Journal of Applied Botany and Food Quality 92, 343 - 354 (2019), DOI:10.5073/JABFQ.2019.092.046

[1]Department of Beverage Research, Geisenheim University, Geisenheim, Germany
[2]Department of Vegetable Crops, Geisenheim University, Geisenheim, Germany
[3]Department of Biometry and Population Genetics, Justus Liebig University, Giessen, Germany
[4]Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany

Chemical composition of field grown radish (*Raphanus sativus* L. var. *sativus*) as influenced by season and moderately reduced water supply

Christine Schlering[1, 2]*, Helmut Dietrich[1], Matthias Frisch[3], Monika Schreiner[4], Ralf Schweiggert[1], Frank Will[1], Jana Zinkernagel[2]

(Submitted: June 25, 2019; Accepted: October 25, 2019)

Summary

Seasonal variations in water availability as increasingly provoked by climate change pose severe challenges for vegetable production, particularly for crops requiring reliable and high water supply for achieving satisfactory quality. In contrast to most previous studies applying severe water deficits, we examined the effects of moderate water deficits on the chemical composition of red radish tubers during three consecutive years with variable climatic conditions. Radish were cultivated in open field, applying two different water supply treatments and following a randomized block design comprising four sets of six plots each. The resulting water reductions of 3-20% led to a significant increase of dry matter-based *myo*-inositol levels, whereas those of selected minerals and trace elements, phenolics and glucosinolates decreased. Anthocyanin levels remained unchanged. Fresh-matter related levels of most analytes increased upon reduced water treatments due to higher dry matter contents. While pigment levels in radish remained unchanged, mild water deficit affected other quality-related parameters such as pungency-related glucosinolates.

Keywords: Vegetables, Polyols, Glucosinolates, Anthocyanins, Drought, Water Deficit

Introduction

Plant responses to changing environmental conditions are complex and vary between cultivation season and year. Abiotic stress factors such as water deficiency induce several changes in various physiological plant processes (PRASAD et al. 2008), generally related to negative effects on plant growth, yield and quality. Future scenarios suggest that increasing global surface temperatures will very likely lead to higher incidences of precipitation-free periods such as for example in Germany (SCHMIDT and ZINKERNAGEL, 2017) or South and West European countries (NIKULIN et al., 2011), particularly because rainfall patterns are shifting upon temperature changes. For example, 2018 proved to be the warmest and one of the driest years in Germany since weather records began. As a result of climate change, growing conditions undergo changes and inadequate water supply might become an increasingly important agricultural challenge for fruit and vegetable production, and drought-stress tolerant cultivars might be desired. Climate change conditions including uncommon temporal limitations or excess in water supply, increased regional mean temperatures, and enhanced radiation intensity induces numerous physiological reactions in plants that could change their chemical composition of valuable phytochemicals, clearly influencing the quality of their harvest products (WANG and FREI, 2011). While the effects of considerable drought stress events on plant physiology have been investigated in detail (TAIZ and ZEIGER, 2010; AKINCI and DOROTHY, 2012), the impact of a mild reduced water supply on the quality of vegetables has only been insufficiently studied so far. Moreover, even less is known about the effects of moderate water shortage on the plant's chemical composition in open field cultivation, caused by precipitation shifts from summer to winter as expected for many parts of Europe in the context of climate change (BINDI and OLESEN, 2011). Depending on its duration and intensity, reduced water supply will affect vegetable crop production in different ways and levels from physiology to crop quality. While perennial crops such as grapevine and fruit trees seem to be well adapted for mild water deficits, vegetable crops – particularly freshly marketed products such as spinach as well as tomato and cucumber – appear to be more susceptible to water shortage (COSTA et al., 2007; NISHIHARA et al., 2001). The drought-induced responses modulate large parts of the plant metabolism and the resulting effects are highly crop-specific (WANG and FREI, 2011) and often hardly predictable. Root vegetables might be affected in a particular way, especially if water deficits occurring during tuber or root initiation (DARYANTO et al., 2016), since drought increases the rigidity of the soil and, thus, might affect growth of the produce. For instance, red radish (*Raphanus sativus* L. var. *sativus*) might be particularly affected due to their short tuber growth periods. Red radish belongs to the botanical family Brassicaceae and occurs in many different color variations and shapes (PAPETTI et al., 2014). Economically important cultivars often include those with red round hypocotyl tubers, which are eaten predominantly as raw food and are being harvested at a very early stage of development long before flowering. They provide a range of nutrients as well as quality-related and bioactive compounds, which also have been associated with physiological benefits for human health (MANCHALI et al., 2012). For instance, they were reported to contain considerable amounts of vitamin C (28 mg/100 g fresh weight), glucosinolates, and phenolic compounds like anthocyanins (SCHREINER et al., 2002; SOUCI et al., 2008). Glucosinolates are important for the often desired mild, moderate or strong pungency of radish, additionally their breakdown products being associated with health promoting such as anti-carcerogenic effects (HIRATA et al., 2018). Besides their importance for the color and consumer acceptance of radish, anthocyanins have been related to health-promoting properties due to their high antioxidant capacity (YAO et al., 2004). Furthermore, radish is rich in dry matter, soluble sugars, proteins, and minerals (SOUCI et al., 2008). Moderate water reduction may support or affect the accumulation of quality-related and health promoting compounds in red radish. Earlier studies have shown that inadequate water supply can lead to higher concentrations of primary and secondary metabolites in vegetables other than red radish (KOYAMA et al., 2012; SCHREINER et al., 2009; ZHANG et al., 2008). Moreover, varying climate conditions also contribute to alterations in the chemical composition of the product, as shown for bioactive compounds

* Corresponding author

in different *Brassica* vegetables (Aires et al., 2011). Therefore, we sought to study the effect of a mild reduced water supply on quality relevant morphological and physio-chemical traits of the red radish tubers in open field cultivation during three successive years including four cultivation sets, thus resembling more practically relevant conditions as compared to experiments in controlled environments like greenhouses or growth chambers.

Material and methods

Chemicals

All reagents and solvents used were at least of analytical or HPLC quality unless specified differently. Folin-Ciocalteu's phenol reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany); L-(+)-ascorbic acid from Carl Roth (Karlsruhe, Germany); D-(+)-catechin-hydrate, *myo*-inositol (> 99.5%, HPLC) and anhydrous glycerol from Sigma Aldrich (Steinheim, Germany). Pelargonidin-3-*O*-glucoside obtained from Extrasynthese (Genay Cedex, France). L-aspartic acid (Ph. Eur., USP) was received from AppliChem (Darmstadt, Germany).

Plant material and experimental setup

Red radish cv. Celesta F1 (Enza Zaden, Dannstadt-Schauernheim, Germany) were grown from seeds sown in the field. Cultivation experiments were conducted under open field conditions on a sandy loam in a plot installation at Geisenheim University, Germany (49°59′ N, 7°58′ E). A total of four cultivation sets were grown during the years 2015 (1 set), 2016 (2 sets) and 2017 (1 set) as shown in Tab. 1. Each set consisted of six circular plots (marked by A, C, F, H, M and P) with a diameter of 11.9 m. Each plot was subdivided into four quarters (subplots) for the simultaneous cultivation of three different vegetable crops with an annual crop rotation. Each subplot quarter was again divided into three further segments for conducting the different irrigation treatments following a randomized block design, while the outer segment of each subplot was excluded from harvest (Fig. 1). The irrigation levels remained assigned to the subplots during the full duration of the experiments, irrespective of the annual crop rotation. The size of each segment was at least 6.5 m^2, respectively.

Cultivation and water supply

The red radish seeds were sown in rows with a spacing of ca. 0.125 m between the rows and a sowing distance of ca. 0.033 m in the rows. Fertilization was carried out uniformly with calcium ammonium nitrate according to commercial standard specifications for radish cultivation (109 kg/N ha^{-1}) based on mineralized N (NO_3^--N) in 0-15 cm soil depth. Crop protection was applied equally to all plots, whereby application depended on the growing set. The insecticide Karate Zeon (Syngenta, Maintal, Germany) was used against flea beetles (*Psylliodes* spp.) and Goldor Bait (BASF, Ludwigshafen, Germany) against wireworms (Elateridae) once per cultivation set. The removal of weeds was conducted manually. Water supply was carried out as follows: Drip irrigation was initiated when the soil moisture tension fell below -20 kPa in 10 cm depth and the well-irrigated segments (CTR) were provided with 100% water supply (6.2 L/m^2 per irrigation), as controlled by a tensiometer with electronic pressure sensor (Tensio-Technik, Bambach GbR, Geisenheim, Deutschland). Segments assigned to reduced water supply (RWS) were provided with 63% (2015, 2016-2), 68% (2016-1) and 51% (2017), respectively, of the aforementioned water amounts by irrigation. Since natural precipitation dominated the moisture tension, the total amount of irrigation events and, consequently, the total amount of supplied water was highly variable from set to set. As a result, the extent to which water supply had been reduced was variable, amounting to a reduction of 10% (2015), 15% (2016-1), 20% (2016-2) and 3% (2017), respectively, when considering both irrigation and precipitation (Tab. 1). Leaf water potential was measured two days before harvest (2016-1 and 2016-2) or just before harvest (2017) by using a Scholander PWSC 3000 (Soil Moisture Equipment Corp., USA). To ensure uniform germination and seedling growth, the soil of both the full and reduced water supply treatments was kept moderately moist by receiving the same, sufficiently high water amounts during initial stage. Depending on the growing period, this initial stage lasted for 9 days (2015), 7 days (2016-1, 2016-2) and 12 days (2017). Tab. 1 summarizes further detailed information on cultivation data and water supply.

Harvest

A total of 120 plants per segment were harvested randomly when tuber diameter reached 25-30 mm, representing the commercially targeted maturation stage for harvest. To avoid border effects, outer rows were omitted from harvest. Specifically, harvest dates were 26, 23, 23 and 33 days after sowing for the cultivation sets 2015, 2016-1, 2016-2 and 2017, respectively (Tab. 1). After harvest and shoot removal with a knife, tubers were cleaned twice manually with fresh tap water to remove adhering soil particles. Then, radish tubers were frozen at -80 °C until analyses.

Climatic data

Local climate data were supplied by the local weather station, which was located at 100 meters distance to the experimental field site in Geisenheim. Climatic parameters for each cultivation period are summarized in Tab. 1. Based on detailed weather situations of daily temperature and air humidity profile (supporting information, Fig. A.1 and A.4), the different growing periods were characterized

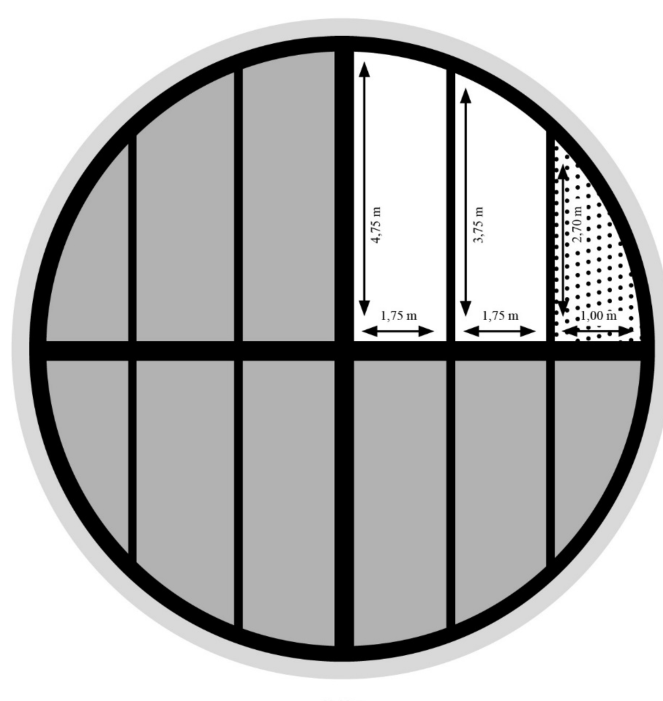

Fig. 1: Circular structure of the open field experimental setup. Each quarter of the circle represents a plot divided into three subplots. Red radish was grown in one of the four plots. Subplots differed in the irrigation regimes. The outer subplot (marked by dot pattern) was excluded from the harvest.

Tab. 1: Cultivation and climate data during the different experimental periods. DAS: days after sowing. Irrigation (mm): total irrigation amount including irrigation during initial stage. Total water amount (%) RWS: water amount (%) of the reduced variants including watering after sowing.

Growing season	2015	2016-1	2016-2	2017
Sowing date (Year-month-day)	2015-08-06	2016-06-29	2016-08-29	2017-04-19
Beginning of water supply differentiation (DAS)	15	7	7	28
Harvest date (DAS)	26	23	23	33
Temperature sum (°C)	565.1	488.1	483.6	408.9
Daily mean air temperature (°C)	21.7	20.3	20.2	12.0
Mean relative air humidity (%)	67.1	83.8	84.2	68.4
Global radiation sum (MJ/m^2)	444.1	480.5	372.8	590.9
Daily mean global radiation (MJ/m^2)	16.4	20.0	15.5	17.4
Wind speed sum (m/s) at height of 2 m	38.4	32.1	27.4	43.8
Evaporation sum (mm)	92.4	86.6	61.1	94.0
Precipitation sum (mm)	21.2	9.2	12.8	62.9
Number of differentiated irrigation events	2	6	5	1
Irrigation (mm)	93	56	43	48
Total water amount (mm) incl. precipitation	125	65	56	111
Total water amount (%) RWS	**90%**	**85%**	**80%**	**97%**

and assigned as follow: 2015 (warm & moist), 2016-1 and 2016-2 (warm & dry) and 2017 (cold & moist). Strictly speaking, the cultivation periods 2015 and 2017 were characterized by strong variations of global radiation and air humidity (supporting information, Fig. A.2 and A.3), whereas the conditions in 2016 were much more constant.

Sample preparation

For all analyses, except for the determination of ascorbic acid and anthocyanins, 25 radish tubers of each sample were lyophilized (BETA 2-8 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) prior to grinding with a laboratory mill (IKA M 20; IKA-Werke, Staufen, Germany). For the analyses of anthocyanins, the colored external tissues of 15 radish tubers were carefully separated with a vegetable peeler, frozen immediately at -80 °C, and ground into a fine powder under liquid nitrogen with the aforementioned laboratory mill. Dry matter content was determined from freeze-dried material. D-Glucose, D-fructose, titratable acidity, L-malic acid and fumaric acid as well as inorganic anions like nitrate, phosphate, sulfate and chloride were determined from aqueous extracts prepared from the lyophilized powder. For this purpose, an aliquot of 8-10 g of lyophilized radish powder was thoroughly homogenized with 500 mL ultrapure water at room temperature for approx. 10 s at high level (setting 2) using a stainless steel food blender (Waring Blender, Waring Commercial, Torrington, CT 06790, USA). After transferring the extract into an 800 mL beaker using 150 mL ultrapure water, extraction was continued for 10 min under continuous magnetic stirring, followed by a single ultrasound-assisted extraction step in an ultrasound water bath for another 5 min. After centrifuging for 5 min at 4596 × *g* to separate liquid and solid phases, the supernatants were collected, filtered, and stored at -25 °C until analyses. Extraction procedures for all other target analytes are given below.

Chemical analysis

Unless otherwise noted, IFU-methods (International Fruit Juice Union, Paris) were used for determination of routine parameters in aqueous extracts, such as sugars, total acidity, organic acids and inorganic anions.

Sugars and polyols

D-glucose and D-fructose were determined with enzymatic kits (R-Biopharm, Darmstadt, Germany) using a Konelab 20 Xti analyzer (ThermoFisher, Dreieich, Germany). Determination of polyols was carried out in duplicates as follows. An aliquot of 400 mg of lyophilized powdered radish was extracted twice with 12 mL ultrapure water in a shaking water bath for 1 h at 45 °C. After centrifuging for 10 min at 12.857 × *g*, supernatants were collected, made up to a volume of 25 mL and filtered through a 0.2 µm membrane (PES filter; VWR, Darmstadt, Germany). Extracts were injected (10 µL) into a HPAEC-PAD system (Dionex/ThermoFisher BIOLC system, ICS 5000+, ICS 3000 SP, Thermo Fisher Scientific, Dreieich, Germany) equipped with an anion-exchange Carbopac MA 1 column (250 × 4 mm, Thermo Fisher Scientific, Dreieich, Germany) and a guard column of the same material, both operated at 15 °C. Separation was achieved by isocratic elution with 500 mmol/L NaOH at a flow rate of 0.5 mL/min. Quantitation was conducted with linear external calibrations of *myo*-inositol and glycerol.

Organic acids

Titratable acidity, calculated as citric acid, was measured potentiometrically after titration to pH 8.1 with 0.3 M NaOH (Titroline alpha, Schott, Mainz, Germany). L-malic acid was determined using enzymatic kits (R-Biopharm, Darmstadt, Germany). Fumaric acid was determined on a Summit HPLC-UV system (Dionex, Idstein, Germany) using a serial column connection of a Luna C18 250 × 4.6 mm and a Rezex Fast Fruit 100 × 7.8 mm (Phenomenex, Aschaffenburg, Germany). An isocratic elution profile with diluted aqueous phosphoric acid (1 mL H_3PO_4 (0.6%)/100 mL) and a flow rate of 0.7 mL/min was used. Quantitation was carried out by UV detection at 230 nm using an external calibration. Determination of ascorbic acid was conducted by iodometric titration. For this, an aliquot of 100 g of fresh frozen radish were homogenized in 200 g of oxalic acid (1%, w/v) using a Waring Blender. After centrifuging at 4596 × *g* for 10 min at 4 °C, the supernatant was acidified with aqueous sulfuric acid (10%) prior to ascorbic acid determination by automatic potentiometric titration (Schott-Titrator, Titrinoline Alpha Plus; software, Titrisoft) with 1/128 mol L^{-1} iodide-iodate solution. Results were expressed as ascorbic acid in mg g^{-1} fresh weight.

Inorganic anions

Nitrate, sulfate and phosphate were analyzed by ion chromatography on an ICS 2100-system on an IonPac AS 17 column using a KOH-gradient (4-45 mmol/L in 20 min, flow rate 0.5 mL/min) and suppressed conductivity detection (Dionex, Idstein, Germany).

Quantitation was based on linear calibrations with a multi-ion reference solution (Bernd Kraft, Duisburg, Germany). Chloride was analyzed by potentiometric titration with an AgCl-electrode using a Schott Titrator TitroLine alpha plus combined with a sample changer TW alpha plus and a piston burette Titronic universal (Schott, Mainz, Germany).

Total carbon and nitrogen

Elemental analyses of radish samples for carbon and nitrogen were carried out in duplicate by the Dumas combustion method (Vario MAX CNS, Elementar Analysensysteme GmbH, Langenselbold, Germany), combusting 300 mg of freeze-dried radish powder at 950 °C. Aspartic acid was used as reference substance.

Minerals and trace elements

For minerals and trace elements analyses, the principle of the Kjeldahl digestion with the Gerhardt Turbotherm rapid digestion unit (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) was applied as follows: An aliquot of 250 mg of freeze-dried powdered plant material was mixed with 10 mL of a specifically designed digestion solution (420 mL 18 M H_2SO_4, 330 ml aqueous 30% H_2O_2 p.a., 0.48 g Se (black powdered), 14.0 g lithium sulfate (suprapur)). After digestion at 100 °C for 100 min, the extract was made up to 50 mL with ultrapure water. Elemental analyses for P, K, Ca, Mg, Mn, Zn and Cu were executed with an ICP-OES (SPECTRO ARCOS, SPECTRO Analytical Instruments, Kleve, Germany).

Total phenols

An aliquot of 500 mg of freeze-dried powdered radish was extracted twice with 12 mL of 80% aqueous methanol under ultrasonication for 30 min and consecutive centrifugation for 10 min at 12.857 × *g*. The combined supernatants were made up to 25 mL with extraction solvent and stored at -28 °C until spectrophotometric analyses with the Folin-Ciocalteu reagent and a linear (+) catechin calibration as described previously (Singleton and Rossi, 1965).

Anthocyanins

Separation, identification and quantitation of single anthocyanins in the radish skin was carried out by HPLC-PDA-MS using the procedure described by Will and Dietrich (2006). In brief, an aliquot of 5 g of powdered fresh radish skin was extracted twice with 12 mL of aqueous 85% methanol acidified with 1% formic acid (v/v), using a cooled ultrasonic bath for 30 min in the dark. After centrifugation, supernatants were collected and combined, made up to a volume of 25 mL with extraction solvent and filtered through a 0.2 µm membrane (Chromafil O-20/25 PTFE syringe filter; Macherey-Nagel, Düren, Germany) prior to injection. Chromatographic and mass spectrometric parameters, settings and equipment was used as reported earlier (Will and Dietrich, 2006). Injection volume was 4 µL and flow rate 250 µL/min at 40 °C. Anthocyanins were identified by comparison of retention times, UV/Vis absorption maxima, and mass spectra to those of authentic reference substances or literature data (Jing et al., 2012; Wu and Prior, 2005). Quantitation was carried out at 520 nm using an external calibration with pelargonidin-3-*O*-glucoside.

Glucosinolates

Identification and quantitation of glucosinolates were determined according to Wiesner et al. (Wiesner et al., 2013).

Statistical analysis

Compositional data was analyzed with regard to both dry and fresh matter. Dry matter-related data should allow evaluations irrespective of different water contents in the plant material due to reduced irrigation treatments, while fresh matter-related data should represent the nutritional composition of the harvested edible plant material. Data were analyzed by fitting a linear mixed-effect model using the lmer-function within the lme4-package (Bates et al., 2015) of the statistical software R (R Core Team, 2016). The evaluation of the single cultivation sets based on a model with respect to the random plot-effects (eq. 1), while the total dataset was evaluated with respect to the interaction of plot and year (eq. 2):

y = water supply + plot (eq. 1)

y = water supply + plot:year (eq. 2),

where y represents the analyzed parameter, water supply (control, reduced) was a fixed factor and plot as well as the interaction plot:year were random factors. Comparisons of means derived from different treatments were considered significantly different if p-values < 0.10. Pairwise comparison of least-squares means was carried out with lsmeans-package (Lenth, 2016) to estimate fixed-effects of the treatment as well as random effects for the plot and season. Significances of random effects were calculated by the lmerTest-package (Kuznetsova et al., 2016). The results for the treatment (control, reduced) were evaluated on basis of adjusted data generated from the model mentioned above. Principal Component Analysis (PCA) was carried out by using the R-packages FactoMineR (Le et al., 2008) and factoextra (Kassambara and Mundt, 2017). To avoid an overweight of single anthocyanins and glucosinolates, only total amounts were considered for Principal Component Analysis (PCA). Three missing values were calculated by the missMDA-package (Josse and Husson, 2016).

Results and discussion

Evaluation of cultivation sets and season

The entire data set was visualized by PCA after correcting for random plot-effects (cf. eq.1) by the least-square means method. The corresponding loading plot revealed a strong differentiation of the cultivation sets by their chemical composition and points out the seasonal effects on the quality of the produce. The first two principal components (PCs) accounted for 66.4% of the total variance in case of dry matter evaluation (Fig. 1) and 62.1% in relation to fresh matter evaluation (data not shown). Consequently, the chemical composition was strongly dependent on the season with all its individual climatic conditions, which was already shown to affect selected substances in *Brassica* varieties (Aires et al., 2011; Schreiner, 2005). Cultivation sets from 2015 and 2017 partly showed an overlap, while both sets from 2016 were clearly separated within the PCA loading plot (Fig. 1). The total water amounts were higher in 2015 (125 mm) and 2017 (111 mm) compared to 2016-1 (65 mm) and 2016-2 (56 mm), respectively. These years clusters also differed in mean relative air humidity, which were 67.1% (2015) and 68.4% (2017) as compared to the datasets 2016-1 (83.3%) and 2016-2 (84.2%) (Tab. 1). The resulting higher leaf-air vapor pressure difference in 2015 and 2017, respectively, might have altered parameters such as transpiration and tissue temperature, which in turn may affect ion uptake, carbon assimilation and water transport (Grantz, 1990). Nevertheless, both sets from 2016 were clearly separated within the PCA loading plot (Fig. 1).

Effects of reduced water supply on the chemical composition of radish biomass

When comparing chemical parameters across the full dataset by

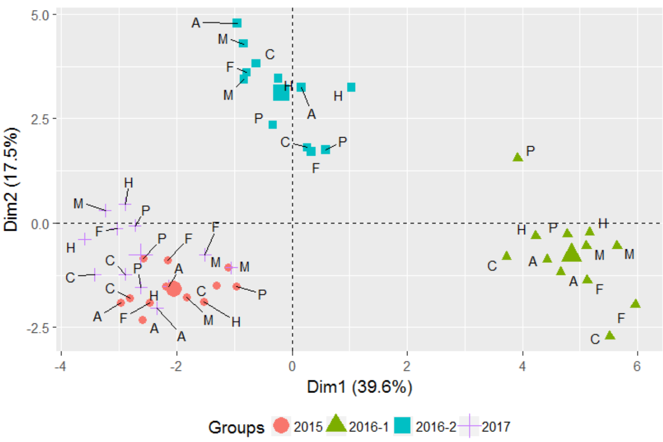

Fig. 2: Principal Component Analysis of the original, non-adjusted dataset including all radish cultivation sets (2015 (red circles), 2016-1 (green triangles), 2016-2 (blue squares) and 2017 (violet crosses)). Score plot represents the individual samples of each cultivation set (separated by color) and plot (marked by the letters A, C, F, H, M and P) based on dry matter-related chemical analyses.

PCA, the data points of the well-irrigated radish samples were mainly clustered in sectors with positive PC2, while those of the less irrigated radish samples were mainly clustered in sectors with negative PC2 (Fig. 2A). Most of the individuals were separated by the second principal component PC2 (Dim2), which accounted for 11.4% of the total variance. Accordingly, the first two principal components (Dim1+Dim2) accounted for 38.2% of the total variance in the dataset. The corresponding variables are represented in the loading plot (Fig. 2B). The most important variables with high contribution to Dim2 were contents of anions like phosphate and chloride as well as content of phosphorous (P), carbon (C), malic acid and total phenols. Dim1 was mainly influenced by contents of minerals like Ca, Mg, N, K, P and Zn as well as nitrate and anthocyanins. Apart from these multivariate results, univariate statistical evaluation shown in Tab. 2 was carried out to support the multivariate approach. While the content of malic acid, phosphate, P and Mn as well as total phenols and glucosinolates decreased in samples derived from reduced water supply, the content of polyols like inositol and glycerol as well as Cu increased in the same group. In brief, except for polyols and Cu, all other targeted constituents remained constant or were diminished under reduced water supply (Tab. 2). When subjecting the fresh matter-related data to PCA, the distinction of both treatments was substantially clearer (Fig. 3A) than when using dry matter-related data (Fig. 2A). The second principal component (Dim2) explaining 17% of the total variance. Similarly to the dry matter-related evaluation, the first two principal components accounted for 47.3% of the total variance. However, discrimination of both treatments was mainly based on primary metabolites like sugars and polyols, total carbon, total phenols, ascorbic acid as well as sulfate and copper (Fig. 2A-B). Univariate evaluation (Tab. 2) confirmed these results. Total glucosinolates decreased under reduced water supply. In contrast, contents of many other constituents such as polyols showed a significant increase. This is possibly being related to a concomitantly significantly increase in dry matter content (Tab. 2), which was also shown for carrot tap roots subjected to drought stress just prior to harvest (SØRENSEN et al., 1997).

Sugars and polyols

The dry matter-related content of sugars such as glucose and fructose was similar irrespective of the water supply applied ($0.1072 < p < 0.2932$ and $0.1992 < p < 0.6536$, resp., Tab. 2). Similarly, differences in fresh matter-related contents of glucose and fructose were insignificant, except for the set 2016-1, where glucose and fructose were significantly higher under reduced water supply cultivation conditions ($p = 0.0129$ and $p = 0.058$, respectively). In this latter set (2016-1), the overall glucose and fructose levels (1.40-1.48 and 0.99-1.03 g/100g FW) were higher than in all other sets (1.11-1.34 and 0.74-0.89 g/100 g FW). As shown in Tab. 1, the set of 2016-1 was grown under comparably warm and high radiation conditions, thus possibly evoking highest sugar accumulation as a result of enhanced photosynthetic rates. In agreement with our results, SCHREINER et al., (2002) found that contents of glucose and, in particular, fructose as metabolites of photosynthesis were highest in radish grown at high mean light intensities and high mean temperatures.

By analogy to glucose and fructose, highest levels of polyols were also found in the warm and well-irradiated growing period 2016-1 (Tab. 2). However, in contrast to glucose and fructose, both dry and fresh matter-related concentrations of polyols like glycerol and *myo*-inositol were significantly increased in 2016 and 2015 upon reduced water supply (Tab. 2 and 3), except for the set 2017 grown under cold

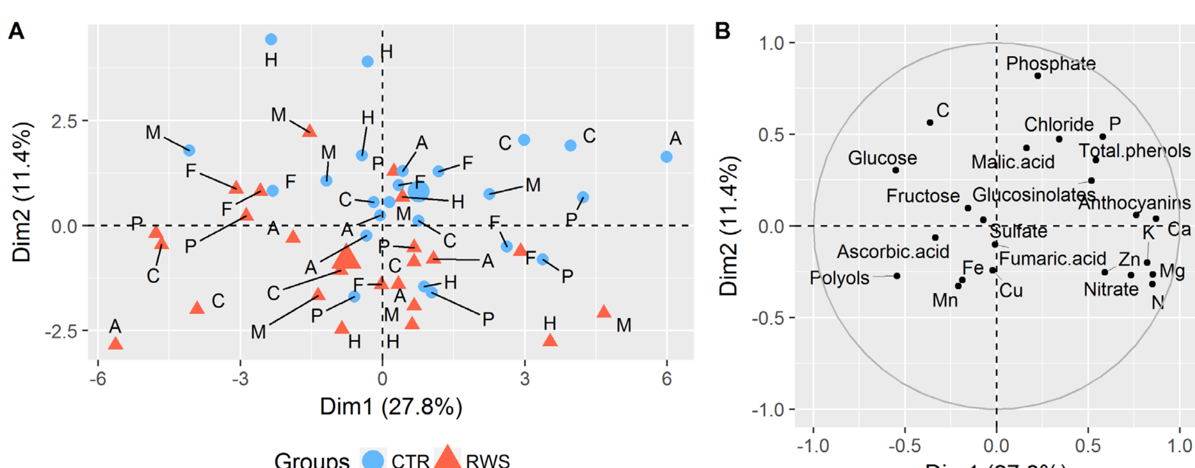

Fig. 3: Multivariate evaluation by PCA (PC1+PC2) of the total radish dataset including all cultivation sets (2015, 2016-1, 2016-2 and 2017) based on dry matter-analyses. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according control CTR (blue circles) and reduced water supply RWS (red triangles). The corresponding loading plot (B) shows the related variables determined by the chemical analyses.

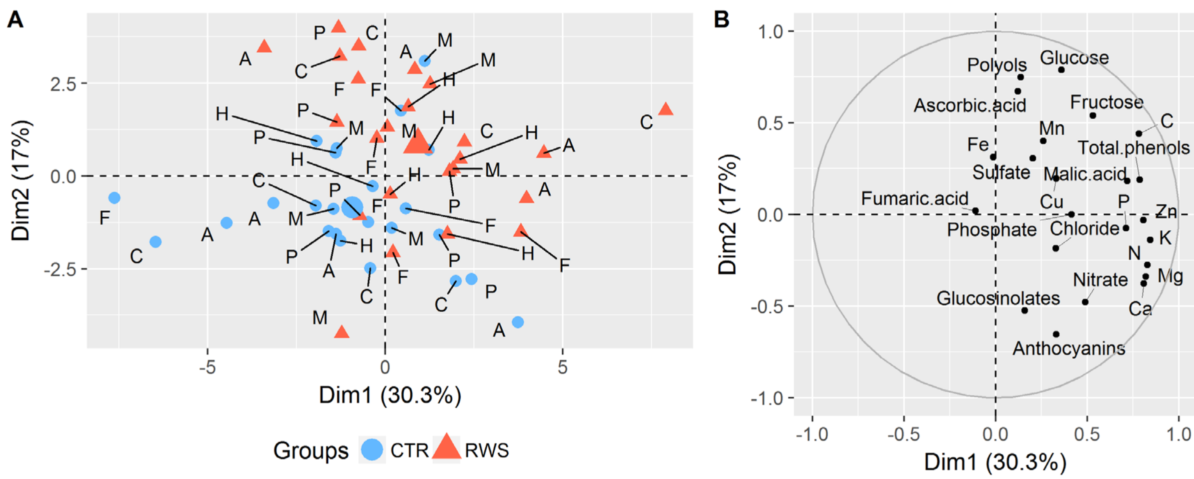

Fig. 4: Multivariate evaluation by PCA (PC1+PC2) of the total radish dataset including all cultivation sets (2015, 2016-1, 2016-2 and 2017) based on fresh matter-analyses. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according control CTR (blue circles) and reduced water supply RWS. The corresponding loading plot (B) shows the related variables determined by the chemical analyses.

(daily mean air temp.: 12 °C) and comparably well-watered (97% relative to control, Tab. 1) conditions. Supporting the hypothesis that water supply might have played a role, the reduced water supply in the set of 2015 was at 90% relative to full supply, already reaching a marginal statistical significance ($p = 0.1303$). Those of 2016-1 and 2016-2 were at a more intensely reduced water supply, i.e. at 85% and 80% rel. to full water supply, consequently yielding statistically significant differences (Tab. 2 and 3). Our data also shows that the climatic conditions in the set of 2016-1 resulted in an increase of total polyols, mainly that of inositol (Tab. 2). Polyols have been reported to act as compatible solutes in the osmotic adjustment and as osmoprotectants in plants, being accumulated to osmotically significant levels without adverse effects on the plant's metabolism (Nuccio et al., 1999). In this context, Dietrich et al. (2007) found strong increases of sorbitol in pear and apple juice exposed to drought stress. In agreement with our findings, strong increases of *myo*-inositol were observed in drought-stressed pea (*Pisum sativum* L.) in both the field experiment and under greenhouse conditions (Charlton et al., 2008). In our study, we show for the first time that the accumulation of *myo*-inositol and glycerol in radish might be elicited at already comparably small reductions in water supply, i.e., at ≤ 10% of the full water supply as indicated in our study.

Organic acids

The organic acids studied in our experiment were hardly affected by moderate water reduction, except for malic acid, which was negatively affected in 2015, but not in 2016-1, 2016-2 and 2017. The reason for these observations remains unclear. Similarly, ascorbic acid levels remained unchanged by mildly reducing water supply, suggesting that the nutritional value with regard to vitamin C remained unaffected irrespective of the water supply. However, by analogy to glucose, ascorbic acid levels were highest in the sets 2016-1 and 2016-2 grown in the warm and well-irradiated conditions, possibly being associated as glucose represents the biosynthetic precursor of ascorbic acid. In contrast to mildly reducing water supply, stronger drought stress was earlier shown to lead to an increase of ascorbic acid in fresh matter of hydroponically grown leafy vegetables like lettuce, spinach and turnip leaf (Koyama et al., 2012). In agreement with these results, we also found higher ascorbic acid levels in radish under mildly reduced water supply with statistical significance ($p = 0.0891$ and $p = 0.0517$, respectively) for the set 2016-1 as well as the total dataset.

Anions

Nitrate (NO_3^-) contents were only insignificantly influenced by reduced water supply (Tab. 2 and 3, Fig. 2A-B). However, NO_3^- contents were substantially lower in 2016-1 (11.1-13.8 mg/g DM) and 2017 (17.95-18.96 mg/g DM) in contrast to other cultivation sets (26.5-32.8 mg/g DM) as shown in Tab. 1. High radiation and evapotranspiration rates might have led to balanced conditions between absorption and assimilation of NO_3^-, resulting in lower NO_3^--levels. However, frequent stress-related conditions for the respective radish plants just before harvest might have affected NO_3^--levels in the set of 2016-1 (Tab. 1). In agreement with this hypothesis, Sørensen et al. (1997) found lowered nitrate levels in carrots (*Daucus carota* L.) even when drought stress occurred just prior to harvest. In contrast to 2016-1, where irrigation was finished two days prior to harvest, irrigation in 2016-2 was finished 6 days before harvest due to adequate soil humidity, probably having led to more continuous NO_3^- uptakes in contrast to 2016-1. Further study is needed though.

Although the dry matter-related content of phosphate (PO_4^{3-}) was significantly decreased by water supply reduction when considering the overall dataset, the significant decline of PO_4^{3-} was only observed in 2016-2, when water reduction was highest, i.e., to 80% of full water supply. Since phosphate mobility in the soil depends on moisture (Taiz and Zeiger, 2010), reduced soil moisture might have diminished the uptake into the tubers (da Silva et al., 2010).

Similarly to the other anions, sulfate (SO_4^{2-}) and chloride (Cl^-) levels were hardly affected by reduced water supply, except for the set 2017, where a significant increase in Cl^- from 0.89 to 0.96 mg/100 g DM ($p = 0.0375$) was observed (Tab. 2). While the levels of SO_4^{2-} and Cl^- were highest in 2016-1 and 2016-2, when irrigation events were more frequent compared to the other sets, the mild water deficit evoked in our study appeared to be insufficient to significantly affect sulfate and chloride uptake.

Carbon

Water supply reductions did not influence dry matter-related total carbon (C) as shown by multivariate and univariate analyses. Fresh matter-related total carbon showed to be significantly higher in samples grown under reduced water supply (1.75-1.95 g/100 g FM) than in control samples (1.67-1.91 g/100 g FM). These findings correlate well to significantly higher contents of dry matter in radish grown under reduced water supply. In agreement, multivariate evaluation confirmed a considerable contribution of C to the separation of both variants on fresh matter basis (Fig. 3A-B).

Nitrogen

Mild reductions in water supply had no significant impact on dry matter-related total nitrogen (N) in radish (Tab. 2, Fig. 2A-B), while it was associated with an apparently significant increase of fresh matter-related nitrogen contents in the warm and moist year 2015 and the cold and moist year 2017 (Tab. 3). In these years, the total nitrogen levels were substantially higher (94.5-104.0 mg/100 g FW) than in 2016 (72.1-81.6 mg/100 g FW). Because soil N availability is negatively affected by drought events and this strongly influences nutrient absorption and uptake by plants, it might be assumed that N mobility had been temporarily restricted due to insufficient total water amounts in 2016 (56-65 mm) in contrast to 2015 (125 mm) and 2017 (111 mm). In addition, lower air humidity in 2015 (67.1%) and 2017 (68.4%), in contrast to 2016 (83.3 to 84.2%), might have led to higher N levels. GISLERÖD et al. (1987) found decreased levels of selected macronutrients in greenhouse plants when exposed to higher air humidity. Further studies showed that negative effects on the plants' nitrogen level are alleviated with drying-rewetting cycles (HE and DIJKSTRA, 2014), possibly explaining the absent treatment effect in 2016-1 and 2016-2, although effects were expected due to several short-time drought events.

Potassium

By analogy to our observations on nitrogen levels, water supply limitations exerted only insignificant effects on dry matter-related contents of potassium according to both PCA analysis and univariate statistical evaluation (Fig. 2A-B, Tab. 2). In contrast, significantly increased fresh matter-related contents were observed in the sets of 2015 and 2017 with reduced water supply (Tab. 3), possibly being a result of generally increased dry matter, which in turn resulted from reduced moisture content. Since potassium plays multiple important functional roles in plant cells (TAIZ and ZEIGER, 2010), its level should be strictly regulated by plant metabolism. In agreement, AFZAL et al. (2014) also found only insignificant effects of drought stress on dry matter-related K^+ concentrations in leaves and roots of mungbean (*Vigna radiata* (L.) Wilczek). In our study, radish showed highest absolute K^+ levels in 2017 (232.6-255.6 mg/100 g FW; 51.5-53.2 mg/g DW) when precipitation was most frequent and abundant, as compared to the sets of other years (198.6-222.3 mg/100 g FW; 39.0-47.4 mg/100 g DW). Under these conditions in 2017 (cold and moist), however, K^+ levels notably increased upon water supply limitation ($p = 0.0405$, fresh matter; $p = 0.0711$, dry matter).

Phosphorous

The content of phosphorous (P) in radish dry matter was significantly decreased by moderately reduced water supply. By analogy to PO_4^{3-}, the decline of P was highly significant for the total dataset as well as for the single cultivation sets 2015 and 2016-2 (Tab. 2). Multivariate analyses by PCA support this result (Fig. 2A-B). GREENWAY et al. (1969) found that even a small decrease in water potential reduces P uptake by tomato plants (*Lycopersicon esculentum* Mill.), while water uptake and transport were generally lower in gradually treated plants in contrast to plants suddenly exposed to low water potential. However, P uptake was much less for a considerable time even if plants were exposed to stress for only one hour. Further studies in agreement with these and our findings have been reported (SARDANS and PEÑUELAS, 2004; SÁNCHEZ-RODRÍGUEZ et al., 2010).

Calcium

Reduced water supply led to lower calcium (Ca^{2+}) contents in radish dry matter, being significant in 2016-1 ($p = 0.0266$) and 2016-2 ($p = 0.0907$), but not in the cultivation sets of 2015 and 2017. Contrary to K^+ or N, absolute Ca^{2+} content was higher in reducibly watered samples in 2017, but water reduction was very low (Tab. 1). In general, Ca^{2+} acts as important constituent of the middle lamella of the cell wall and it is required in numerous metabolic processes (TAIZ and ZEIGER, 2010) including responses to abiotic stress like drought (HU and SCHMIDHALTER, 2005; KNIGHT et al., 1997). However, SÁNCHEZ-RODRÍGUEZ et al. (2010) found no differences in the Ca^{2+} accumulation of tomato leaves when plants were treated by moderate drought stress, although its uptake was significantly affected. HU and SCHMIDHALTER (2005) concluded that even if its uptake is reduced, the accumulation of Ca^{2+} declines very slightly. In low-transpiring organs like tubers or fruits, which are supplied predominantly via the phloem, its concentration is considerably lower than in leaves (MARSCHNER, 1986). In agreement, we did not observe any consistent effect of reduced waters supply on the Ca^{2+} content in red radish.

Magnesium

Similarly to other minerals, effects on magnesium (Mg^{2+}) with regard to reduced waters supply on radish were apparently insignificant as shown by multivariate analyses (Fig. 2A-B). However, univariate evaluation demonstrated an increase in the Mg^{2+} content due to reduced water supply in 2016-1 ($p = 0.0946$) (Tab. 2). In agreement, PULUPOL et al. (1996) found only insignificant effects of deficit irrigation on the dry weight-based levels of magnesium in tomato fruits grown in a glasshouse. However, fresh weight-based Mg^{2+} contents have been found to be higher in fruits of deficit irrigated plants than in fruits of well-watered plants (PULUPOL et al., 1996), which also applies to radish fresh matter grown under moist conditions in 2015 ($p = 0.0642$) and 2017 ($p = 0.0387$) (Tab. 3).

Micronutrients (Fe, Zn, Mn, Cu)

Dry matter-related iron (Fe) contents were reduced in 2015 ($p = 0.0846$), whereas zinc (Zn) and copper (Cu) were increased in 2017 ($p = 0.0297$ and $p = 0.0523$, respectively) by slight water reduction (Tab. 2). These findings might be related to the involvement of these micronutrients in photosynthesis and their catalytic role in the antioxidant systems of plants (HÄNSCH and MENDEL, 2009). Evaluation of the total dataset indicated a significant dry matter-related decrease of manganese (Mn) and a significant increase in Cu upon water supply limitation. Similar observations have been reported earlier (SÁNCHEZ-RODRÍGUEZ et al., 2010). PCA analyses supported the contribution of these micronutrients to the variants' differentiation (Fig. 2B). Because micronutrient transport through the soil and towards the root is governed by diffusion, diminished levels were expected when limiting water supply. Considering the total fresh matter-related dataset (Tab. 3), significantly higher contents of Zn and Cu were found for samples with limited water supply in 2017. Our results showed generally reduced contents of Fe in 2016-1 and 2017 (Tab. 2), when global radiation was highest. The reduced contents of Fe in the set of 2016-1 are in agreement with the trend found for most macronutrients. However, this results are contradictory to ZANCAN et al. (2006), who found iron accumulation in roots and leaves of maize provoked by high UV-B radiation.

Phenolic compounds

Reduced water supply led to significant reductions ($p = 0.0523$) in the dry matter-related content of total phenols (Tab. 2), while fresh matter-related contents remained unchanged (Tab. 3). Previous studies reported variable effects of drought stress on levels of polyphenols including anthocyanins in the respective plants (ROBBINS et al., 2005).

In agreement with literature, the quantitatively most abundant anthocyanins in radish skin were acylated derivatives, namely pelargo-

Tab. 2: Influence of reduced water supply on the dry matter (DM)-related levels of constituents of radish tubers from different cultivation sets (2015, 2016-1, 2016-2 and 2017). Linear mixed model, t-test, p-values: < 0.1, < 0.05 (*), < 0.01 (**) and < 0.001 (***), nd = not determined. CTR: Control treatment with full water supply. RWS: Reduced water supply treatment.

	Radish 2015			Radish 2016-1			Radish 2016-2			Radish 2017			Radish total		
	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value
Leaf water potential															
Ψ [-MPa]	nd	nd	nd	0.15	0.21	**0.0123** **	0.16	0.24	**0.0002** ***	0.07	0.09	**0.0006** ***	nd	nd	nd
Dry matter [%]	4.79	4.97	**0.0337** *	5.00	5.17	**0.0886**	4.64	4.71	**0.0035** **	4.61	4.75	0.1736	4.76	4.90	**0.0001** ***
Sugars and Polyols															
Glucose [mg/g]	254.5	244.6	0.2932	281.1	286.5	0.1577	272.9	283.7	0.1072	247.06	239.92	0.1846	269.5	271.6	0.9550
Fructose [mg/g]	184.3	178.9	0.3452	197.3	199.2	0.6387	160.2	162.3	0.6536	168.39	162.01	0.1992	180.6	180.1	0.3963
Polyols total [mg/g]	2.07	2.18	0.1303	3.66	4.40	**0.0009** ***	1.71	1.85	**0.0072** **	1.97	2.01	0.5062	2.48	2.81	**0.0009** ***
Inositol [mg/g]	1.75	1.85	0.1467	3.15	3.81	**0.0019** **	1.37	1.51	**0.0071** **	1.41	1.43	0.6012	2.09	2.39	**0.0010** **
Glycerol [mg/g]	0.32	0.34	0.2722	0.51	0.60	**0.0021** **	0.35	0.34	0.4549	0.56	0.58	0.5521	0.39	0.42	**0.0197** *
Organic acids															
Total acidity [mg/g]	15.0	14.6	0.3111	11.2	10.1	0.3701	12.3	11.8	0.6768	10.60	10.09	**0.0816**	12.8	12.2	0.1109
Malic acid [mg/g]	36.1	34.7	**0.0454** *	30.5	29.7	0.1487	34.9	34.3	0.1865	35.66	35.70	0.9680	33.8	32.9	**0.0235** *
Fumaric acid [mg/g]	3.39	3.49	0.4969	4.86	4.63	0.2960	4.81	6.39	0.3158	2.52	2.70	0.8172	4.35	4.34	0.8718
Ascorbic acid [mg/g]	5.64	5.47	0.2969	5.80	6.03	0.2339	6.45	6.45	0.9872	5.21	5.30	0.3032	5.96	5.98	0.6362
Anions															
Nitrate [mg/g]	32.8	32.3	0.8200	13.8	11.1	0.1077	27.8	26.5	0.5418	17.95	18.96	0.4586	24.77	23.29	0.3540
Phosphate [mg/g]	11.48	11.16	0.4993	12.90	12.12	0.1668	11.14	10.17	**0.0211** *	10.04	9.89	0.6674	11.84	11.15	**0.0092** **
Sulfate [mg/g]	2.52	2.75	0.5239	3.99	4.13	0.2328	4.00	4.05	0.7945	3.47	3.32	0.5819	3.51	3.64	0.5680
Chloride [mg/g]	0.88	0.98	0.5387	1.90	1.92	0.8835	1.79	1.27	0.1402	0.89	0.96	**0.0375** *	1.52	1.38	0.3948
Elements															
Carbon [mg/g]	370.7	369.9	0.5456	382.9	382.6	0.7838	372.8	371.7	0.6534	368.53	367.43	0.4987	375.5	374.8	0.2969
Macronutrients															
Potassium [mg/g]	44.95	44.76	0.7944	40.02	38.95	0.2718	47.35	46.05	0.4133	51.54	53.18	**0.0711**	44.10	43.26	0.6644
Nitrogen[mg/g]	20.24	20.36	0.6406	14.69	14.14	0.1497	17.62	16.72	0.1060	20.93	21.60	0.1840	17.52	17.07	0.4893
Phosphorous [mg/g]	4.15	4.03	**0.0241** *	4.29	4.17	0.3220	4.67	4.35	**0.0081** **	4.51	4.48	0.6585	4.37	4.18	**0.0056** **
Calcium [mg/g]	3.57	3.59	0.7281	3.01	2.86	**0.0266** *	3.54	3.22	**0.0907**	2.91	3.07	**0.0824**	3.37	3.22	0.2259
Magnesium [mg/g]	1.30	1.33	0.3591	0.97	1.03	**0.0946**	1.26	1.19	0.2125	1.29	1.35	0.1756	1.20	1.16	0.5856
Micronutrients															
Iron [µg/g]	90.27	76.85	**0.0846**	68.57	70.29	0.7039	94.70	97.00	0.7962	84.02	70.76	0.2825	84.84	81.38	0.1873
Zinc [µg/g]	22.65	22.32	0.4337	20.27	20.14	0.8511	23.43	23.25	0.6763	22.11	23.11	**0.0297** *	22.12	21.90	0.7166
Manganese [µg/g]	11.19	10.87	0.2130	9.45	9.47	0.9389	10.85	10.51	0.2925	9.91	8.98	0.1312	10.52	10.28	**0.0355** *
Copper [µg/g]	4.29	5.39	0.1907	5.83	6.11	0.6488	5.68	5.77	0.6223	5.21	5.86	**0.0523**	5.27	5.76	**0.0397** *
Phenolic compounds															
Total phenols [mg/g]	11.0	10.8	0.4305	11.2	11.0	**0.0849**	11.59	11.16	0.1487	10.17	10.17	0.9750	11.25	10.99	**0.0523**
Total anthocyanins [mg/g peel]	20.36	21.32	0.1570	26.32	23.96	**0.0909**	33.99	31.51	0.1040	29.19	29.53	0.3814	26.89	25.60	0.1217
Glucosinolates															
Total glucosinolates [µmol/g]	11.97	12.30	0.7959	28.52	21.49	**0.0159** **	26.32	23.67	0.4396	16.06	16.60	**0.0528**	22.27	19.16	**0.0670**
Aliphatic glucosinolates [µmol/g]	11.22	11.53	0.8026	27.88	21.00	**0.0153** **	25.55	23.09	0.4627	15.51	16.01	**0.0523**	21.55	18.54	**0.0691**
Indolic glucosinolates [µmol/g]	0.75	0.79	0.7086	0.64	0.49	0.2062	0.76	0.58	**0.0260** *	0.55	0.59	0.2419	0.72	0.62	**0.0887**

Tab. 3: Influence of reduced water supply on the fresh matter (FM)-related levels of constituents of radish tubers from different cultivation sets (2015, 2016-1, 2016-2 and 2017). Linear mixed model, t-test, p-values: < 0.1, < 0.05 (*), < 0.01 (**) and < 0.001 (***), nd = not determined. CTR: Control treatment with full water supply. RWS: Reduced water supply treatment.

	Radish 2015			Radish 2016-1			Radish 2016-2			Radish 2017			Radish total		
	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value
Leaf water potential															
Ψ [-MPa]	nd	nd	nd	0.15	0.21	**0.0123** *	0.16	0.24	**0.0002** ***	0.10	0.15	**0.0006** ***	nd	nd	nd
Dry matter [%]	4.79	4.97	**0.0337** *	4.64	4.71	**0.0886**	4.78	4.98	**0.0035** **	4.61	4.75	0.1736	4.76	4.90	**0.0001** ***
Sugars and Polyols															
Glucose [g/100 g]	1.22	1.22	0.9732	1.40	1.48	**0.0129** *	1.27	1.34	**0.0734**	1.11	1.15	0.4301	1.25	1.30	**0.0315** *
Fructose [g/100 g]	0.88	0.89	0.8612	0.99	1.03	**0.0580**	0.74	0.76	0.3632	0.76	0.78	0.6191	0.84	0.87	0.1060
Polyols total [mg/100 g]	9.93	10.84	**0.0640**	18.26	22.47	**0.0012** **	36.93	39.23	**0.0494** *	42.24	42.79	0.7267	26.84	28.83	**0.0011** **
Inositol [mg/100 g]	8.39	9.18	**0.0681**	15.70	19.44	**0.0019** **	29.46	32.02	**0.0299** *	30.19	30.45	0.8205	20.93	22.77	**0.0006** ***
Glycerol [mg/100 g]	1.55	1.65	0.1056	2.56	3.03	**0.0017** **	7.47	7.21	0.2660	12.05	12.34	0.6387	5.91	6.06	0.3452
Organic acids															
Total acidity [mg/100 g]	71.67	73.33	0.5623	55.00	56.67	0.7926	56.67	53.33	0.3632	48.33	48.33	1.0000	58.33	57.50	0.6643
Malic acid [mg/100 g]	171.66	175.00	0.3632	153.33	153.33	1.0000	163.33	160.00	0.1747	160.00	170.00	0.2031	162.09	164.58	0.2656
Fumaric acid [mg/100 g]	16.20	16.83	0.3615	24.73	23.89	0.3358	23.59	23.63	0.9488	10.73	12.30	0.5945	18.81	19.16	0.6467
Ascorbic acid [mg/100 g]	26.98	27.17	0.7796	28.95	30.98	**0.0891**	29.94	30.35	0.6099	24.43	24.86	0.3860	27.57	28.34	**0.0517**
Anions															
Nitrate [mg/100 g]	156.61	160.59	0.6672	68.85	57.17	0.1567	129.17	124.49	0.6269	80.46	90.94	**0.0869**	108.77	108.30	0.9165
Phosphate [mg/100 g]	54.92	55.28	0.8917	64.34	62.56	0.5315	51.69	47.92	**0.0603**	45.17	47.58	0.1262	54.03	53.34	0.5312
Sulfate [mg/100 g]	12.04	13.58	0.3944	19.97	21.34	**0.0693**	18.55	19.07	0.5622	15.57	15.73	0.8995	16.53	17.43	0.1153
Chloride [mg/100 g]	4.23	4.84	0.3983	9.52	9.63	0.8613	8.32	5.88	0.1462	4.03	4.53	**0.0675**	6.53	6.22	0.5244
Elements															
Carbon [g/100 g]	1.77	1.84	**0.0392** *	1.91	1.95	**0.0207** *	1.73	1.75	0.1962	1.67	1.77	0.1770	1.77	1.83	**0.0046** **
Macronutrients															
Potassium [mg/100 g]	215.16	222.30	**0.0155** *	199.39	198.61	0.8630	219.88	216.72	0.6784	232.64	255.56	**0.0405** *	216.77	223.30	**0.0759**
Nitrogen [mg/100 g]	96.77	101.06	**0.0088** **	73.18	72.13	0.5282	81.85	78.69	0.2546	94.52	104.01	**0.0469** *	86.58	88.97	0.1311
Phosphorous [mg/100 g]	19.88	20.03	0.3780	21.37	21.24	0.8066	21.69	20.51	**0.0534**	20.21	21.69	0.1323	20.79	20.87	0.8006
Calcium [mg/100 g]	17.04	17.81	0.1188	15.01	14.57	0.1292	16.42	15.15	0.1631	13.15	14.80	**0.0169** *	15.41	15.58	0.5958
Magnesium [mg/100 g]	6.20	6.59	**0.0642**	5.14	4.96	0.1969	5.85	5.58	0.3290	5.84	6.50	**0.0387** *	5.76	5.91	0.2440
Micronutrients															
Iron [µg/100 g]	433.98	382.79	0.1748	342.23	358.24	0.4520	451.42	456.74	0.8885	377.74	341.80	0.5064	384.89	402.16	0.3976
Zinc [µg/100 g]	108.22	110.80	0.2798	101.03	102.71	0.6131	108.78	109.39	0.7859	99.99	111.04	**0.0199** *	104.50	108.49	**0.0162** *
Manganese [µg/100 g]	53.56	54.02	0.7145	47.09	48.28	0.3652	50.37	49.42	0.4395	44.75	43.26	0.6470	48.96	48.74	0.8279
Copper [µg/100 g]	20.50	26.73	0.1436	29.09	31.11	0.4915	26.35	27.19	0.3222	23.54	28.14	**0.0152** *	24.87	28.29	**0.0094** **
Phenolic compounds															
Total phenols [mg/g]	53.34	54.41	0.4228	55.87	56.66	0.4263	57.93	57.63	0.8291	46.01	49.08	0.2009	53.29	54.45	0.1364
Total anthocyanins [mg/g peel]	128.35	139.14	**0.0349** *	131.17	122.13	0.1553	158.04	148.32	0.1993	136.99	138.30	0.3581	138.64	136.97	0.5703
Glucosinolates															
Total glucosinolates [µmol/g]	57.07	61.04	0.5404	142.23	109.68	**0.0253** *	122.07	111.04	0.4724	74.19	76.63	**0.0635**	98.89	89.60	0.1067
Aliphatic glucosinolates [µmol/g]	53.48	57.18	0.5545	139.05	107.19	**0.0245** *	118.53	108.31	0.4968	72.75	74.96	**0.0801**	95.95	86.91	0.1076
Indolic glucosinolates [µmol/g]	3.59	3.86	0.3901	3.19	2.50	0.2458	3.54	2.72	**0.0299** *	1.44	1.67	**0.0628**	2.94	2.69	0.2019

nidin-3-*O*-(*p*-coumaroyl-)-diglucoside-5-*O*-(malonyl-)-glucoside and pelargonidin-3-*O*-(feruloyl)-diglucoside-5-*O*-(malonyl)-glucoside, accounting for approx. 80% of the total anthocyanins (Wu and Prior, 2005) (data not shown). Limited water supply led to only insignificant effects on total anthocyanins in radish. Univariate analysis indicated a dry matter-related decline of total anthocyanins in radish skin upon limited water supply in 2016 (Tab. 2), whereas a fresh matter-related increase was found in 2015. In agreement, multivariate analyses did not indicate a strong contribution of anthocyanins to the differentiation of samples grown under full or reduced water supply (Fig. 2A-B). Interestingly, both fresh and dry matter-related contents of anthocyanins were highest in 2016-2 followed by 2017 (Tab. 2 and 3). In the context of seasonal effects on radish tuber color, Schreiner et al. (2002) found most intense red coloration at high photosynthetic photon flux density, hypothesizing its production to be dependent on light intensity, quality and duration (Mazza and Miniati, 1993). In addition, an earlier study revealed that radish gained more distinctive red shades at lower mean temperatures (Schreiner et al., 2002). In our study, lowest mean temperature and highest global radiation occurred in 2017, which also showed high amounts of anthocyanins apart from the set 2016-2 (Tab. 2 and 3). In brief, mild water supply reduction did not significantly affect anthocyanin contents, representing a most important commercial quality trait due to its direct correlation with the tubers' color.

Glucosinolates

Reduced water supply led to decreased dry matter-related contents of total, aliphatic and indolyl glucosinolates. Most abundant glucosinolates in radish were aliphatic derivatives such as glucoraphasatin (4-methylthio-3-butenyl glucosinolate) and glucoraphenin (4-methylsulfinyl-3-butenyl glucosinolate). In earlier studies, drought stress has been shown to more likely increase glucosinolate concentration in *Brassica* species such as *Brassica carinata* (Schreiner et al., 2009), *B. rapa* ssp. *rapifera* L. (Zhang et al., 2008) and *B. napus* L. (Jensen et al., 1996). However, the intensity of drought appears to be a decisive impact factor in glucosinolate increase, because, under minor drought, glucosinolate concentrations did not increase in *Brassica napus* plants (Jensen et al., 1996) and decreased in *Brassica oleracea* var. *italica*. (Robbins et al., 2005) Thus, Robbins et al. (2005) also observed decreasing concentrations of mainly indolyl-glucosinolates in broccoli (*B. oleracea* var. *italica*) induced by a slightly reduced water supply. Our results showed that total glucosinolates concentration was highest in 2016 (21.49-28.52 µmol/g DM) as compared to the other years (11.97-16.60 µmol/g DM). In 2016, precipitation was lowest and, thus, irrigation events were frequently released, but total water amounts were consideratly lower compared to 2015 and 2017. Zhang et al. (2008) also proved significant interactions of growing season and water supply for turnip, which underlines the seasonal-dependent effects on glucosinolates.

Conclusion

In conclusion, slightly reduced water supply situations, as expected in the future due to climate change, were shown to alter the chemical composition of red radish grown in open field cultivation. The effects expectedly appeared to vary in dependence of the respective environmental conditions. Dry matter contents were mainly enhanced by moderate water reduction and the same was found for polyols, which seem to be highly sensitive to slight water lowering. Being important for color and pungency of the produce, anthocyanin and glucosinolate levels were shown to be only marginally affected. In brief, our results indicate that red radish plants are able to cope with moderately reduced water supply, only marginally affecting the overall quality of its commercialized tubers. However, limitations of water supply in areas of vegetable production may increase by the on-going climate change and thus exceed those exposed in our simulation, ultimately aggravating the effects observed in our study and thus still impacting crop quality.

Funding sources

The LOEWE excellence cluster FACE2FACE from the Hessian State Ministry of Higher Education, Research and the Arts (Wiesbaden, Germany) funded this study.

Supplementary data

Information on the daily mean weather conditions during the different cultivation periods 2015, 2016-1, 2016-2 and 2017 is supplied in Fig A.1-4 as supporting information.

References

Afzal, A., Gulzar, I., Shahbaz, M., Ashraf, M., 2014: Water deficit-induced regulation of growth, gas exchange, chlorophyll fluorescence, inorganic nutrient accumulation and antioxidative defense mechanism in mungbean (*Vigna radiata* (L.) Wilczek). J. Appl. Bot. Food Qual. 87, 147-156. DOI: 10.5073/JABFQ.2014.087.022

Aires, A., Fernandes, C., Carvalho, R., Bennett, R.N., Saavedra, M.J., Rosa, E.A.S., 2011: Seasonal effects on bioactive compounds and antioxidant capacity of six economically important brassica vegetables. Molecules 16, 6816-6832. DOI: 10.3390/molecules16086816

Akinci, S., Dorothy, L.M., 2012: Plant Water-Stress Response Mechanisms. In: Rahman, I.M.M., Hasegawa, H. (eds.), Water Stress, 15-42. InTech.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015: Fitting Linear Mixed-Effects Models Using {lme4}. J. Stat. Softw. 67, 1-48. DOI: 10.18637/jss.v067.i01

Bindi, M., Olesen, J.E., 2011: The responses of agriculture in Europe to climate change. Reg. Environ. Change 11, 151-158. DOI: 10.1007/s10113-010-0173-x

Charlton, A.J., Donarski, J., Harrison, M., Jones, S., Godward, J., Oehlschlager, S., Arques, J., Ambrose, M., Chinoy, C., Mullineaux, P., Domoney, C., 2008: Responses of the pea (*Pisum sativum* L.) leaf metabolome to drought stress assessed by nuclear magnetic resonance spectroscopy. Metabolomics 4. DOI: 10.1007/s11306-008-0128-0

Costa, J.M., Ortuño, M.F., Chaves, M.M., 2007: Deficit irrigation as a strategy to save water. Physiology and potential application to horticulture. J. Integr. Plant Biol. 49, 1421-1434. DOI: 10.1111/j.1672-9072.2007.00556.x

da Silva, E.C., Nogueira, R.J.M.C., Silva, M., Albuquerque, M., 2010: Drought Stress and Plant Nutrition. Plant Stress 5.

Daryanto, S., Wang, L., Jacinthe, P.-A., 2016: Drought effects on root and tuber production. A meta-analysis. Agr. Water Manage. 176, 122-131. DOI: 10.1016/j.agwat.2016.05.019

Dietrich, H., Kruger-Steden, E., Patz, C.D., Will, F., Rheinberger, A., Hopf, I., 2007: Increase of sorbitol in pear and apple juice by water stress, a consequence of climatic change? Fruit Processing 6, 348-355.

Gisleröd, H.R., Selmer-Olsen, A.R., Mortensen, L.M., 1987: The effect of air humidity on nutrient uptake of some greenhouse plants. Plant Soil 102, 193-196. DOI: 10.1007/BF02370702

Grantz, D.A., 1990: Plant response to atmospheric humidity. Plant Cell Environ. 13, 667-679. DOI: 10.1111/j.1365-3040.1990.tb01082.x

Greenway, H., Hughes, P.G., Klepper, B., 1969: Effects of water deficit on phosphorus nutrition of tomato plants. Physiol. Plantarum 22, 199-207. DOI: 10.1111/j.1399-3054.1969.tb07856.x

Hänsch, R., Mendel, R.R., 2009: Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant Biol. 12, 259-266. DOI: 10.1016/j.pbi.2009.05.006

He, M., Dijkstra, F.A., 2014: Drought effect on plant nitrogen and phosphorus. A meta-analysis. New Phytol. 204, 924-931. DOI: 10.1111/nph.12952

Hirata, T., Cho, Y.-M., Suzuki, I., Toyoda, T., Akagi, J.-I., Nakamura, Y., Numazawa, S., Ogawa, K., 2018: 4-Methylthio-3-butenyl isothiocyanate mediates nuclear factor (erythroid-derived 2)-like 2 activation by regulating reactive oxygen species production in human esophageal epithelial cells. Food Chem. Toxicol. 111, 295-301. DOI: 10.1016/j.fct.2017.11.022

Hu, Y., Schmidhalter, U., 2005: Drought and salinity. A comparison of their effects on mineral nutrition of plants. J. Plant Nutr. Soil Sci. 168, 541-549. DOI: 10.1002/jpln.200420516

Jensen, C.R., Mogensen, V.O., Mortensen, G., Fieldsend, J.K., Milford, G.F.J., Andersen, M.N., Thage, J.H., 1996: Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. Field Crop Res. 47, 93-105. DOI: 10.1016/0378-4290(96)00026-3

Jing, P., Zhao, S.-J., Ruan, S.-Y., Xie, Z.-H., Dong, Y., Yu, L., 2012: Anthocyanin and glucosinolate occurrences in the roots of Chinese red radish (*Raphanus sativus* L.), and their stability to heat and pH. Food Chem. 133, 1569-1576. DOI: 10.1016/j.foodchem.2012.02.051

Josse, J., Husson, F., 2016: missMDA: A package for handling missing values in multivariate data analysis. J. Stat. Softw. 70, 1-31. DOI: 10.18637/jss.v070.i01

Kassambara, A., Mundt, F., 2017: factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.4. https://CRAN.R-project.org/package=factoextra.

Knight, H., Trewavas, A.J., Knight, M.R., 1997: Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. Plant J. 12, 1067-1078. DOI: 10.1046/j.1365-313X.1997.12051067.x

Koyama, R., Itoh, H., Kimura Syuji, Morioka, A., Uno, Y., 2012: Augmentation of antioxidant constituents by drought stress to roots in leafy vegetables. HortTechnology 22.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016: lmerTest: Tests in linear mixed effects models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest.

Le, S., Josse, J., Husson, F., 2008: FactoMineR: An R package for multivariate analysis. J. Stat. Softw. 25, 1-18. DOI: 10.18637/jss.v025.i01

Lenth, R.V., 2016: Least-Squares Means: The R Package lsmeans. J. Stat. Softw. 69, 1-33. DOI: 10.18637/jss.v069.i01

Manchali, S., Chidambara Murthy, K.N., Patil, B.S., 2012: Crucial facts about health benefits of popular cruciferous vegetables. J. Funct. Foods 4, 94-106. DOI: 10.1016/j.jff.2011.08.004

Marschner, H., 1986: Mineral nutrition of higher plants. Academic Press, London.

Mazza, G., Miniati, E., 1993: Anthocyanins in fruits, vegetables, and grains. CRC Press, Inc.

Nikulin, G., Kjellström, E., Hansson, U.L.F., Strandberg, G., Ullerstig, A., 2011: Evaluation and future projections of temperature, precipitation and wind extremes over Europe in an ensemble of regional climate simulations. Tellus A 63, 41-55. DOI: 10.1111/j.1600-0870.2010.00466.x

Nishihara, E., Inoue, M., Kondo, K., Takahashi, K., Nakata, N., 2001: Spinach yield and nutritional quality affected by controlled soil water matric head. Agr. Water Manage. 51, 217-229. DOI: 10.1016/S0378-3774(01)00123-8

Nuccio, M.L., Rhodest, D., McNeil, S.D., Hanson, A.D., 1999: Metabolic engineering of plants for osmotic stress resistance. Curr. Opin. Plant Biol. 2, 128-134. DOI: 10.1016/S1369-5266(99)80026-0

Papetti, A., Milanese, C., Zanchi, C., Gazzani, G., 2014: HPLC–DAD–ESI/MSn characterization of environmentally friendly polyphenolic extract from *Raphanus sativus* L. var. "Cherry Belle" skin and stability of its red components. Food Res. Int. 65, 238-246. DOI: 10.1016/j.foodres.2014.04.046

Prasad, P.V.V., Staggenborg, S.A., Ristic, Z., Ahuja, L.R., Reddy, V.R., Saseendran, S.A., Yu, Q., 2008: Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Ahuja, L., Reddy, V.R., Saseendran, S.A., Yu, Q. (eds.), Response of crops to limited water. Understanding and modeling water stress effects on plant growth processes, 301-355. American Society of Agronomy; Crop Science Society of America; Soil Science Society of America, Madison, Wisconsin.

Pulupol, L.U., Behboudian, M.H., Fisher, K.J., 1996: Growth, yield, and postharvest attributes of glasshouse tomatoes produced under deficit irrigation. HortScience 31, 926-929.

R Core Team, 2016: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robbins, R., Keck, A., Banuelos, G., Finley, J., 2005: Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. J. Med. Food 8. DOI: 10.1089/jmf.2005.8.204

Sánchez-Rodríguez, E., del Mar Rubio-Wilhelmi, M., Cervilla, L.M., Blasco, B., Rios, J.J., Leyva, R., Romero, L., Ruiz, J.M., 2010: Study of the ionome and uptake fluxes in cherry tomato plants under moderate water stress conditions. Plant Soil 335, 339-347. DOI: 10.1007/s11104-010-0422-2

Sardans, J., Peñuelas, J., 2004: Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. Plant Soil 267, 367-377. DOI: 10.1007/s11104-005-0172-8

Schmidt, N., Zinkernagel, J., 2017: Model and growth stage based variability of the irrigation demand of onion crops with predicted climate change. Water 9, 693. DOI: 10.3390/w9090693

Schreiner, M., 2005: Vegetable crop management strategies to increase the quantity of phytochemicals. Eur. J. Nutr. 44, 85-94. DOI: 10.1007/s00394-004-0498-7

Schreiner, M., Beyene, B., Krumbein, A., Stützel, H., 2009: Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in *Brassica carinata* leaves as affected by water supply. J. Agric. Food Chem. 57, 7259-7263. DOI: 10.1021/jf901076h

Schreiner, M., Huyskens-Keil, S., Peters, P., Schonhof, I., Krumbein, A., Widell, S., 2002: Seasonal climate effects on root colour and compounds of red radish. J. Sci. Food Agric. 82, 1325-1333. DOI: 10.1002/jsfa.1189

Singleton, V.L., Rossi, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144.

Sørensen, J.N., Jørgensen, U., Kühn, B.F., 1997: Drought effects on the marketable and nutritional quality of carrots. J. Sci. Food Agric. 74, 379-391. DOI: 10.1002/(SICI)1097-0010(199707)74:3<379::AID-JSFA814>3.0.CO;2-Y

Souci, S.W., Fachmann, W., Kraut, H., Kirchhoff, E., 2008: Food composition and nutrition tables. Die Zusammensetzung der Lebensmittel, Nährwert-Tabellen. La composition des aliments, Tableaux des valeurs nutritives. Medpharm Scientific Publ, Stuttgart.

Taiz, L., Zeiger, E., 2010: Plant Physiology. Fifth Edition. Das Original mit Übersetzungshilfen. Ainauer Associates Inc., Sunderland, Massachusetts USA.

Wang, Y., Frei, M., 2011: Stressed food – The impact of abiotic environmental stresses on crop quality. Agr. Ecosyst. Environ. 141, 271-286. DOI: 10.1016/j.agee.2011.03.017

Wiesner, M., Zrenner, R., Krumbein, A., Glatt, H., Schreiner, M., 2013: Genotypic variation of the glucosinolate profile in pak choi (*Brassica rapa* ssp. *chinensis*). J. Agric. Food Chem. 61, 1943-1953. DOI: 10.1021/jf303970k

Will, F., Dietrich, H., 2006: Optimised processing technique for colour- and cloud-stable plum juices and stability of bioactive substances. Eur. Food Res. Technol. 223, 419-425. DOI: 10.1007/s00217-005-0222-8

Wu, X., Prior, R.L., 2005: Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts, and grains. J. Agric. Food Chem. 53, 3101-3113. DOI: 10.1021/jf0478861

Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R., Chen, S.S., 2004: Flavonoids in food and their health benefits. Plant Food Hum. Nutr. 59, 113-122. DOI: 10.1007/s11130-004-0049-7

Zancan, S., Cesco, S., Ghisi, R., 2006: Effect of UV-B radiation on iron content and distribution in maize plants. Environ. Exp. Bot. 55, 266-272. DOI: 10.1016/j.envexpbot.2004.11.004

Zhang, H., Schonhof, I., Krumbein, A., Gutezeit, B., Li, L., Stützel, H., Schreiner, M., 2008: Water supply and growing season influence glucosinolate concentration and composition in turnip root (*Brassica rapa* ssp. *rapifera* L.). J. Plant Nutr. Soil Sci. 171, 255-265. DOI: 10.1002/jpln.200700079

ORCID
Christine Schlering https://orcid.org/0000-0003-3605-9875
Helmut Dietrich https://orcid.org/0000-0002-0335-247X
Matthias Frisch https://orcid.org/0000-0003-4234-8972
Monika Schreiner https://orcid.org/0000-0002-5629-4429
Ralf Schweiggert https://orcid.org/0000-0003-0546-1335
Frank Will https://orcid.org/0000-0003-0369-3087
Jana Zinkernagel https://orcid.org/0000-0001-6817-091X

Address of the corresponding author:
Christine Schlering, Department of Soil Science and Plant Nutrition, Department of Microbiology and Biochemistry, Geisenheim University, Germany
E-mail: Christine.Schlering@hs-gm.de

Supplementary material

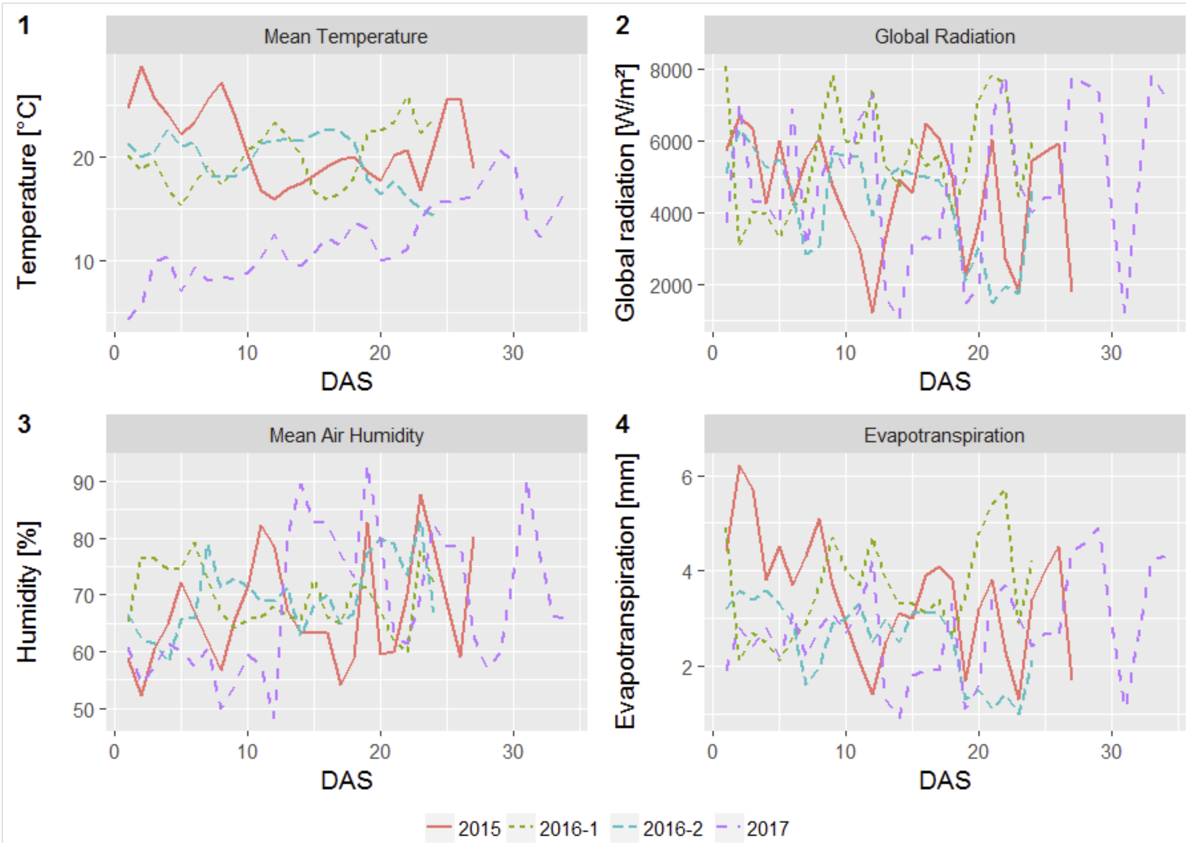

Fig. A.1-4: Daily mean weather conditions during the different cultivation periods 2015, 2016-1, 2016-2 and 2017: (1) Mean air temperature [°C], (2) Global radiation sum [W/m²], (3) Daily mean relative humidity [%] and (4) Daily mean evapotranspiration [mm]; DAS = days after sowing.