



Diurnal water fluxes and growth patterns in potato tubers under drought stress

Katharina Huntenburg¹ · Daniel Pflugfelder² · Robert Koller² · Ian Charles Dodd¹ · Dagmar van Dusschoten¹

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Abstract

Background and aims Potato tubers comprise 83% water at harvest, but surprisingly few studies address tuber water relations in drying soil. This study aims to understand whether soil drying alters tuber water fluxes and their effect on tuber volume growth.

Methods Tuber water content and volume growth were investigated every 4 h using magnetic resonance imaging (MRI) during soil drying and re-watering, with leaf gas exchange, leaf water potential and foliar abscisic acid (ABA) concentration measured concurrently.

Results Tubers of well-watered plants showed a diurnal growth pattern with their volume and average water content (TWC) increasing overnight.

Withholding irrigation caused typical shoot drought stress responses (e.g. stomatal closure), dampened fluctuations in total TWC and paused nocturnal volume growth. Irrespective of soil moisture, tubers lost water (likely to the shoot) during the daytime when the plant transpires, while tuber water loss to the soil was minimal. Re-watering restored tuber volume growth and average TWC due to root water uptake and transport to the tuber.

Conclusions Potato tubers can supply water to the shoot. Nocturnal water influx needs to exceed daytime water efflux for net tuber volume growth, which should be considered in irrigation management.

Keywords Drought stress · MRI · Plant physiology · Phenotyping · Tuber volume · Water potential

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K. Huntenburg (✉) · I. C. Dodd
Lancaster Environment Centre, Lancaster University,
Farrer Avenue, Lancaster LA1 4YQ, United Kingdom
e-mail: Katharina.huntenburg@wur.nl

K. Huntenburg
NIAB, Lawrence Weaver Road, Cambridge CB3 0LE,
United Kingdom

D. Pflugfelder · R. Koller · D. van Dusschoten
Institute of Bio- and Geosciences, IBG-2: Plant Sciences,
Forschungszentrum Jülich, Jülich 52428, Germany

Abbreviations

ABA	Abscissic acid
HAS	Hours after onset of the stress
MRI	Magnetic resonance imaging
SWaP	Soil water profiler
TWC	Tuber water content
Ψ	Water potential

Introduction

Potatoes are the 4th most important staple crop in the world, being produced on 361 million ha worldwide

in 2018 (FAO 2020). Climate change is expected to decrease tuber yields on all continents (e.g. in Eastern Europe, northern America and the lowlands of Africa) by the end of this century (Raymundo et al. 2018). Since potatoes are very water demanding, drought stress limits yields of rainfed crops even in countries with high annual rainfalls such as England (AHDB Potatoes 2013; Haro-Montegudo et al. 2017; Huntенburg et al. 2021). To mitigate drought effects, improved irrigation management strategies and breeding more drought tolerant varieties is necessary. Both these approaches rely on better understanding potato responses to drought stress.

Above- and below-ground plant organs react differently to drought stress in the field. Partial rootzone drying decreased potato stomatal conductance, with photosynthesis rates restricted early in the season but later similar to the well-watered treatment (Liu et al. 2006). Deficit irrigation decreased shoot growth, but stomatal conductance, photosynthesis rates and leaf water potential were similar to well-watered plants throughout the whole season (Huntенburg et al. 2021). Below ground, long term drought stress increases root growth (Stalham and Allen 2004; Puértolas et al. 2014), which might increase water extraction from deeper soil layers (Blanchy et al. 2020). In tubers, drought stress increases soluble sugar content even at mild stress levels (irrigation to 75% of field capacity) while tuber dry matter content is only reduced at high stress levels (irrigation to 50% of field capacity) (Faradonbeh et al. 2022). An increased content in soluble sugars increases the osmotic potential of the tuber and will therefore influence water dynamics between the organs. Curiously, although potatoes are grown for their tubers which comprise 83% water at harvest, irrespective of the irrigation regime (Saeed et al. 2008), very little is known about water flux to or from the tuber under drying soil and whether that affects tuber growth. To better understand drought-induced yield losses, we need to better understand the water relations of tubers in drying soil.

Potato tubers gain weight during the night and lose weight throughout the day (Baker and Moorby 1969). In field-grown potatoes, the tuber always had a higher (less negative) water potential (Ψ) than the leaf during three days and nights without irrigation or rainfall and the Ψ_{tuber} to Ψ_{leaf} gradient was greater during the day than during the night (Gandar and Tanner 1976). This indicates water could move from the tuber to the leaf

along the water potential gradient, especially during the day. The tuber is linked to the vascular system of the plant through the stolons, which transport water and nutrients in xylem and phloem vessels (Nelson et al. 1990). Xylem flow volume and velocity through the lower stem (as measured with magnetic resonance imaging) was higher during the day than at night in potatoes due to greater transpiration during the day (Aliche et al. 2020a). Nocturnal stomatal closure decreases transpiration, but day-time transpiration probably magnifies the difference in water potential between tuber and leaf. However, both Ψ_{tuber} and Ψ_{leaf} decrease during the day, and recover in the evening and overnight with a water potential difference between Ψ_{tuber} and Ψ_{leaf} of 0.3 – 0.4 MPa during the day and 0.1 – 0.2 MPa at night (Gandar and Tanner 1976), with corresponding patterns of high xylem flux during the day and lower flux at night (Aliche et al. 2020a). Interestingly, in the drought tolerant potato cultivar ‘Biogold’, xylem transport volume in the lower stem is similar in drought stressed and well-watered plants during the day, while xylem transport volume of drought-stressed plants decreases at night (Aliche et al. 2020a). Thus, water supply to the leaves is the same under well-watered and drought stressed conditions during the day when the leaf transpires, but is lower under drought stress at night, when leaf water status recovers. Tuber contributions to the water flux towards the leaves overnight were hypothesized based on fluctuating tuber weight (Baker and Moorby 1969), but not considered in more recent literature on stolon formation or fluxes of water and carbohydrates in drought-stressed potato plants (Lahlou and Ledent 2005; Aliche et al. 2020a,b). To determine whether tuber water content (TWC) and tuber growth depend on soil water availability, an *in-vivo* study of the same tubers at several timepoints throughout the day and the night, with accompanying shoot physiological measurements, is necessary.

In-vivo measurements of below-ground plant organs are inherently difficult. The opaque nature of the soil impedes visual methods thereby encouraging rhizotron studies (Parker et al. 1991; Smit and Groenwold 2005), but these fail to account for the three-dimensional structure of the tuber. Since mechanical methods (e.g. dendrometers) to determine organ growth can get blocked by soil particles, impeding measurements, these have not been attempted in potato. Magnetic resonance imaging (MRI) was a suitable technique

to image bulky and complex structures like sugar beet with high accuracy and detail (Metzner et al. 2014). Moreover, MRI technique visualises ^1H -nuclei (protons), which are abundant in water molecules. Hence, it is possible to deduce the water content, shape and volume of below ground organs using images obtained with MRI. To our knowledge, such techniques have not been applied to study the short-term dynamics of potato tuber growth as the soil dries.

MRI allowed non-destructive diurnal measurements of tuber growth and TWC, at differing soil water availabilities. Moreover, shoot physiology (stomatal conductance, photosynthesis rates, leaf water potential and ABA content) were monitored concurrently to determine perceived severity of the drought stress above- and below-ground. We hypothesised that tubers take up water during the night and lose water throughout the day, which increases and decreases tuber growth rate respectively, with such fluctuations expected to be higher in well-watered plants.

Material and methods

Plant material

Potatoes (*Solanum tuberosum* cv. 'Maris Piper') were grown in cylinders of 40 cm height, 11.2 cm inner diameter and 3.9 L volume. Sandy loam was demagnetized (strong magnets filtered out strongly ferro-magnetic particles prior to planting as described previously (van Dusschoten et al. 2016)) and 200 ml fertilizer solution added (5% Hakaphos Rot, content: 8% N (5% NO_3 , 3% NH_4), 12% P_2O_5 , 24% K, 4% Mg, 0.01% B, 0.02% Cu, 0.05% Fe, 0.05% Mn, 0.001% Mo, 0.02% Zn). The moistened soil mixture was filled into the cylinders until 9 cm below the edge, to a bulk density of 1.5 g/cm^3 . Seed potatoes of 18–20 mm diameter (TLC potatoes, Banchory, Scotland) were then placed on the settled soil and the pots were filled with loose soil until 2 cm below the edge. Plants were grown in a controlled environment at 14 h light period (500–600 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ at plant level, white LED, Cree LED, Durham, NC, USA), 20/ 16 °C day/ night temperature and 56–66% relative humidity (RH, setpoint 60%) at Forschungszentrum Jülich, Germany. Every 2–3 days, pots were weighed to determine water loss (evapotranspiration) and re-watered to approx. 20% (v/v) soil water content. Plants emerged 11 to

18 days after planting. When they were 30 cm high, the apical bud was removed to limit height growth and therefore damage from robot handling during the experiment. Six weeks after planting (4 weeks after emergence), water was withdrawn from 4 plants for 2 days which were re-watered on the 3rd day, while 4 plants were watered daily with 150 ml from the top after the second block of physiological measurements. This irrigation volume was the mean daily water loss by evapotranspiration calculated over the week prior to the experiment. Drought stressed plants were re-watered basally by being placed in a tray of water for 20 min to ensure sufficient water uptake and prevent introducing systematic errors in the tuber water content measurements.

Physiological measurements

Physiological measurements started 2 h after supplementary lights were switched on and were carried out 30 min before each plant underwent MRI measurements in the morning and afternoon.

Whole plant transpiration rate was measured by weighing the plants at 30-min intervals. Two pots with soil, but without a plant, were weighed at a 4 h interval to estimate the evaporation rate from the soil without plant. Whole plant transpiration rate is estimated from the difference between evapotranspiration and soil evaporation. Furthermore, stomatal conductance and photosynthesis rates of a young, fully expanded leaf were measured using an infrared gas analyser (LI 6400XT, LI-COR, Lincoln, USA) with 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR (10% blue), 400 ppm CO_2 , 300 $\mu\text{mol/s}$ flow rate, a Block Temperature of 23 °C and 50% RH inside the cuvette.

In the morning measurements (8.00 – 11.30am), the same leaf was measured as the previous afternoon. After leaf gas exchange measurements, one leaflet of this leaf was sampled and directly frozen in liquid nitrogen for subsequent ABA radioimmunoassay (Quarrie et al. 1988). The apical three leaflets of the same leaf were subsequently used to measure leaf water potential with a pressure chamber (Plant Water Status Console, Soil Moisture Equipment Corp., Santa Barbara, USA). On day 4, only the morning measurements were taken. Fresh weight and dry weight of all tubers with a diameter larger than 5 mm were measured.

Soil moisture was measured twice daily at 5, 20 and 35 cm below the soil surface using a simplified soil water profiler (SWaP, see van Dusschoten et al. 2020) at 180 MHz and with a Span of 60 MHz (Supplementary Fig. 1). The 5 cm measurement includes the tubers as indicated in Fig. 3 and therefore shows generally higher values than the lower two measurements. Mean soil moisture was calculated as the mean of three measurements (top, middle, bottom) per pot and timepoint. Since plants were re-watered from the base of the pot, capillary rise was calculated using the water flow model HYDRUS-1D v4.17 (Šimůnek et al. 2012). Parameters in the model were determined as explained previously (Müllers et al. 2022) using the HYPROP setup (Meter group, Munich, Germany). Namely, the parameters were: $Q_{\text{sat}}=0.42$, $Q_{\text{res}}=0.02$, $\alpha=0.051 \text{ cm}^{-1}$, $n=0.588$, $K_s=4 \text{ cm h}^{-1}$.

MRI measurements

MRI measurements were taken from all eight plants every 4 h over 4 days and nights with a dedicated 4.7 T magnet (Magnex Scientific Ltd., Oxford, England) with $400 \text{ mT} \cdot \text{m}^{-1}$ gradient coils (MR Solutions, Guildford, England) and a 14 cm RF-coil (Doty Scientific, Columbia, SC, USA). Measurement setup and image processing were similar to that described (van Dusschoten et al. 2016), but with the following adaptations (FOV = $132 \times 132 \text{ mm}$, slice thickness was 1.2, Echo Time (TE) = 10 ms, image matrix = 256×256 using 2 averages). A 5 mm tube filled with a 5 mM NiCl_3 solution was inserted into the soil as internal reference to correct for non-biologically relevant signal fluctuations. Each measurement took about 30 min. We verified whether observed changes of potato water amounts could be attributed to a shortening of the relaxation time (T_2), but observed no significant changes to the extent that these would affect our findings. A handling robot (MiniLiner 3.0, Geiger Handling GmbH & Co. KG, Jülich, Germany) picked the pots from a holding magazine and placed them into the magnet for measurements (Fig. 1A). To prevent mechanical damage due to handling eight plants in a confined space, all plants were wrapped in foil from three sides during the night (8 pm – 6am) (Fig. 1B). In the morning, the foil was removed and the plants were placed under a light panel ($500 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 14 h/day, white LED, Cree LED, Durham, NC, USA) 5 m away from the magnet to keep environmental conditions

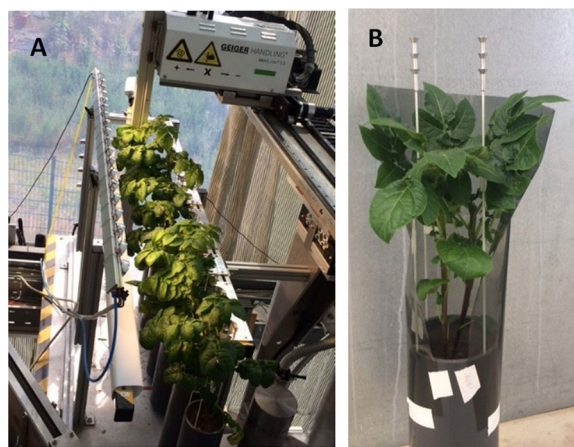


Fig. 1 Plants positioned in the holding magazine (A), treatments were alternated well-watered and drought stressed. Since moving the plants from the rear to the magnet in the front can cause leaf damage, plants were wrapped in foil on three sides overnight (B)

similar to the measurement conditions (on average: $\text{rH}=40\%$, $T=25^\circ\text{C}$). In the daytime plants were individually placed into the magazine shortly before the measurement. To determine water losses through the periderm, 16 unsprouted tubers (similar size to the tubers in the main experiment) were placed into soil at 2.5%, 5%, 7.5% and 15% water content (4 tubers per water content) and individually measured twice with 12 h difference using the same MRI setup as explained for whole plants with tubers.

Data analysis and statistics

To visualise and analyse images, the software package Mevislab (version 2.2.3, MeVis Medical Solutions, Bremen, Germany) was used. With this software, single tubers were separated from noise, roots and other tubers as follows: First a binary mask is generated using a signal intensity threshold. Holes within this binary mask were eliminated using the binary closing algorithm, whereas the kernel size was manually adapted to completely eliminate holes in the mask. To discriminate touching tubers and to remove roots, stolons and stems, this mask is eroded by a manually chosen distance. This leads to isolated clusters. The cluster within the tuber of interest is then selected manually and dilated by the previously selected distance to regain the

original shape of the tuber. For tubers with close neighbours, this procedure can overestimate the volume by reaching into the neighbouring tuber. Therefore, as a final step, we removed parts of the dilated volume which are closer to a non-selected cluster. From these generated masks, tuber volume and TWC (signal intensity) for each tuber and timepoint were extracted and normalised for temperature effects using the aforementioned reference tube. Total TWC is calculated from the signal intensity of the whole tuber, whereas average TWC is signal intensity per unit volume (voxel). Tuber volume, total TWC and average TWC were normalised to the initial value before further analysis. This procedure was carried out for every single detectable tuber of each plant and the average of all tubers per plant was used for statistical analysis.

For statistical analysis and graphs, the software package R (Version 4.0.3, 2020, The R Foundation for Statistical Computing, Vienna, Austria) was used. Tubers were treated as pseudo-replicates, while plants were real replicates when calculating treatment means. Two-way repeated measures ANOVAs were carried out to evaluate the effects of drought stress and time after starting the treatment on all repeatedly measured variables. Assumptions of independent and identically (as Normal) distributed data were met. The assumption of sphericity is necessarily met, because the factor drought stress has only 2 levels. For normalised TWC and normalised tuber volume, the start of the experiment (-5 h) is not considered in the statistical analysis, since all values were equal (=1).

If significant differences occurred in the repeated measures ANOVA, pairwise comparisons were made using plant mean values (averaging across all tubers of one plant) for single time points.

The experiment was repeated in the summers of 2019 and 2020 with similar results. For clarity only results of the experiments 2020 are shown.

Results

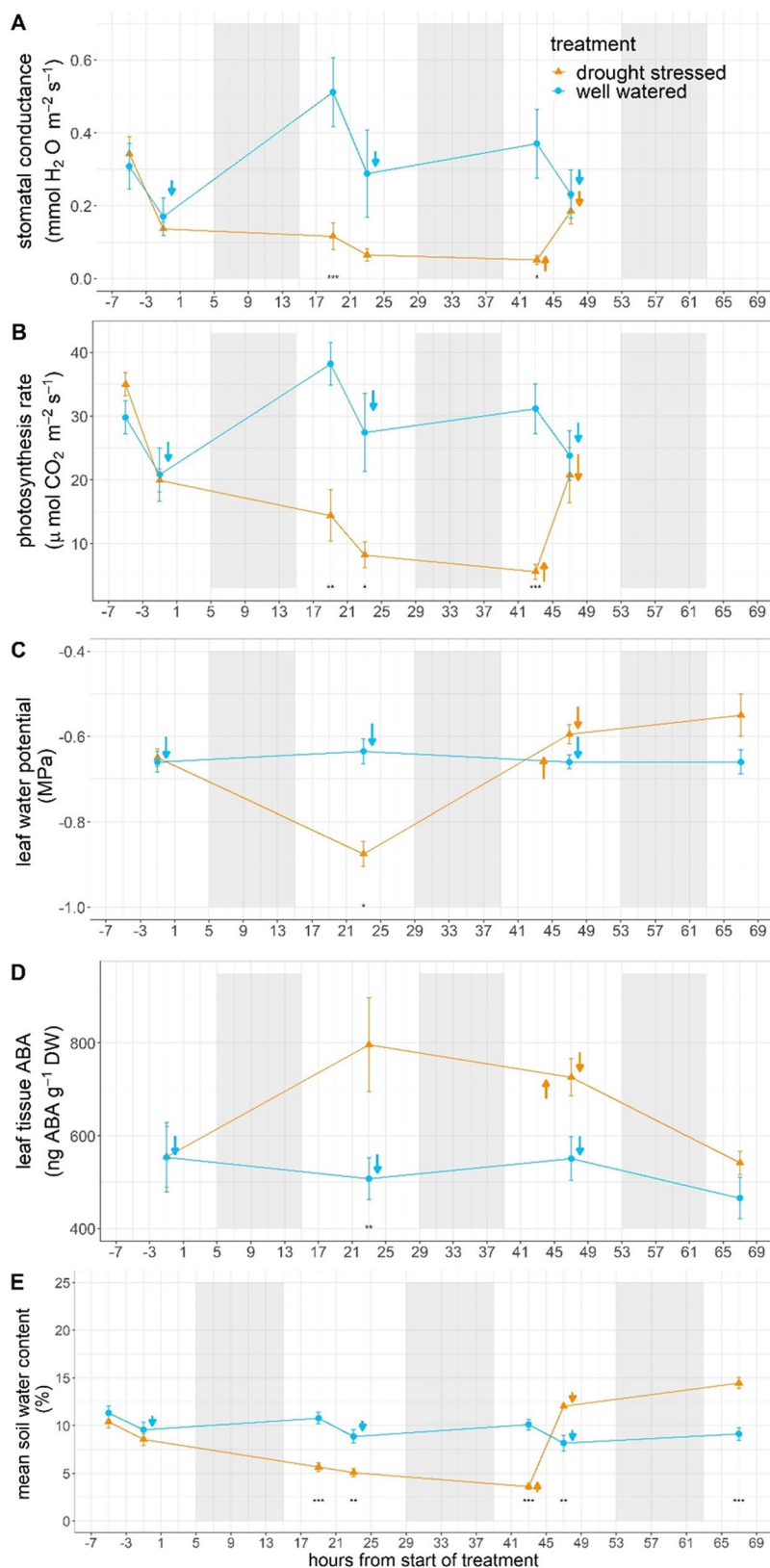
Soil drying decreased stomatal conductance and photosynthesis rates in drought stressed plants (Fig. 2A and B), both of which recovered to well-watered levels upon re-watering ($p=0.56$ and $p=0.62$

for stomatal conductance and photosynthesis rate respectively at 47 h after onset of stress, HAS). Furthermore, afternoon leaf water potential was similar between treatments on day 1 (-1 HAS), but was 0.24 MPa lower in drought stressed than well-watered plants on day 2 (23 HAS, $p=0.001$). After re-watering, there were no significant treatment differences ($p=0.06$ and $p=0.31$ for 47 and 67 HAS respectively, Fig. 2C). Leaf tissue ABA concentration was 57% higher in drought stressed plants than in well-watered plants on day 2 ($p=0.0011$, Fig. 2D), but not different to well-watered plants upon re-watering ($p=0.056$ and $p=0.31$ for 47 HAS and 67 HAS respectively). Thus, soil drying decreased leaf water status and gas exchange and increased foliar ABA concentration on day 2. Re-watering at 44 HAS restored leaf gas exchange to the level of well-watered plants within 3 h, when measurements were made at 47 HAS.

Mean soil moisture was similar in both treatments at the beginning of the experiment and was maintained between 8.2–11.3% for well-watered plants ($\Psi_{\text{soil}} = -0.07$ – -0.03 MPa respectively, Supplementary Fig. 2). These values did not significantly differ over time (1-way ANOVA, $p=0.057$) due to intermittent irrigation. Nineteen hours after start of the treatment (HAS), drought stressed plants experienced 5.65% soil moisture (approximately $\Psi_{\text{soil}} = -0.9$ MPa, Supplementary Fig. 2) and thus soil moisture almost halved compared to well-watered plants ($p<0.001$, Fig. 2E). After re-watering the drought stressed plants, the soil held 3.8% and 5.4% more water than the well-watered plants at 47 HAS ($p=0.004$) and 67 HAS ($p=0.0009$) respectively. Hence soil moisture differed between the treatments during the drying period, and re-watering was sufficient to increase soil moisture in drought stressed plants to levels higher than well-watered plants.

Since mean soil moisture was markedly higher in drought stressed plants after re-watering than in well-watered plants, moisture distribution in the pot was examined more closely. Moisture was always highest close to the soil surface (Fig. 3A), because tubers in that layer influenced the measurement. Treatment differences were obvious in the lower layers starting from 0 HAS, when well-watered plants were re-watered and had a higher soil moisture than drought stressed plants until drought stressed plants were re-watered (individual t-tests, $p<0.05$, Fig. 3B and C). After re-watering drought stressed

Fig. 2 Stomatal conductance (A), photosynthesis rate (B), afternoon leaf water potential (C), leaf tissue ABA content (D) and mean soil moisture (mean of 3 positions per pot) (E) of well-watered (blue circles) and drought stressed (orange triangles) plants. Symbols are means \pm SE of 4 plants. Grey bars indicate hours without light, arrows indicate when plants were watered from the top (downward arrow) or the bottom (upward arrow) in the treatment corresponding to the colour. Asterisks indicate significant differences between treatments at that timepoint according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



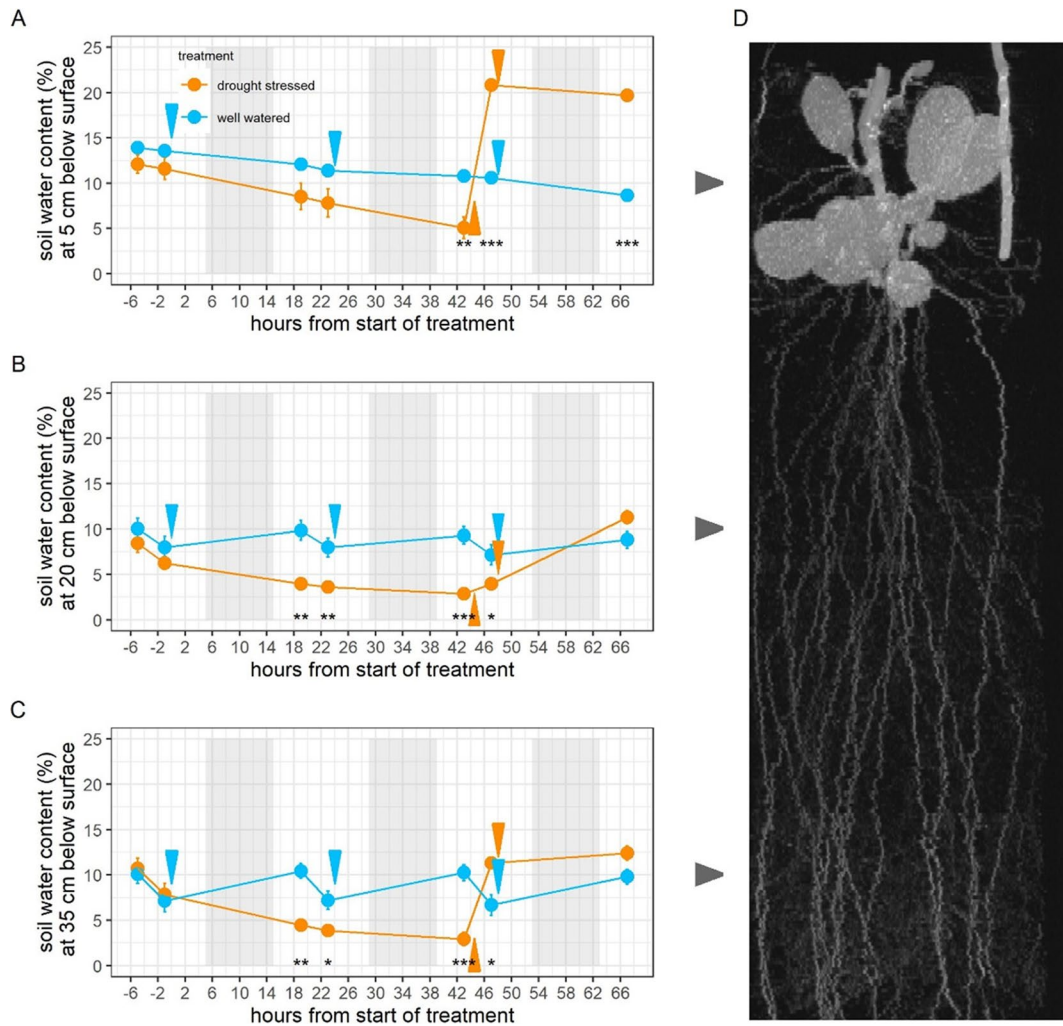


Fig. 3 Soil moisture of well-watered (blue) and drought stressed (orange) plants measured at 5 cm (A, also contains tubers), 20 cm (B) or 35 cm (C) below the soil surface and image of root and tuber distribution of a sample plant (D). (A – C) Symbols are means \pm SE of 4 plants. Grey bars indicate hours without light, arrows indicate when plants were watered from the top (downward arrow) or the bottom (upward arrow)

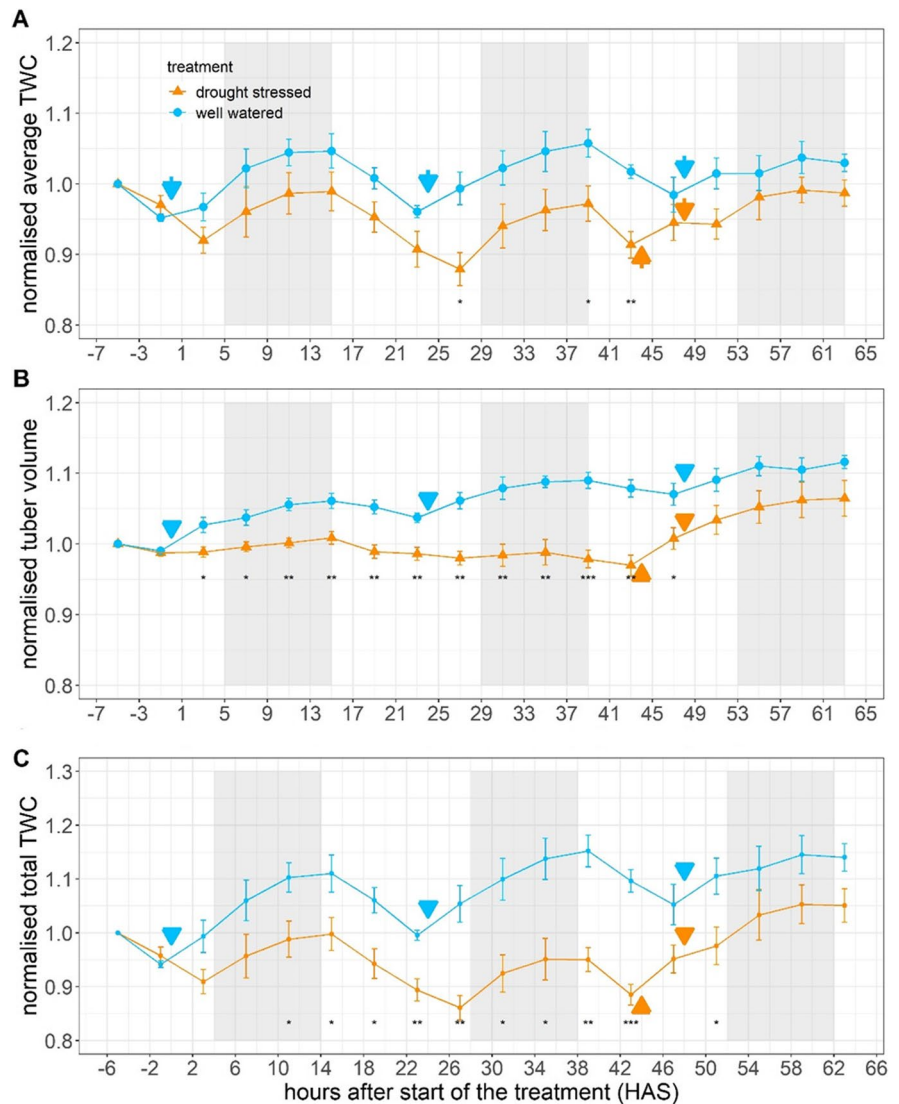
in the treatment corresponding to the colour. Asterisks indicate significant differences between treatments at that timepoint according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (D) Grey arrows at right indicate locations of soil moisture measurements. Reference tube visible in the top right corner of the image

plants from the bottom of the pot at 43 HAS, the water content at the bottom and at the top of the pot increased drastically, reaching values higher than in well-watered plants (Fig. 3A and C), while the soil moisture in the middle of the pot remained lower than in the well-watered plants (Fig. 3B). Capillary rise in Kaldenkirchen soil at -0.9 MPa was calculated to be less than ca 5 cm within 20 min of basal re-watering (Supplementary Fig. 4). We suggest that water redistribution from the bottom to the top of the

pot did not result from capillary rise in the soil, but instead resulted from water redistribution through the roots into the tubers at the top of the pot.

Normalised average TWC (tuber water content per volume unit) fluctuated diurnally (Fig. 4A), but differently in well-watered and drought stressed plants (RM ANOVA $p = 0.01$, Supplementary Table 1). In well-watered plants, normalised average TWC decreased significantly over the day until re-watering and then increased significantly

Fig. 4 Normalised average TWC (tuber water content) (A), normalised tuber volume (B) and normalised total TWC (C) of well-watered (blue circles) and drought stressed (orange triangles) plants over the time course of three days (white background) and nights (light grey bars). The starting point of the experiment is set to 1 for each tuber (normalised). Symbols are means \pm SE of 4 plants per treatment and time point. Asterisks indicate significant differences between treatments at that timepoint according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



overnight at a higher rate than it decreased in the day (Table 1). In contrast to well-watered plants, tuber water loss of drought stressed plants exceeds subsequent water uptake (Table 1). For comparison, tubers of well-watered plants lost ca. 10% of their average water content over 8 h in the daytime (Fig. 4), while unsprouted tubers in dry soil lost 4% of the average water content in 12 h (Supplementary Fig. 3). Re-watering increased the normalised average TWC of drought stressed plants, which was subsequently not significantly different from well-watered plants (47 – 63 HAS, $p > 0.05$). This confirms that some of the water taken up by the roots was indeed transferred into the tubers.

To understand water dynamics of the tubers, it is important to also consider changes in tuber volume over time. In well-watered plants, tuber volume increased by 11.6% over 68 h (ANOVA, $p = 0.0011$) and tubers were significantly bigger than drought stressed tubers from 3 HAS until 47 HAS (individual ANOVAs per time point, $p < 0.05$, Fig. 4B). Drying soil paused tuber volume growth (Tukey HSD, $p > 0.05$ for all individual comparisons until 43 HAS). Similarly, there was no tuber growth in unsprouted tubers in dry soil over a 12 h period (Supplementary Fig. 3). Re-watering the drought stressed plants substantially increased their tuber volume, resulting in values that did not significantly

Table 1 Diurnal differences in normalised average tuber water content (Δ norm. average TWC) of well-watered and drought stressed plants. Values refer to the comparison between the timepoint above and below the respective entry

	well-watered			drought stressed		
	time (HAS)	Δ norm. average TWC	t-test	time (HAS)	Δ norm. average TWC	t-test
start of the experiment	-5			-5		
<i>day</i>		- 4.8%	$p=0.0029^{**}$		- 8.0%	$p=0.022^{*}$
lowest value day 1	-1			3		
<i>night</i>		9.5%	$p=0.027^{*}$		6.9%	$p=0.086$
highest value day 2	15			15		
<i>day</i>		- 8.6%	$p=0.033^{**}$		- 11%	$p=0.023^{*}$
lowest value day 2	23			27		
<i>night</i>		8.5%	$p=0.0101^{*}$		9.3%	$p=0.035^{*}$
highest value day 3	39			39		
<i>day</i>		-7.3%	$p=0.062$		- 5.8%	$p=0.11$
lowest value day 3	47			43		
<i>night</i>		3.1%	$p=0.17$		7.3%	$p=0.033^{*}$
end of the experiment	63			63		

Asterisks indicate significant difference (Δ) between lowest and highest values of the day according to t-test with $^{*} p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$

differ from well-watered plants from 51 HAS onwards (individual ANOVAs per time point, $p > 0.05$). Thus, sufficient water supply is necessary to facilitate tuber growth. However, if plants are re-watered after a drought period as short as two days to soil water potentials ≥ -0.2 MPa (after re-watering), compensatory growth by tubers of drought stressed and re-watered plants can recover volume to the level of well-watered plants.

Normalised total TWC is the product of the normalised average TWC and the tuber volume. Hence, in tubers of well-watered plants, the water influx overnight (Fig. 4A) together with the tendency of increased volume overnight (Fig. 4B) leads to 14% more water being stored in the tubers during the whole experiment (ANOVA, $p=0.012$; Fig. 4). In drought stressed plants the decrease in normalised average TWC combined with no change in tuber volume before re-watering lead to a 11.5% less water in the tuber at the end of the drought stress period (ANOVA, $p=0.0095$; Fig. 4C) and no significant change in normalised total TWC between beginning (-5 HAS) and end (63 HAS) of the experiment (ANOVA, $p=0.20$).

Diurnal rhythm

In addition to the changes in normalised average and total TWC and tuber volume over a drought stress period of two days and subsequent watering, diurnal patterns in average and total TWC suggest water (re-) distribution within the potato plant. Average and total TWC decreased in both treatments throughout the day and increased overnight (Table 1, Fig. 4A and C). For well-watered plants, this increase started after re-watering the plants every afternoon (Fig. 4A and C). For drought stressed plants, the water loss continued until the end of the light period and average TWC increased in the dark period without additional water (Fig. 4A and C). Note that the rate of relative water loss from the tubers throughout the day is similar in well-watered and drought stressed tubers (Fig. 4A, Day 2: -0.011 and -0.010 units per hour in well-watered and drought stressed plants respectively, $p=0.91$) as is the rate of water uptake throughout the night (Fig. 4A, Night1: 0.0031 and 0.0036 units per hour in well-watered and drought stressed plants respectively, $p=0.85$; Night 2: 0.0044 and 0.0040 units per hour in well-watered and drought stressed

plants respectively, $p=0.87$; Night 3: 0.0018 and 0.0007 units per hour in well-watered and drought stressed plants respectively, $p=0.73$). Thus, the rate of water uptake and loss per unit volume was similar in well-watered and drought stressed plants. However, drought stressed tubers lost water for a longer period and took up water for a shorter period, which lead to an overall decrease in average and total TWC compared to well-watered plants.

Although drought stressed plants had lower average and total TWC, their water influx and efflux rates were similar to well-watered plants. Nevertheless, the diurnal pattern of tuber volume differed between the treatments (Fig. 4B, repeated measures ANOVA, $p=0.02$, Supplementary Table 1). In well-watered plants, tuber volume tended to increase overnight and decrease throughout the day, while these changes were not present in drought stressed plants. However, when comparing the highest and lowest values of a 24 h period (as done for tuber volume in Table 1), no significant differences occurred within a single treatment (ANOVA, $p>0.05$). This indicates for example, that in well-watered plants the highest normalised average TWC at night was not significantly different from the lowest normalised average TWC in the day before or after that night. Thus, the number of replicates is too low to discriminate diurnal patterns in sequential comparisons, but the repeated measures ANOVA can detect differences in the patterns between the treatments.

Despite their lack of formal statistical significance, tuber volume changes affected normalised total and average TWC. More pronounced changes in normalised total and average TWC occurred in well-watered plants, whereas these were smaller in drought stressed plants (Fig. 4C). In summary, potato tubers under well-watered conditions increased their normalised total and average TWC overnight, while this response was attenuated or did not occur in tubers of drought stressed plants. Furthermore, tubers of drought stressed plants lost water throughout the day.

Discussion

Potato tubers comprise around 80% of the total plant biomass (Fasan and Haverkort 1991), accumulate soluble sugars under drought stress (Faradonbeh et al. 2022), and therefore play an integral part

in understanding whole plant water relations and drought responses. However, tubers are difficult to study *in vivo* and previous studies could only measure water potential of different tubers at the beginning and the end of the night period (Gandar and Tanner 1976) or continuously measure the same tuber growing in an artificial (non-soil) environment (Baker and Moorby 1969). More recent studies employed MRI technology to measure stem xylem fluxes (Aliche et al. 2020a) or investigated the impact of drought on root and stolon formation and tuber yield (Lahlou and Ledent 2005) or on tuber dry matter content and soluble sugar content (Faradonbeh et al. 2022), but surprisingly do not consider tuber water relations. In this study, we used MRI to frequently (every 4 h) measure all tubers of multiple plants per treatment in intact soil columns, to understand how soil drying affects diurnal tuber volume growth and water relations. Total and average TWC decreased during the day, when the plant transpired, and increased at night, leading to nocturnal tuber volume growth in well-watered plants (Fig. 4). Soil drying dampened these diurnal fluctuations in total and average TWC, and paused tuber volume growth (Fig. 4), thus confirming previous hypotheses of tuber water flux based on tuber weight (Baker & Moorby 1969). At night, when transpiration is minimal, total and average TWC of drought stressed tubers slightly increased. In addition, unsprouted tubers in dry soil hardly lost any water over 12 h (Supplementary Fig. 3). Since water loss through the periderm into the drying soil is minimal, tuber water loss during the daytime must therefore be transported within the plant to the organ with the lowest water potential, the leaves.

Soil drying induced typical physiological responses in the shoot, by decreasing leaf gas exchange and increasing foliar ABA concentration from 23 HAS (Fig. 2), with re-watering restoring these variables to well-watered values. Since the roots were distributed throughout the soil column (Fig. 3D), all soil layers dried. Basal re-watering of the drought stressed plants increased soil moisture strongly at the top and the bottom of the pot (Fig. 3A, C). However, re-watering had only a minor effect in the middle of the pot (Fig. 3B, 47 HAS), since capillary rise of water in the soil column is about 5 cm for this soil type (Supplementary Fig. 4). The SWaP measures total moisture in the pot at a given height and does not distinguish

between water in the soil and water in tubers. Hence, measurements at the top of the pot reflect a combined moisture value of tubers and surrounding soil. The increased water content at the top of the pot is therefore a result of water being relocated via the roots to the top of the pot and into the tubers. After re-watering the drought stressed plants, average TWC and tuber volume increase significantly (Fig. 4, Table 1). For the SWaP measurements, the increase in tuber volume magnifies the effect of water influx into the tubers so that the total moisture measured in the top of the pot after re-watering accounts for bigger tubers with a higher water content per volume unit (average TWC) than before re-watering. Compared to well-watered plants, the significantly higher moisture values in the top of the pots of drought stressed plants after re-watering can be explained by water influx into the tubers. In addition, potato tubers accumulate soluble sugars even under mild drought stress (Faradonbeh et al. 2022), which decreases their osmotic potential and thus water is likely to be distributed to the tubers.

Mild drought stress impaired tuber volume growth, but subsequent re-watering substantially increased tuber growth rate such that both treatments had similar tuber volumes at the end of the experiment (Fig. 4B). Potato plants exposed to combined drought and heat stress for 15 days and then re-watered recovered to well-watered levels of weight gain (as measured via CT scan) within 15 days after re-watering (van Harsselaar et al. 2021). Tuber dry matter content did not differ between deficit irrigation treatments and controls in the field (Huntenburg et al. 2021). While increased cell division would lead to an accumulation of dry matter, tuber volume growth requires cell expansion. Ultimately, cell and plant organ growth have been related to cell wall relaxation and turgor thus:

$$G = m(P - Y)$$

with G =cell elongation rate, m =yielding coefficient, P =cell turgor, Y =yield threshold turgor (turgor over which irreversible cell extension occurs) (Lockhart 1965; Passioura and Fry 1992). Overnight, average TWC increases in well-watered plants, while tuber volume shows positive growth rates (Fig. 4). Hence, we assume that the water influx in tubers of well-watered plants after re-watering and overnight was

high enough to adequately increase cellular turgor, or that sufficient relaxation of cell walls occurred overnight, to facilitate growth ($P > Y$). In drying soil, less water is available to increase cell turgor and therefore, cell growth is reduced. In drought stressed plants, the water efflux from the tuber in the day exceeded the influx into the tuber at night (Fig. 4). This means even though average TWC increased from the lowest value in the day to the highest value in the next night, nocturnal turgor levels likely did not reach the yield threshold turgor ($0 < P < Y$) required for cell growth (Fig. 4, Table 1, (Hohl & Schopfer 1992)). Thus, tuber volume growth in drought stressed plants paused in drying soil. However, re-watering drought stressed plants initially substantially increased tuber growth rates, which later returned to similar growth rates as in well-watered plants (Fig. 4). This behaviour reflects the underlying interdependence of P , m and Y (Hohl and Schopfer 1992; Passioura and Fry 1992) and shows that short-term drought stress (withholding water for 120 h, then re-watered) may not impair cell wall extensibility (Durand et al. 1995), but that turgor is indeed the main driving force for tuber growth.

Watering the plants in the evening could have reinforced the effect of nocturnal tuber growth. Tubers of well-watered plants and of drought stressed plants after re-watering started growing as soon as sufficient water was available (Fig. 4). However, normalised total TWC increased over night and declined throughout the day in drought stressed plants without any irrigation events in the first two days. While we are confident that diurnal rhythms (rather than when watering occurred) regulate tuber growth, further research is needed to understand whether the timing of irrigation might affect tuber volume growth at a defined soil water content.

To maximise tuber yield, water influx into the tuber overnight should not be restricted and daytime water efflux should be minimal. In the absence of other considerations, irrigation in the late afternoon or evening may be a practical solution to adjust soil moisture dynamics to the periodicity of tuber growth. During times of low transpirational losses (evening, night), water is directed into the tubers allowing cell extension (Fig. 4). This process is irreversible and tuber shrinkage due to the subsequent loss of water in the daytime is unlikely assuming mild to moderate drought stress and re-watering of the plants after

a short stress period (Green et al. 1971; Passioura and Fry 1992). Prolonged and/or more severe drought stress may irreversibly constrain tuber volume growth or cause tuber shrinkage (Płodowska et al. 1989), such that re-watering would not allow full recovery of tuber volume. Further research is needed to assess the effects of severe or prolonged drought stress on tuber cell wall properties and tuber growth *in vivo*.

While the timing of irrigation may affect the influx of water into tubers, restricting daytime water efflux from the tuber is more difficult because it is not fully understood where the water is lost to. Direct water loss to surrounding soil is unlikely, as the tuber periderm is an effective water barrier under drying conditions (Vogt et al. 1983) and unsprouted tubers in dry soil only lost minimal amounts of water over a 12 h period (Supplementary Fig. 3). However, xylem and phloem vessels within the stolons may act as conduits for water exchanges between the tuber and the rest of the plant, driven by water potential or osmotic gradients. Diurnal fluctuations in total and average TWC may thus be determined by processes in other plant organs, rather than local soil moisture availability. In the phloem, soluble sugars are transported from the leaves (source) to the tubers (sink) (Aliche et al. 2020b), while xylem flow is mainly driven by differences in water potential (Holbrook and Zwienicki 2005). The water potential gradient between tubers and leaves suggests daytime water flow from the tuber to the leaves, while the water potential difference is close to zero during the night (Gandar and Tanner 1976), which is reflected in xylem flow rates in potato stems under well-watered conditions (Aliche et al. 2020a). However, acropetal xylem flow in the lower stem of potato under drought stress decreased significantly in a cultivar that hardly produced any tubers under drought stress, while xylem flow rates were maintained in a cultivar that did produce tubers under drought stress (Aliche et al. 2020a,b). This means the tuber could supply water to the shoot during the daytime, when the roots cannot extract sufficient water from the soil to support shoot demands. During the night, with a minimal water potential gradient between tuber and leaf, water extracted by the roots is transported to the tuber. Low soil water availability may restrict water flow to the leaves, because the water would be shared between tubers and leaves at night. Studying these hypotheses requires the use of deuterium labelled water (Hafner et al. 2017) to

understand the pathway of water through a potato plant under restricted soil water availability. Screening different genotypes could give insights into whether tuber number, stolon size or osmotic potential affect daytime water efflux and possibly discriminate future markers for drought tolerance breeding.

To conclude, magnetic resonance imaging allowed repeated measurements of water content and volume of all tubers of a potato plant. Tuber water content shows similar diurnal fluctuations to the shoot (Gandar and Tanner 1976), but in contrast to the root or shoot, they do not directly exchange water with their environment (Vogt et al. 1983; Taiz and Zeiger 2010). Since tubers form a large percentage of the total plant biomass and contain around 83% water at harvest, they may influence other plant physiological responses to drying soil. While further experiments with dyes or tracer molecules are desirable, we suggest that tubers are not just sink organs, but might provide water to the shoot as the soil dries out.

Author contributions KH, DvD and RK conceptualised the work. KH wrote the manuscript with editorial contributions from ICD, DvD, DP and RK. KH and DvD carried out the experiments and DP performed MRI data curation and helped with image analysis.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no conflict of interest.

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