Ben Gairn’ (BG) blackcurrant cultivars. 1. obsv: 0 days of recovery, 2. obsv: 7 days of recovery, 3. obsv: 14 days of recovery. Bars represent average (n=4), with standard error bars.

Total root length showed that ‘Ben Gairn’ had a better root recovery than ‘Narve Viking’, which had only half the length of roots after drought treatment and recovery phase compared to after full irrigation. For fully irrigated plants ‘Narve Viking’ showed a significantly higher root length compared with ‘Ben Gairn’. There was no statistical difference in root length for ‘Ben Gairn’ between the treatments in comparison with ‘Narve Viking’. The length of the main root system clearly indicated that fully irrigated plants had a more branched main root system compared with plants that were exposed to drought stress treatment. Specific root length didn’t show any difference within cultivar or treatments (Table 6).

Table 6. Effects of drought treatment after the recovery phases on root length, length of main root and specific root length in two blackcurrant cultivars grown in the greenhouse. Data are means of 4 plants. Values with the same letters within a column are not significantly different at $P < 0.05$ according to Tukey's test.

Cultivar/treatment	Root length (m)	Length of main root (m)	Specific root length (m g^{-1})
BGFI	7.14 ^b	0.18 ^a	8.55 ^a
BGNI	5.74 ^b	0.11 ^b	5.93 ^a
NVFI	12.68 ^a	0.18 ^a	7.23 ^a
NVNI	6.26 ^b	0.13 ^b	5.47 ^a

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4. Course portfolio		
COURSE TITLE	DATE	ECTS
<i>Modelling Climate Effects on Crops and Cropping Systems</i>	4.10-11.10.2010.	5
<i>Nutrient use efficiency</i>	8.11.2010.	2
<i>Introduction to Statistical Modelling in Agriculture, Biological and Environmental Science</i>	Nov/Dec 2010.	6
<i>Plant breeding for adaptation to climate change</i>	24 -29.01. 2011.	6
<i>Scientific writing and communication</i>	Jan/Feb 2011.	3
<i>Applied statistics with R for the agricultural, life and veterinary sciences</i>	14 -19.08.2011.	6
<i>Visual display of quantitative information in applied plant science</i>	29.11-01.12.2011.	2
TOTAL ECTS		30

5. Dissemination and teaching activities

March 15, 2011 – Journal club presenting a paper A.Sønsteby and O.M. Heide. (2011). Elevated Autumn temperature promotes growth cessation and flower formation in black currant cultivars (*Ribes nigrum L.*) Journal of Horticultural Science & Biotechnology 86: 120–127.

June 10, 2011 - Friday seminar held at the Department of Food presenting a PhD thesis and preliminary results from first experiment.

Janury 2011 – Poster presentation PhD presentation and relation of my project to breeding in climate change, NOVA PhD course participant – Iceland.

June 7, 2011 - Poster presentation at the 8.th International Horticulture, Technology & Machinery Exhibition in Aarslev „Plasticity of blackcurrant in a changing climate; focus on water efficiency,,.

June 22 to 26, 2011 - Zlatibor, Serbia, two posters presentation „Effect of water stress on the ability of blackcurrant cultivars to grow under potted conditions,, and „Plasticity of blackcurrant in a changing climate; focus on water efficiency.

September, 21 -22, 2011.ClimaFruit 2 year planning and reporting meeting, held in Bioforsk, Apelsvoll, Norway. Presentation of preliminary results and talk for the Clima Fruit web site describing the project.

October, 3-4, 2011.Folum and Aarlev, Integration day, workshop.

October 25, 2011. Journal club presenting the paper Chaves et al., (2010) Grapevine under deficit irrigation: hints from physiological and molecular data. Annals of Botany 105: 661–676.

September, 29 – 30, 2011, PhD Seminar 2011 held in Sandbjerg, Denmark. PhD poster presentation with preliminary results.

26-29 June 2012 Dundee, Scotland, poster presentation „Root recovery in two blackcurrant cultivars after a period of drying out,,.

6. Conferences, seminars and events

June 22 to 26, 2011 - Zlatibor, Serbia

10 International Rubus and Ribes Symposium, International Society for Horticultural Science (ISHS)

Two posters presentation „Effect of water stress on the ability of blackcurrant cultivars to grow under potted conditions,, and „Plasticity of blackcurrant in a changing climate; focus on water efficiency.

26-29 June 2012 Dundee, Scotland

The eighth symposium of the International Society of Root Research (ISRR)

Poster presentation „Root recovery in two blackcurrant cultivars after a period of drying out,,

7. Thesis structure

Thesis structure

1. General introduction
 - Rationale
 - Hypotheses and objectives
2. General literature review

Blackcurrant

- botany
- growth and development
- biology, flower induction and flowering
- cultivation
- cultivars and breeding

Response of blackcurrant to water deficit

-water relations and stomatal conductance

Genetic control/gene expression

Plant growth and biomass production

Candidate genes associated with water deficit

3. Material and methods
 - short descriptions of own experiments
4. Results and discussion
 - plasticity of blackcurrant to water stress
 - effect of drought stress on flower initiation and flowering
 - physiological adaptation to drought stress
 - conclusions and perspectives
5. References
 - Appendix I paper 1
 - Appendix II paper 2
 - Appendix III paper 3

8. Paper 1

Working title: Effects of water stress during flowering of two pot grown blackcurrant (*Ribes nigrum* L.) cultivars

The work has been discussed in details with my supervisor Karen Koefoed Petersen, Hanne Lindhard and Hanne L. Kristensen. The first draft of introduction, material and methods and results and discussion part and conclusions are in process.

Hypothesis of the article

Water stress during flowering will induce changes in growth, dry matter distribution and reduce the potential fruit yield, but to which degree depend of the capacity to adjust physiologically during water stress.

Dry soil conditions will reduce the amount of fine root growth relative to full irrigation and this will affect the root's ability for regrowth.

Root nitrogen uptake activity differs between stressed and non-stressed plants. Nitrogen uptake will be increased after 12 days of drought stress and 14 days of recovery due to shoot growth.

Abstract

Introduction

Materials and methods

Treatments

- Two blackcurrant cultivars ('Ben Gairn' and 'Narve Viking')
- Fully irrigated and none irrigated plants.

Experimental outline of experiment 1.

Measurements and calculations

Daily evapotranspiration

Stomatal conductance

Leaf water potential

Leaf water potential

Turgor

Leaf area after 12 days of drought

FW and DW of leaves, flowers and stems after drought and recovery

Shoot length before and after 12 days of drought
Root window observations during recovery
¹⁵N and the end of the recovery period
WinRHIZO analyses of roots after recovery (root length, distribution between root classes and dry weight of the roots)
Specific leaf area

Results

Figure 1 - Accumulated evapotranspiration
Figure 2 - Stomatal conductance
Figure 3 - Water potential, osmotic potential and turgor
Figure 4 - Root regrowth after 0, 7 and 14 days
Figure 5 - ¹⁵N uptake over 14 days of recovery
Table 1. Leaf area, leaf water content, specific leaf area and WUE
Table 2. DM distribution between plant parts after 12 days of drought and 17 days of recovery
Table 3. Stomatal conductance - statistic
Table 4. Water potential, osmotic potential and turgor - statistic
Table 5. Root recovery measurements – statistic
Table 6. Root length, length of main root and specific root length

Discussion

Evapotranspiration and water relations during drought stress to blackcurrant
Plant growth and dry matter distribution after drought and recovery
Root activity (¹⁵N) and root re-growth
Cultivar differences in response to drought stress during flowering

Conclusions

9. Paper 2

Working title: Influence of drought stress during flower induction in two cultivars of blackcurrant

The plan is going to be discussed in details with my supervisors Karen Koefoed Petersen, Lillie Andersen and Hanne Kristensen (Aarhus University, Denmark) and Rex Brennan (James Hutton Institute

The plan is that in April 2012 top cuttings of cultivars ‘Ben Hope’ and ‘Titania’ will be rooted and grown under optimal water and nutrient conditions in greenhouse. Plants will be trimmed to only one shoot until the period of harvest in late July. Plants will be kept in the vegetative stage by long day (>18 hours).

One period of deficit irrigation (50% of fully irrigated) are going to be investigated during the initial stages of flower induction which will be induced by transfer to short day conditions (10 h). During water stress and the following recovery period evapotranspiration, stomatal conductance, plant water status, leaf ABA content and top and root growth and flower initiation will be studied. The following year a monitoring of bud burst and flowering are going to be investigated.

Hypothesis

Water stress during flower induction will reduce the number of flower strigs and number of flowers per strig the following year and thereby reduce yield but to which degree depend on cultivar and the ability to adjust physiologically during and after water deficit.

Root growth is generally thought to occur in two peaks, one around flowering and a second after harvest. Therefore we expect root growth to be reduced during, immediately after and also the following spring when plants are subjected to drought stress during flower initiation.

Abstract

Introduction

Materials and methods

Treatments

- Two blackcurrant cultivars (‘Ben Hope’ and ‘Titania’)
- Fully irrigated and deficit irrigated plants.
- Days of deficit irrigation before recovery (0,3, 5, 7 and 10 days)

Measurements during deficit irrigation July/August 2012

- ET_c and ET_o manually weighting
- stomatal conductance
- Leaf water potential,
- Leaf osmotic potential
- ABA
- Relative water content
- Leaf growth (individual leaves)
- Root window observations

Measurements during recovery March 2013-

- Root window observation
- First flower open
- Number of strigs
- Flower per strigs
- Length of new shoot growth, one shoot per plant
- Fresh and dry weight of flowers, leaves and stems

Results

Figure 1 - Daily evapotranspiration, reference evapotranspiration, crop coefficient

Figure 2 - Stomatal conductance during drought and recovery

Figure 3 - Water potential, osmotic potential and turgor

Figure 4 - ABA

Figure 5 - Root regrowth after 0, 7 and 14 days and after overwintering

Figure 6 - DM distribution between plant parts after overwintering

Figure 7- Bud burst, flowering and fruiting performance

Table 1 - Extension growth, no of flower striges per bush and per strig, date of first flower opening in the following year

Discussion

Water relations during drought stress in the flower induction period and during the following recovery phase

DM distribution after drought and recovery

Changes in root growth as affected by period of deficit irrigation during flower initiation

Extension growth, bud burst and flowering

Conclusions

10. Paper 3

Working title: Differential regulation of genes in two blackcurrant cultivars subjected to drought stress during flowering using microarray technology

The plan is going to be discussed in details with my supervisors Karen Koefoed Petersen (Aarhus University, Denmark) and Rex Brennan (James Hutton Institute, Scotland).

Most physiological processes are affected from the experiment I (evapotranspiration, stomatal conductance, water potential, osmotic potential and turgor, etc). The knowledge of environment is crucial for planning any breeding study. From the first greenhouse study, two cultivars were chosen due to days of drought stress in order to see the differences in gene expression between the stressed and unstressed samples, and identify some of the key genes that show differential expression. A very large number of genes are up- or down- regulated by drought stress, but is going to be a challenge to identify and screen the key genes or groups of genes.

Hypothesis of the article

1. Sensing and signalling of water deficit will activate or deactivate genes which can be identified by microarray analyses of total RNA.
2. Depending on drought tolerance blackcurrant cultivars will show differences in gene expression when irrigation is withheld.

Abstract

Introduction

Materials and methods

Treatments (samples from exp. 1)

- Two blackcurrant cultivars ('Ben Gairn' and 'Narve Viking')
- Fully irrigated and none irrigated plants.
- Three time points (3, 5, 7 days)
- Four biological replicates

Measurements

- Total RNA extraction according to Hedley et al., 2010 (May-July in Aarslev).
- Prepare, run and interpret microarrays (November, Dundee) and identification of candidates genes
- RTPCR to verify up and down regulation of candidates genes

Results

Figure 1 – Overview of gene expression profiling for two blackcurrant cultivars.

Figure 2 - Microarray expression data based upon days of drought stress

Figure 3 - Frequency of significant differentially expressed genes between sample time points, days of sampling and number of differentially expressed genes (up-regulated and down-regulated)

Figure 4 - Validation of microarray expression profiles for selected Ribes gene by RT-PCR.

Discussion

Insight of genetic components associated with drought stress during flowering

The differences in gene expression between stressed and unstressed plants

Candidate genes that show differential expression.

Insight into the genetic control of drought stress during a flowering time in two North Region blackcurrant cultivars.

The potential development and use of genetic markers for drought stress for future breeding

The use of microarrays over a time course leading up to drought stress key changes in gene expression at flowering time.

Conclusions