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Chemical composition of field grown vegetables as influenced by season and moderately reduced water supply

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*„Wer A sagt, der muss nicht B sagen.
Er kann auch erkennen, dass A falsch war“.*

Berthold Brecht, Der Jasager und Der Neinsager (1930)

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Preliminary Remarks

The work presented within this thesis is a collection of papers published in international peer reviewed journals, which are listed below. Each of them deals with one of three different vegetable crops, which have been investigated within the LOEWE excellence cluster FACE₂FACE with regard to future climate effects of moderate water reduction on vegetable production in open field cultivation.

Full Papers

SCHLERING, C.; DIETRICH, H.; FRISCH, M.; SCHREINER, M.; SCHWEIGGERT, R.; WILL, F.; ZINKERNAGEL, J. (2019). Chemical composition of field grown radish (*Raphanus sativus* L. var. *sativus*) as influenced by season and moderately reduced water supply. *Journal of Applied Botany and Food Quality*, 92, 343–354, doi:10.5073/JABFQ.2019.092.046.

SCHLERING, C.; ZINKERNAGEL, J.; DIETRICH, H.; FRISCH, M.; SCHWEIGGERT, R. (2020). Alterations in the chemical composition of spinach (*Spinacia oleracea* L.) as provoked by season and moderately limited water supply in open field cultivation. *Horticulturae*, 6, 25, doi:10.3390/horticulturae6020025.

SCHLERING, C.; SCHWEIGGERT, R.; DIETRICH, H.; FRISCH, M.; ZINKERNAGEL, J. (2020). Effects of moderately reduced water supply and picking time on the chemical composition of pickling cucumber (*Cucumis sativus* L.) in open field cultivation. *Agronomy*, 10, 1097; doi:10.3390/agronomy10081097.

General Introduction

Climate change and abiotic stress

Agricultural value added is most severely threatened by climate change (Rosenzweig et al., 2014), particularly regarding horticultural production in open field cultivation. Climate change-driven alterations of environmental conditions as mainly characterized by rising temperatures and substantial shifts in precipitation patterns may cause less favorable conditions for the cultivation of currently grown crops. In open field cultivation, a large number of abiotic factors including temperature, water availability, carbon dioxide (CO₂) concentration as well as light and relative humidity continuously exert an impact on the growth performance of cultivated plants. Though, the effect each abiotic factor has on the plants' growth depends on its quantity or intensity (Schulze et al., 2019; Koyro et al., 2012). Besides rising atmospheric CO₂ concentration, which itself influences plant growth by serving as substrate for photosynthesis, future scenarios suggest simultaneous increases in global surface temperatures and changing precipitation patterns, frequently being associated with drier periods due to absent rainfall. Future projections indicate that 'dry and hot' extreme conditions may become more severe in the future climate for almost the entire European continent (Kundzewicz et al., 2006). Most of the climate models indicate a shift in rainfall distribution from summer to winter such as for example in Germany (Schmidt & Zinkernagel, 2017) or South and West European countries (Nikulin et al., 2011). Such changes of the environmental conditions will very likely lead to higher incidences of precipitation-free periods. In addition, fluctuations in temperature as well as the resulting adverse distribution of rainfall consequently influence soil evaporation and plant transpiration. Thus, water availability and crop production are likely to decrease in future (Kang et al., 2009). Thus, climate change poses a major challenge for the global crop production by entail higher incidences of abiotic stress situations for plants, likely accompanied by constrains in production and quality. Abiotic stress factors such as drought, elevated temperature, salinity, rising CO₂ as well as alterations in radiation and humidity pose a growing threat to the production of agricultural and horticultural crops, and growers already struggle with their consequences. For example, the past years 2015-2019 proved to be the warmest years in Germany since weather records began. Accompanied by below-average precipitation and high evapotranspiration rates, the climatic water

balance has developed significantly negative on a large scale and soil moisture was abnormally low (Meinert et al., 2019). Thus, adverse distributions of rainfall already create short-term water deficiencies in open field cultivars, which very likely lead to sub-optimal conditions for plant growth. Such temporary stress-related conditions can induce complex interactions in the plant's metabolism, especially under open field conditions, when a combination of different stresses such as for example heat and drought frequently occurs. However, the resulting effects of the combined stressors cannot be predicted from individual stressors (Pereira, 2016; Suzuki et al., 2014). According to Koyro et al. (2012), especially drought and salt stress are becoming more and more a serious threat to the agricultural crop production and, they are collaterally catalyzed by global climate change and increasing population. In brief, global climate change will very likely boost the occurrence of such abiotic stressors, and agricultural food production might become increasingly impaired. Furthermore, crop production may be limited by affecting the global land area by constraint (Cramer et al., 2011). Nevertheless, the overall outcome might not necessarily be negative in all regions. For instance, while the northern European areas may have primarily positive effects of climate change due to increased productivity and in the "cultivable" range of species grown, disadvantages will predominate in southern European areas because of lower harvestable yields, higher yield variability and a reduction in suitable areas for traditional crops (Bindi & Olesen, 2011). However, uncertainties and variations of the projected impacts entailing by climate change will take much effort to adapt agricultural and horticultural practices to future environmental conditions. Finally, abiotic stresses will remain a challenge not only to agricultural and horticultural crop production, but also to the natural environment (Pereira, 2016).

Therefore, the aims of this doctoral thesis were to study the impact of moderate water reduction on the chemical composition of different vegetable crops being cultivated in open field, varying in their harvestable organs. In order to address this problem, spinach (*Spinacia oleracea* L.), radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.) were selected according to their harvest organ (leaf, root and fruit) and crops were cultivated during three consecutive years using an open-field randomized block design including six replications per cultivation set. Each crop was grown under well-watered and moderately or mildly reduced water supply in open field, while cucumber was

partly cultivated in a greenhouse experiment. The vegetables harvest organs were analyzed for their chemical composition and results were evaluated according to both the effect of water supply treatment and the effect of year or season. For better interpretation, the data were discussed based on both dry and fresh matter evaluation. While dry matter-based results help to evaluate the plant material in consideration of plant physiology and irrespective of varying water contents, fresh matter-based data reflects the nutritional values of the edible harvest organ. In summary, the purpose of this work was to explore the extent to which a moderate or slight reduction of total water supply alters the nutritional composition of the harvest products, and how much these are being affected by prevailing environmental conditions. In addition, detailed chemical analysis generally contributes to the nutritional characterization of the aforementioned vegetable crops.

Overview on types of abiotic stress

Plants growing in open field cultivation are exposed to highly variable environmental conditions. Different types of abiotic stress factors occur, originating from exceeding or deficient physical, chemical, and energetic conditions to which they are subjected. In general, such stress is caused by suboptimal environmental conditions, which keep plants away from achieving their full genetic potential (Bhatla, 2018). Practically, plants almost never have the optimal quantities or intensities of all abiotic factors available and, thus, suboptimal or even damaging quantities represent deviations from their physiological optimum (Schulze et al., 2019). Abiotic stress factors such as high temperature, drought or excessive light affect the plants' growth and development, associated with multiple plant responses ranging from changes in the cellular metabolism and gene expression to the modulation of growth rate and crop yield (Bhatla, 2018). In open field cultivation, plants are being influenced by several abiotic stress factors such as temperature, water, radiation, chemical stress, and mechanical stresses (Schulze et al., 2019), visualized in Figure 1. Moreover, plants are usually subjected to different stressors at the same time, occurring simultaneously in the field. For example, dry periods are often connected to relatively high temperatures, resulting in multiple stressors for the plant. However, the plants' response to the combination of different stresses is unique and cannot be extrapolated from their response to the individually applied stresses (Mittler, 2006). For example, drought or heat stress applied

individually were shown to hamper the morphological growth and grain yield of maize (*Zea mays* L.), while the negative effects were more severe upon simultaneous application of drought and heat stress (Hussain et al., 2019). Besides that, Bethke et al. (2009) found a strong interaction between drought stress and its time of application on the levels of sugars and organic acids in potato tubers (*Solanum tuberosum* L.), which, however, were apparent solely in combination with high daytime temperatures. These results highlight the importance of the interactive effects between a stress event, the timing of its occurrence, and the environmental conditions prevailing at the time. In contrast, effects of drought stress were predominant over heat stress in tomato cultivars (*Solanum lycopersicum* L.) (Zhou et al., 2017). However, the plants' response to the imposed stress depends not merely on the severity and duration of a particular stress, but also on factors such as developmental stage, species, and the type of organ or tissue affected by the stress. Anyway, the incidence of combined stressors such as drought and heat is likely to increase under global climate change, why experiments focusing on the impact of a specific abiotic stress should be carried out in open field cultivation with all its corresponding environmental factors.

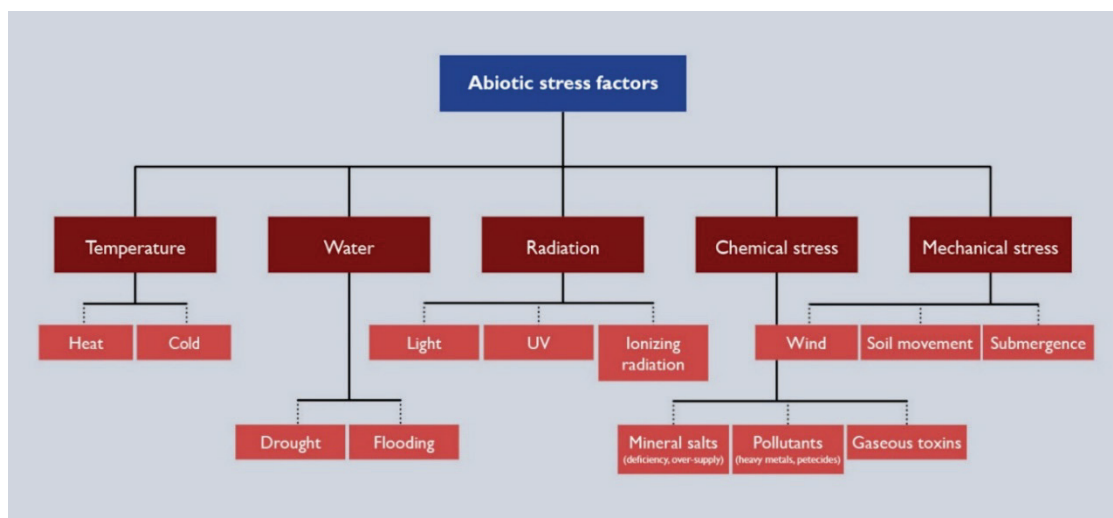


Figure 1: Overview on types of abiotic environmental factors creating stress for plants (Schulze et al., 2019).

Nowadays, one of the major concern in the changing climate is drought, affecting virtually every region worldwide and represents one of the most destructive natural hazards faced by society (Schubert et al., 2016). There is a scientific evidence that drought situations will increase upon global warming, because atmospheric moisture demand increases by warming and therefore, altered atmospheric circulation patterns

become more likely, both contributing to the drying (Dai, 2011). Such high temperatures are again accompanied by increased evapotranspiration, which could lower or deplete the water reservoir in soils and therefore, causing a lack in water supply (Moretti et al., 2010). This again may result in a reduced nutrient uptake by the plant. Apart from that, the relative air humidity determines the vapor pressure gradient between the leaf stomatal cavity and the atmosphere, which is the driving force for transpirational water loss. As a consequence, low relative humidity cause a large vapor pressure gradient, which can provoke water deficits in plants, even at adequate soil water (Taiz & Zeiger, 2010). In any case, the most important physiological activities for crop yield, growth and photosynthesis, are adversely affected by water deficit. Depending on the severity, the reaction lasts from a decrease in the photosynthetic rate coincides with stomatal closure, which again can result in nutrient shortage, degeneration of the photosystem and an accompanied decline in protein level as well as in the accumulation of reactive oxygen species (Bhatla, 2018). For example, canola (*Brassica napus* L.) grown under drought stress at reduced field capacity of 75% and 50%, respectively, showed a significant reduction of fresh and dry weight, shoot and root length as well as in the levels of potassium (K^+), calcium (Ca^{2+}), nitrogen (N) and phosphorous (P) (Ashraf et al., 2013). Finally, climate change will likely provoke higher frequencies of such water deficit situations, evoking several changes in various plant processes, as for example in physiological, biochemical, and molecular components of the photosynthesis (Prasad et al., 2008). It is expected that climate change will cause increasing water shortages along the Mediterranean, in the south-west Balkans and in the south of European Russia (Bindi & Olesen, 2011), and already today, drought is one of the major limitations to food production worldwide. Even high productive agricultural regions experience short periods of drought within almost any year (Barnabas et al., 2008).

Limited water supply

Water is the central molecule in all the physiological plant processes by being the major medium for transporting metabolites and nutrients (Lisar et al., 2012). A plant water deficit occurs, if transpiration rate exceeds water uptake, and it is involved in several different stresses such as drought (Bray, 1997). In general, drought is characterized by below-normal precipitation over a certain period, resulting in a temporary dry period in

contrast to permanent aridity in arid areas. Drought events occur worldwide, even in wet and humid regions, because it is defined as a dry spell relative to its local normal condition (Dai, 2011). According to Dai (2011), drought can be classified into three different types: (1) ‘Meteorological drought’, which is characterized by a period with below-normal precipitation, often accompanied with above-normal temperatures. (2) ‘Agricultural drought’ is defined by a period with dry soils resulting from below-average precipitation, intense but less frequent rain events, or above-normal evaporation, and (3) ‘Hydrological drought’, which occurs when water storages in lakes, or reservoirs and river streamflow fall below long-term mean levels. Apart from the resulting type of drought, climate change will likely promote such drought situations due to altered precipitation patterns accompanied by higher temperatures. Depending on the type of plant and the timing, duration, intensity and its frequency of occurrence during the growth period, drought events affect plants at different levels. In general, cell expansion and growth are considered to be the first processes hampered under water deficit, whereas photosynthesis and assimilate partitioning is only adversely affected with prolonged water deficit (Artlip & Wisniewski, 2002). Sugars often accumulate after the onset of stress as a result of inhibited shoot growth, while root growth is stimulated under mild stress situations (Schulze et al., 2019). A decrease in the photosynthetic activity caused by drought stress may result in reduced nutrient uptake, which thereby hinders plant growth. Furthermore, the excess of light energy absorbed due to repressed CO₂-fixation can lead to light-driven accumulation of reactive oxygen species (Bhatla, 2018). Due to the ubiquitous involvement of water in all cellular structures and processes, water deficiency affects almost all processes in plants and, it is difficult to differentiate between primary consequences and secondary effects due to primary damage (Bhatla, 2018).

As a consequence of insufficient precipitation, drought often occurs in conjunction with relatively high temperatures and high radiation, which promote evapotranspiration and affect photosynthetic kinetics, thus intensifying the adverse effects of drought and further reducing crop yields (Mir et al., 2012). The consequential stress based on both, the reduction in soil water potential, which usually arise gradually over a period of time, and the daily based changes in net radiation and humidity, which lead to variations in the evaporation rate (Morgan, 1984). Drought was shown to generally reduce nutrient

uptake by the roots due to reduction of soil moisture, since most of the mineral nutrients are dependent on soil moisture to move through the soil matrix and be taken up by plants (Taiz & Zeiger, 2010; da Silva et al., 2010). Concomitantly, the plants' roots are unable to take up most of the mineral nutrients from the soil because of inadequate root activity as well as slow ion diffusion and water movement rates (Dubey & Pessarakli, 2002). Moreover, microorganism-based soil mineralization processes may be affected by drought (da Silva et al., 2010) and thus, reduce the potential nutrient availability of the soil for the plant. In principle, it is known that drought situations as a result of deficient water supply induce several physiological, biochemical and molecular changes in various plant processes (Prasad et al., 2008). For example, hundreds of genes are being induced under drought conditions in the plant's response to water deficits, however, their functions are often still unknown (Chaves et al., 2003; Cushman & Bohnert, 2000). When soil water potential decreases, plants have to maintain the water potential gradient to the cells to take up water from the soil solution. Therefore, plant cells lower their water potential due to osmotic adjustment by increasing the concentration of solutes, making the osmotic potential of the cell more negative. Such effective osmolytes are soluble sugars, sugar alcohols, oligosaccharides, amino acids, quaternary ammonium compounds or polyamines (Schulze et al., 2019), which are summarized in the following Table 1.

Table 1: Accumulation of different molecules in plant organs under drought stress, and their physiological effects; adopted from a previously published Table of Schulze et al. (2019).

Type of molecule	Examples	Function under drought
Ions	K ⁺	Osmotic adjustment
Proteins	LEA [#] /dehydrins	Membrane and protein protection
	SOD ^z , catalase	ROS ^y detoxification
Metabolites		
Amino acids	Proline	Membrane and protein protection
Sugars	Raffinose	ROS scavenging
Polyols	Mannitol (acyclic)	Osmotic adjustment, ROS scavenging
	Pinitol (cyclic)	
Polyamines	Spermine	ROS scavenging, membrane protection
	Spermidine	
Quaternary amines	Glycine betaine	Membrane and protein protection
	β-Alanine betaine	Osmotic adjustment under hypoxia
Tertiary sulphonium compounds	Dimethyl sulphonopropionate	ROS scavenging
Pigments	Carotenoids	Protection against overexcitation, Photoinhibition
	Anthocyanins	

[#] LEA: late embryogenesis abundant, ^z SOD: superoxide dismutase, ^y ROS: reactive oxygen species

When drought is being perceived by the plants' membrane, molecular signals are transmitted down through multiple signal transduction pathways, resulting in the expression of drought-related genes. In particular, secondary messengers such as Ca^{2+} , reactive oxygen species (ROS), abscisic acid (ABA), phosphoglycerol, diacylglycerol, and transcriptional regulators play an important role in the signal transmitting pathways (Kaur & Asthir, 2017). Such drought-induced gene expression mainly leads to the production of proteins, as for example those involved in signaling cascades and transcriptional regulation (protein kinase, protein phosphatase, and transcription factors), functional proteins that protect cellular membranes, proteins associated with the uptake and transport of water and ions, antioxidants, osmotin (stress responsive protein that is formed at high salt concentrations), and sugar transporters (Kaur & Asthir, 2017; Nakashima et al., 2014). However, with regard to the aforementioned plant responses, it makes a strong difference whether plants are affected by drought in short or long term by being subjected to slowly developing water shortages or short-term water deficits (Chaves et al., 2003). If the water deficit evolves slowly, plants can escape dehydration by shortening their life cycle or by optimize their resource gain in

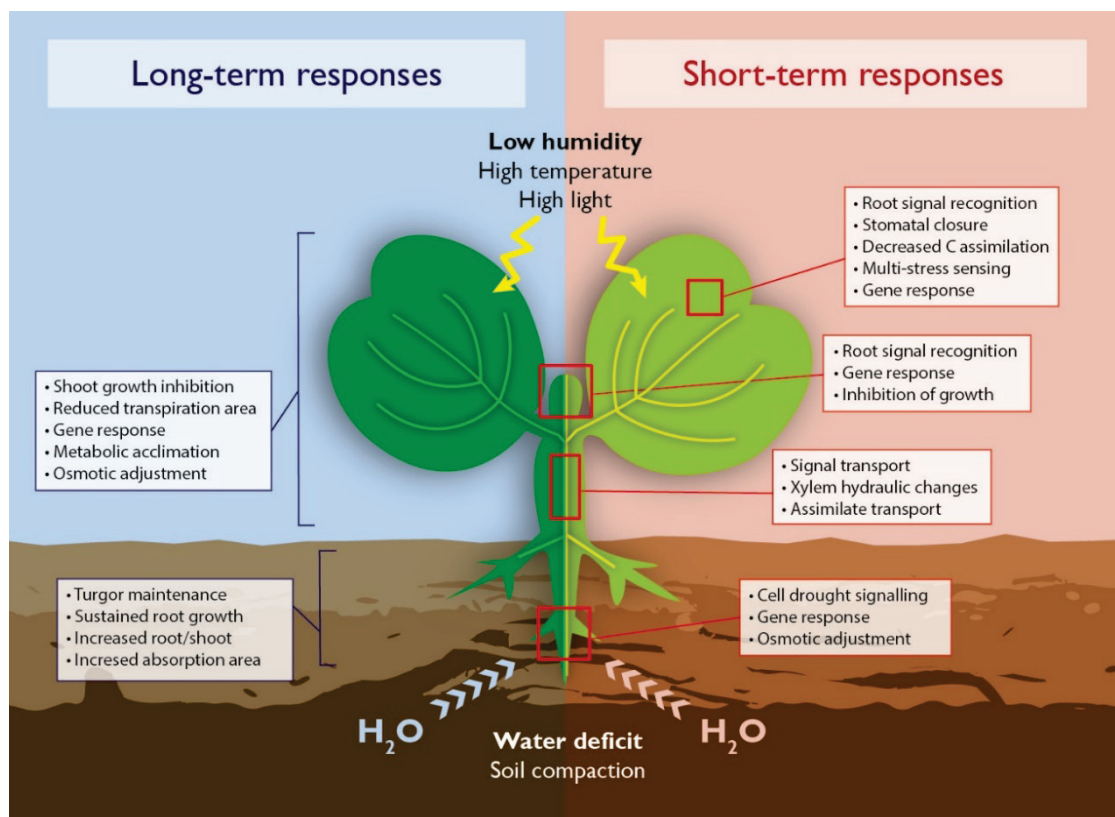


Figure 2: Comparison of short-term (right) and long-term (left) plant responses to drought stress, modified after adoption from Chaves et al. (2003).

the long term through acclimation responses (see Figure 2). In case of expeditiously evolving dehydration, plants react by avoiding or minimizing water loss or exhibiting metabolic protection against the damaging effects of water loss and co-developing oxidative stress (Chaves et al., 2003).

While drought is generally characterized by the aspects of intensity, duration, and spatial coverage (Dai, 2011), drought stress can occur in different types, depending on its duration and intensity. Concerning this matter, He & Dijkstra (2014) analyzed the effects of drought on the plants' nitrogen (N) and phosphorous (P) levels in a meta-analysis, within they classified four different types of drought stress as follows: (1) constant-stressed, (2) drying-rewetting cycle, (3) prolonged drying and (4) intermittent drying. The study revealed different effects of the respective drought type on the plants' N and P content, highlighting once again the significance of the drought type when considering the resulting effects. It was shown that drought situations including drying-rewetting cycles did not show any effect, while negative effects were observed if constant, prolonged, or intermittent drought stress occurred (He & Dijkstra, 2014). The absent effect of drought followed by rewetting can be attributed to enhanced mineralization (Austin et al., 2004), probably related to nutrient release by dead microbial biomass (Borken & Matzner, 2009). However, because the plants' reaction to drought stress and other environmental factors are highly plant-specific and tissue-dependent, the resulting effects of drought or more precisely, of moderate or even mild water deficits on the chemical composition of the harvest product, is difficult to assess. Of course, low water scarcity does not evoke the complex known array of drought-related plant responses such as, for example, stomatal closure and reduced photosynthesis. However, mild or moderate water limitation cause more subtle changes and has earlier been considered to even enhance the formation of health-promoting constituents (Koyama et al., 2012).

Drought effect on the nutritional composition of horticultural products

Water requirements for horticultural crops are generally high (Preece & Read, 2005) and consequently, the production of fruits and vegetables being cultivated in open field is highly prone to be affected by shifting precipitation patterns and water scarcity,

increasingly provoked by climate change. Among various other stress factors, drought is the most common abiotic stress, lowering the yield of many crops (Vadez et al., 2011). In principle, drought is one of the most important side effects of global warming (Ayyogari et al., 2014). Surely, the amplification of climate variability might become an increasingly important challenge for the production of horticultural crops in open field cultivation, and of course, for large-scale agricultural production in any case. Therefore, the effective use of irrigation water has become a key component in the production of field crops and high-quality fruit crops (Kang & Zhang, 2004). Moreover, temperature changes affect the growth and the development of crops by shortening their overall cropping period, defined by the accumulation of thermal degrees above a baseline temperature, which again influence the crops response to limited water supply (Vadez et al., 2011).

The impacts of drought on the physiological and metabolic plant responses have been excessively discussed within various publications (Bhatla, 2018; Akinci & Lösel, 2012; Prasad et al., 2008). Nevertheless, there is comparably little knowledge about the impact of water shortage on the actual nutritional and chemical composition of horticultural crops such as vegetables and, in particular, about the impact of mildly or moderately reduced water supply on different plant organs being utilized as vegetables. Depending on the harvest organ, the reaction to an imposed water deficit might vary significantly. Cramer et al. (2011) already stated that the plants' response to stress is dependent on the organ or tissue affected by the stress. Concerning this, Gargallo-Garriga et al. (2014) demonstrated very well the contrasting response of shoots and roots of two grass varieties (*Holcus lanatus* L. and *Alopecurus pratensis* L.) to drought, which was reflected by altering the biomass allocation and by changing the metabolic activities of their organs. In conclusion, the elemental and nutrient stoichiometry between the organs appeared differently. In principle, any sub-optimal environmental condition causing abiotic stress such as drought, have varying effects on the different plant organs, and besides that, the molecular, cellular and morphological responses vary among the tissues (Gray & Brady, 2016). A study about the effects of water deficits on potato revealed different responses of leaves, roots and tubers concerning their metabolite levels or their expression, including primary metabolites such as glucose and malic acid as well as secondary metabolites such as carotenoids and phenolic compounds (Drapal

et al., 2017). Thus, the quality of horticultural products such as fruits and vegetables, primarily determined by their nutritional composition, is dependent on the reaction of the corresponding organ to the applied stress. Perennial crops such as grapevine and fruit trees seem to be well adapted to mild water deficits, whereas vegetable crops, particularly freshly marketed products such as cucumber, tomato, green leafy vegetables like lettuce or spinach appear to be considerably more susceptible to any type of water shortage (Costa et al., 2007; Nishihara et al., 2001). However, the plant's sensitivity to deficient water supply also differs with developmental stage. The marketable yield of open field processed tomato was shown to diminish under drought stress if applied during early growth stages, while the reduction of irrigation water after flowering did not induce significant losses in biomass and marketable yield (Patanè et al., 2011). Likewise, potted geranium plants (*Pelargonium × hortorum* L.H. Bailey) showed lowest values for both height and flowering, when deficit irrigation was applied during flowering stage (Álvarez et al., 2013). Focusing on the nutritional composition of horticultural crops, the irrigation time was shown to influence not only yield, but also the concentration of valuable substances such as sugars and glucosinolates in cabbage (*Brassica oleracea* L.). There were significantly reduced levels of glucose and fructose but, on the other hand, significantly higher levels of glucosinolates in non-irrigated cabbage compared to irrigated plants (Radovich et al., 2005). In canola, the applied water deficit led to significantly reduced fresh and dry weight of shoots and roots, and at least, to a reduction in the levels of certain minerals such as K^+ , Ca^{2+} , N and P (Ashraf et al., 2013).

Water deficits and vegetable production

The production of foods such as intensively irrigated horticultural crops requires large amounts of water, primarily dictated by the linkage between biomass production and yield (Fereres et al., 2003). From a global perspective, irrigated agriculture is the primary user of diverted water, reaching a proportion that exceeds 70–80% of the total amount in the arid and semi-arid zones (Fereres & Soriano, 2007). Against the background of ongoing climate change and continuing population growth, the reduction of irrigation need in agriculture and horticulture becomes an important issue, especially in regions where water scarcity is predominant. Since horticultural products are usually of high value, their water productivity, which is defined as the ratio of crop yield to the

water amount consumed by the plant (van Halsema & Vincent, 2012), normally exceeds that of field crops (Fereres et al., 2003). Several methods and techniques have been developed to improve irrigation management systems for horticulture. For example, the application of water amounts below the crops' evapotranspiration rate is an important tool to reduce water consumption for biomass production, and for irrigation of annual and perennial crops (Fereres & Soriano, 2007). This is already used by smart irrigation systems to reduce water amounts and even to improve harvest quality of horticultural crops (Costa et al., 2007). For example, the so-called 'regulated deficit irrigation' (RDI) depends on intermittent water deficit during a specific growth period, when the plant or fruit is least sensitive to water reductions. Another technique, the 'partial root-zone drying' (PRD), exposes the roots to alternate drying and wetting cycles. Such treatment enables the plant to grow with reduced stomatal conductance, but without signs of water stress (Kang & Zhang, 2004; Costa et al., 2007). Deficit irrigation regimes have been already successfully applied in fruit orchards and grapevine cultivation (Pérez-Pastor et al., 2009; Loveys et al., 2000). Because cell growth is usually much more susceptible to drought stress than carbon assimilation, mild stress is able to reduce the development of leaf surface area without affecting photosynthesis. Whether dry matter yield is affected by water reduction depends on whether leaf area is limiting the crop's assimilation (Hsiao, 1973). However, the prospects for reducing the evapotranspiration in vegetable crops are limited, because most of the relationships between yield and evapotranspiration are linear (Fereres et al., 2003). For example, the yield of tomato decreased by 60% if plants were grown under deficit irrigation, whereas fruit quality was improved due to higher concentrations of soluble sugars and higher color intensity (Pulupol et al., 1996; Costa et al., 2007). Another study conducted by Koyama et al. (2012) revealed that drought stress supported the accumulation of some quality-related compounds such as ascorbic acid, total polyphenols, and sugar in leaf lettuce (*Lactuca sativa* L.). Because vegetables are an important constituent of a healthy diet, the chemical composition of their harvest organs is widely used as important parameter to evaluate the quality and the overall nutritional value of the produce.

Impact on dry matter

Dry matter is the sum of all residual constituents after water has been removed from the plant tissue. In general, the water content of horticultural products such as fruits and

vegetables is comparably high (70–95%) in contrast to other foods (Benelam & Wyness, 2010; Grandjean & Campbell). It mainly consists of compounds such as dietary fiber and available carbohydrates, proteins, lipids, organic acids and minerals (Souci et al., 2008). Of course, dry matter composition differs between varieties and organs, but especially vegetable products are partly composed of remarkably high amounts of health-related compounds such as dietary fiber, proteins, minerals and trace elements, vitamins, as well as bioactive compounds like flavonoids and glucosinolates. For example, dry matter of leafy green vegetables is known as a rich source of dietary fiber, minerals, trace elements, vitamins and phytochemicals such as polyphenols, terpenoids, sulfur compounds, and alkaloids (Alvino & Barbieri, 2015). Inherently, the production of dry matter varies with genotype, environmental condition and cultivation practice (Quang Duy et al., 2004). Generally, the crops' yield is a result of dry matter production, including accumulation and translocation. Furthermore, dry matter partitioning is the result of the flow of assimilates from source organs (organs with a net export such as leaves) via a transport path to sink organs (organs with a net import such as fruits), primarily regulated by the sink strength (Marcelis & Heuvelink, 1999). Because cell growth is much more susceptible to drought than carbon assimilation, dry matter production can be affected even if stomatal opening and photosynthesis is not reduced (Hsiao & Xu, 2000). For example, as consequence of decreasing water potential and osmotic potential, dry matter contents of fruits and leaves of tomato were significantly higher, when plants had been exposed to water deficit in contrast to well-watered plants. Concomitantly, the specific leaf area was significantly reduced in stressed plants (Ripoll et al., 2016). When leaf area is reduced under drought stress, an increase in the root/shoot dry matter ratio is often observed (Blum, 2011). In contrast, no dry matter accumulation was observed in the aboveground biomass of pear (*Pyrus communis*) under deficit irrigation (Lopez et al., 2013). Whether dry matter production is affected by water deficits or not depends on several factors according to Hsiao & Xu (2000). First, whether accumulation of assimilates in leaves inhibits photosynthesis, second, whether leaf area is low enough to limit total assimilation by the crop, and third, whether assimilates accumulated during the day exceed the requirements for growth at night. In general, water deficits often lead to a reduction of both, fresh and dry weight of a particular plant organ, leading to reduced biomass production, as for example reported for canola exposed to drought stress at different growth stages (Ashraf et al., 2013).

Whereas the water content of tomato fruits grown under moderate water deficit was reduced, dry matter accumulation was less affected (Mitchell et al., 1991). Besides these drought stress-related effects, the content of dry matter is also substantially determined by the plants' growth stage, as shown for spinach, which demonstrated highest values in young leaves at early growth stages (Bergquist et al., 2006).

Impact on sugars and polyols

Drought stress was often shown to increase the content of soluble sugars in plant tissues. For example, the content of total sugars in tomato fruits increased significantly due to the accumulation of both, glucose and fructose, irrespective of whether water deficit was applied at cell division stage, cell expansion stage or maturity stage (Ripoll et al., 2016). A significant increase in the sugar content was also observed in hydroponically grown lettuce subjected to drought stress (Koyama et al., 2012). This might be explained by their role as osmoprotectants as well as by being sources of carbon for maintenance and re-growth during recovery (Chaves et al., 2002). It has been observed that plants from dry habitats contain more soluble sugars than those from moist habitats. Such accumulation is a common phenomenon not only under water deficit but also under high CO₂, low translocation rates or low sink demand (Chaves, 1991). It was shown that drought has an effect on the photosynthetic carbon metabolism by changing the partitioning of newly formed carbohydrates towards sucrose (Quick et al., 1989; Chaves, 1991). The accumulation of sucrose in leaves may result from reduced export rates or increased sucrose synthesis, which is both controlled by enzymatic regulation. Thus, sucrose synthesis can be adjusted either to net photosynthesis or to sink demand (Chaves, 1991). In brief, the increase in the sugar content of leaves as consequence of water deficits seems to be a result of a coordinated regulation established between sucrose synthesis and translocation. Besides that, a study on cabbage revealed an interactive effect of the cultivation period (year) and the irrigation treatment. If cabbage was irrigated during head development, levels of fructose and glucose were found to be higher than those in plants that have not been irrigated during this particular growth period (Radovich et al., 2005). The result clearly shows the dependence between the plants' growth stage and the time of stress application regarding its effect on the chemical composition of the harvest product.

Besides the impact of water deficits on the content of soluble sugars, also polyols are considered to be affected by drought stress. Polyols are known to act as compatible solutes in the osmotic adjustment, being able to accumulate to osmotically significant levels without impairing the plants' metabolism (Nuccio et al., 1999). For example, Dietrich et al. (2007) found a significant accumulation of sorbitol in pear and apple juice from plants exposed to drought stress. Furthermore, strong increases of *myo*-inositol have been observed in pea (*Pisum sativum* L.) exposed to drought (Charlton et al., 2008). According to Bohnert et al. (1995), polyols seem to act via osmotic adjustment and osmoprotection mechanisms, which are difficult to be studied separately. The alteration in the plant metabolism under abiotic stress situations plays an important role in the diversion of carbon to polyol biosynthesis. For example, inositol stirs up the production of compounds involved in stress tolerance such as gums and cell wall-located carbohydrates (Bohnert et al., 1995). Furthermore, plants use inositol to synthesize vegetative storage carbohydrates such as phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), the major storage form of phosphorous comprising 1–5% by weight in cereals, legumes, oil seeds and nuts (Vats & Banerjee, 2004).

Impact on organic acids

Organic acids such as malic and citric acid were shown to be the most abundant organic acids in many fruits and vegetables (Souci et al., 2008). Similar to other solutes, organic acids are known to contribute to the osmotic adjustment (Akinci & Lösel, 2012). Malate, for example, was shown to function as a component of the osmotic adjustment and plays a role in the turgor regulation of guard cells (Dhindsa et al., 1975; Raschke, 1975; Cutler & Rains, 1978). The uptake of low-molecular-weight solutes like organic acids concurs with the movement of water to the roots, a passive process driven by mass flow and diffusion (Rouphael et al., 2012; Marschner, 1986). It was shown that malate levels increased significantly with decreasing water potential in cotton plants (*Gossypium hirsutum* L.), concluding that malate acts as important osmotic agent (Cutler & Rains, 1978). Simultaneously, the levels of citrate decreased significantly. Moreover, tomatoes grown under water deficit conditions contained higher levels of malate, while citric acid was less in contrast to fruits from well-watered plants (Mitchell et al., 1991). Such increase of malic acid in mature tomato fruits grown under water

stress conditions may be a consequence of slowed degradative metabolism of this acid during ripening under drought stress (Mitchell et al., 1991). In contrast, drought was also shown to decrease the content of total organic acids in cocktail tomatoes due to significant decreases in both citric and malic acid, consequently increasing the sugar-to-acid ratio (Ripoll et al., 2016).

The application of drought stress to roots of leafy vegetables such as lettuce, spinach and turnip leaves (*Brassica rapa* L.) led to significantly increased levels of ascorbic acid, which increased steadily with decreasing water level (Koyama et al., 2012). However, the content of ascorbic acid in mung bean (*Vigna radiata* L.) did not alter due to drought stress application (Afzal et al., 2014). In general, antioxidants such as ascorbic acid (vitamin C) are present in high concentrations in fruits and vegetables (Souci et al., 2008) and are considered to be involved in diseases protection (Rice-Evans & Miller, 1995). But the contents of ascorbic acid and vitamin C were also shown to vary significantly in spinach between growth stages, with highest levels in young plants (Bergquist et al., 2006). Moreover, also the day-length was shown to have an impact on the levels of ascorbic acid in spinach. Higher amounts were found in plants being cultivated during winter solstice in contrast to those grown during summer solstice. These findings have been explained by the more stressful environmental conditions during winter solstice requiring the plant to provide increased activity of ascorbate oxidase involved (Lester et al., 2013). Significantly higher levels of ascorbic acid were also found in spinach exposed to low temperature (Proietti et al., 2009), which can be ultimately attributed to more stressful environmental conditions for the plant. Reduced water supply was also shown to increase the content of antioxidants such as ascorbate in field grown chard (*Beta vulgaris* L.), one of the horticultural crops most susceptible to drought stress (Delfine et al., 2003). The study revealed that antioxidant levels were inversely related to the photosynthetic rates, indicating their involvement in the plant mechanisms against the applied stress. Contrary, tubers of native potato plants did not show significant differences of vitamin C after drought stress application (Andre et al., 2009), and no effects were found in the level of ascorbic acid in cocktail tomatoes exposed to different water deficit treatments (Ripoll et al., 2016). In brief, the effect of water deficit on the ascorbic acid levels of horticultural produce appears to be highly

dependent on the plant species and the plant organ, although frequently increased levels have been observed under water deficits.

Impact on inorganic anions

The application of drought stress was shown to lower fresh matter-related levels of nitrate (NO_3^-) in carrot (*Daucus carota* L.), even when drought stress occurred just shortly before harvest (Sørensen et al., 1997). Similar results were found for lettuce subjected to drought (Koyama et al., 2012). It is widely accepted that NO_3^- -fluxes from roots to leaves are reduced under drought stress (Dubey & Pessarakli, 2002), and expression of the nitrate reductase enzyme has been shown to be lowered under sharp drought stress (Morilla et al., 1973). Contrary to the assumption that nitrate reductase activity is related to NO_3^- -level, it has been shown by experiments on corn seedlings (*Zea mays* L.) that such a correlation is not necessarily observed (Morilla et al., 1973). However, if plants grown under stressful water regimes, a decrease in NO_3^- was often observed, as shown in the above-mentioned studies. Nowadays, the content of NO_3^- in selected vegetables is under strong consideration when assessing their quality. Especially leafy vegetables such as spinach, lettuce, rocket (*Eruca vesicaria* L.) and Swiss chard tend to accumulate extremely high NO_3^- levels, reaching final concentrations up to 2500 mg NO_3^- /kg fresh matter (Santamaria et al., 1999). Even if NO_3^- is not inherently toxic, it can be converted to nitrite in the human body, which causes methemoglobinemia and the production of carcinogenic nitrosamines (Chan, 2011; Gangolli et al., 1994). On the other hand, Jockel-Schneider et al. (2016) proofed that dietary nitrate consumption originated from vegetables can be a useful amendment in the control of chronic gingivitis. Focus on NO_3^- -levels, it is important to pay attention on light intensity and light quality during the cultivation period of the vegetable crop, which is known to be the main factor for regulating NO_3^- -concentration in plants (Hua et al., 2014). It was shown by Qiu et al. (2014) that NO_3^- -content and water concentration of leafy vegetables such as rape (*Brassica chinensis* var. *oleifera*), Chinese cabbage (*Brassica rapa* var. *chinensis*) and spinach were positively correlated, indicated that these vegetables accumulates NO_3^- with increasing water content. The mobility of anions such as phosphate (PO_4^{3-}) also depends on soil moisture (Taiz & Zeiger, 2010), thus reduced water supply might diminish its uptake into the roots (da Silva et al., 2010). On the other hand, the mineralization processes of organic soil matter

by microbes, which are responsible for the release of phosphate ions such as H_2PO_4^- and HPO_4^{2-} (Preece & Read, 2005), could have an impact on the overall availability of phosphorous, thus influencing PO_4^{3-} -levels in plants. Besides this, different grasses and legumes showed an accumulation of chloride (Cl^-) as a result of decreasing soil moisture (Abdel Rahman et al., 1971) and the levels of inorganic ions such as NO_3^- and PO_4^{3-} are also known being influenced by seasonal variations. For example, Ćustić et al. (2003) observed increased NO_3^- -accumulation in chicory (*Cichorium intybus*) under warm and wet conditions. It is already known that NO_3^- -accumulation is greatly affected by environmental factors and varies over season (Ćustić et al., 2003; Anjana & Iqbal, 2007). Finally, reduced water supply seems to diminish the levels of anions such as NO_3^- and PO_4^{3-} , while other environmental conditions such as light intensity and temperature were shown to have a strong influence on their uptake and accumulation.

Impact on minerals and trace elements

Plants take up minerals from the soil very effectively due to their large root surface area, being able to absorb inorganic ions even at low concentrations from the soil solution (da Silva et al., 2010). Nonetheless, most minerals are reliant on soil moisture to move through the soil matrix and be taken up by the plants (Taiz & Zeiger, 2010). A study on different plant species conducted by Abdel Rahman et al. (1971) figured out that drought stress generally favored an increase in nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg), but decrease levels of phosphorous (P) and iron (Fe). The element required by plants from the soil in largest amounts is N, which is a constituent of many cell components such as amino acids and nucleic acids (Hu & Schmidhalter, 2005). N is available for the plant in form of nitrate (NO_3^-) and ammonium (NH_4^+) and both are highly mobile in the soil (Maathuis, 2009). If plants are subjected to stress, they accumulate a number of soluble N compounds such as amino acids, which contribute to the osmotic adjustment (Dubey & Pessarakli, 2002), as for example observed in cotton after drought induction (Parida et al., 2007). However, N mobility is severely restricted under drought conditions by the dehydrated soil (da Silva et al., 2010). Experiments on vegetable amaranth (*Amaranthus tricolor* L.) revealed a significant increase of certain minerals and trace elements such as K, Ca, Mg, S, manganese (Mn) and copper (Cu) with incrementing soil water deficit, while a decrease in P, Fe and Zn was observed in the same manner (Sarker & Oba, 2018). On the other

hand, moderate water stress was shown to reduce mineral nutrients such as N, P, S and Mg in leaves of cherry tomato, while no changes were found for K and Ca (Sánchez-Rodríguez et al., 2010). The study also revealed diminished uptake fluxes of most of the macronutrients under drought stress. In contrast, no significant differences were observed for micronutrients such as Fe, Cu and Zn, while Mn significantly decreased under water stress. However, the observed effects were highly cultivar-dependent (Sánchez-Rodríguez et al., 2010). Focus on fruit quality, Mitchell et al. (1991) found decreased ion accumulation in mature tomato fruits grown under water deficit. It was shown that especially the import of K, which account for the major proportion of total ions in tomatoes, was significantly reduced due to drought stress. Similar results were found by Ashraf et al. (2013) and Junjittakarn et al. (2013), who investigated nutrient uptake of canola and peanut (*Arachis hypogaea* L.), respectively. It is known that K availability decrease with decreasing soil moisture due to the reduced mobility of K under dry conditions (Rouphael et al., 2012). The situation is similar for P, whose uptake was reduced in tomato plants by even a small decrease in water potential, even if plants were exposed to stress for only a short time (Greenway et al., 1969). In contrast, no differences were found in the Ca levels of tomato leaves being exposed to drought, although its uptake was significantly reduced (Sánchez-Rodríguez et al., 2010). Against this background, Hu & Schmidhalter (2005) already stated that Ca accumulation is less sensitive to drought than that of K or P. Fresh weight-based Mg levels were shown to increase in tomato fruits of deficit irrigated plants, while the effects were insignificant on dry weight basis (Pulupol et al., 1996). Micronutrients are often reported to decrease in plants subjected to water deficit, because their transport to the roots occurs via diffusion, which is reduced under low soil moisture (Hu & Schmidhalter, 2005). As already stated before, nutrient availability and uptake is less affected if drought is followed by rewetting. For example, while a constant, prolonged and intermittent drying had negative effects on the plants' P level, no effect was observed when plants grown under drying-rewetting cycles (He & Dijkstra, 2014). According to Borken & Matzner (2009), rewetting of dry soils may cause an extra short-term boost in the microbial activity that exceeds those of permanently moist soil. This fact could mitigate the negative effects on the plants' N level evoked by drought stress (He & Dijkstra, 2014). Rewetting of drought stressed cotton plants was shown to recover their protein level to those of control plants, while increased levels of free amino acids had been adjust to the

comparable level of control plants after recovery. Thus, protein synthesis was hardly affected by short-term drought (Parida et al., 2007). Altogether, drought was shown to favor the accumulation of selected minerals such as N, K, Ca or Mg, whereas the levels of P and Fe seem to decrease under drought situations. However, their uptake and accumulation was shown to be highly dependent on the cultivar and harvest organ.

Impact on polyphenols

Polyphenols are plant secondary metabolites, representing one of the most abundant group of bioactive compounds in vegetables. In plant-derived food products, phenolic compounds are relevant to the appearance, taste and flavor as well as to their health-promoting properties (Tomás-Barberán & Espín, 2001). Flavonoids, for example, are a remarkably diverse group of secondary plant products including anthocyanins, flavones and flavonols, which have an apparent role in stress protection, particularly because of their absorbance of ultra-violet radiation (Winkel-Shirley, 2002). However, phenolic compounds also play an important role in the visual appearance of food such as vegetables, being responsible for the blue, purple and red hues of numerous fruits and vegetables (Tomás-Barberán & Espín, 2001). Abiotic stresses are potent elicitors of such bioactive compounds (Toscano et al., 2019), as shown for field grown potato exposed to drought. Indeed, drought stress induced alterations in the proportion of individual phenolic compounds such as phenolic acids, flavonoids and aromatic amino acids, but the polyphenolic profiles did not differ between stressed and non-stressed tubers (Andre et al., 2009). The study revealed that drought-stressed tubers contain higher levels of polyphenols such as caffeic acid, kaempferol-3-*O*-rutinoside and anthocyanins. Nevertheless, the reaction was strongly cultivar-dependent. On the other hand, the content of polyphenols in lettuce significantly increased in response to deficient water supply (Koyama et al., 2012). Similar results were observed in cultivars of cherry tomato differing in their sensitivity to drought (Sanchez-Rodriguez et al., 2012). Thus, deficient water supply can stimulate the metabolism of antioxidants such as polyphenols (Toscano et al., 2019). It is well known that water availability can considerably affect the metabolism of phenolic compounds in plants (Tomás-Barberán & Espín, 2001). Though, no difference in the phenolic production was found in broccoli (*Brassica olearacea* L.), when water supply was reduced to 80% evapotranspiration in open field cultivation (Robbins et al., 2005). However, drought stress-induced increase

of polyphenols in cotton was shown to decrease again upon re-watering to the levels of non-stressed plants, as reported by Parida et al. (2007). Besides such drought-induced effects on plant polyphenols, environmental factors such as light and temperature were also shown to influence the concentration of flavonol aglycones like quercetin, kaempferol, and isorhamnetin as well as their glycosides in field grown kale cultivars (*Brassica oleracea* var. *sabellica*). It has been found that flavonols increased with decreasing temperature and increasing radiation. Thus, the study revealed a significant influence of year, harvest time and cultivar on flavonol contents in kale (Neugart et al., 2012). In this context, significant variations of selected flavonoids within kale cultivars due to the genotypic and climatic conditions had been already described by Schmidt et al. (2010). In brief, deficient water supply was generally shown to increase the levels of polyphenols in many cultivars, although their concentration is highly dependent on environmental factors.

Impact on carotenoids

Carotenoids are widely accepted to play an important role in plant defense against abiotic stress, at least because of their antioxidant properties and their function in the xanthophyll cycle (Schweiggert et al., 2017; Strzałka et al., 2003). Several studies have shown that abiotic stress such as drought affects the carotenoid levels in selected vegetable crops such as tomato (Ripoll et al., 2016) or vegetable amaranth (Sarker & Oba, 2018). For example, decreasing levels of β -carotene and lutein were observed in tomato fruits from plants exposed to water deficit, while the metabolic effects were balanced by concentration, resulting in unchanged levels on fresh weight basis (Ripoll et al., 2016). In contrast, fresh matter-related content of β -carotene significantly increased with increasing soil water deficit in vegetable amaranth (Sarker & Oba, 2018). Similar findings were obtained from experiments on Andean potato tubers exposed to drought stress. It has been shown that the level of carotenoids was affected in a cultivar-dependent manner, while carotenoids increased as a result of drought stress in most of the cultivars (Andre et al., 2009). In addition, Heymann et al. (2014) found higher concentration of *cis*-isomers in field-grown spinach compared to those of greenhouse-grown samples, determined by the illumination conditions during cultivation. It is known, that the concentration of carotenoids as well as the carotenoid profile can be influenced by light quality, which is usually related to growing conditions (Heymann et

al., 2014; Hua et al., 2014). In this context, increasing air temperature was shown to enhance the concentration of lutein and β -carotene in kale (*Brassica oleracea* L.), while increasing temperature led to decreasing levels of such carotenoids in spinach within the same experiment (Lefsrud et al., 2005). Besides those environmental-dependent effects, carotenoid levels were shown to be highly genotype-specific, as shown for example in different spinach genotypes (Kidmose et al., 2001). In general, the consumption of vegetables is one of the best methods for humans to ingest carotenoids (Hua et al., 2014), which are considered to play an important role in preventing the onset of chronic diseases, such as age-related macular degeneration (Johnson, 2014, 2012).

Impact on glucosinolates

Glucosinolates are a group of secondary plant metabolites, which are mainly found in plants of the order Brassicales, including important horticultural crops of the Brassicaceae family (Schreiner et al., 2009). In vegetable crops, glucosinolates are exclusively found in the cruciferous vegetables such as broccoli, cauliflower and radish, while each plant species has its distinct profile (Schreiner, 2005). Glucosinolates are β -thioglucoside N-hydroxysulfates with a side chain and a sulfur-linked β -D-glucopyranose moiety (Fahey et al., 2001). In general, glucosinolates are relatively nonreactive (Fahey et al., 2001). After hydrolytic cleavage of the sulfur-linked β -D-glucopyranose as catalyzed by the enzyme myrosinase, a range of physiologically active compounds such as isothiocyanates, nitriles and thiocyanates is released (Rosa et al., 1996; Rosa & Rodrigues, 1998). Isothiocyanates, for example, are considered being involved not only in the plants' defense against herbivores, but also in the human body protection against cancer. Such breakdown products stimulate the production of cellular antioxidant proteins, and thus, protecting the cell against carcinogens (Traka & Mithen, 2009). In general, glucosinolates tend to increase if plants are exposed to abiotic stress factors such as drought. Several studies have established a drought-induced increase of glucosinolates in *Brassica* species (Schreiner, 2005; Radovich et al., 2005; Jensen et al., 1996). For example, drought was shown to affect the glucosinolate concentration in seeds of field-grown rape (*Brassica napus* L.). While early drought stress during vegetative growth led to substantial increase of glucosinolates, late but mild and prolonged drought did not change glucosinolate levels in rape seeds (Jensen et al., 1996). The study revealed that the production of glucosinolates was related to turgor

loss of both leaves and pods, probably because of a turgor-responsive gene expression (Jensen et al., 1996). As already stated by Schreiner et al. (2013), the accumulation of glucosinolates is induced by the plants' osmotic adjustment, since the resulting shift from primary to secondary metabolism promotes the synthesis of glucosinolates (Schreiner et al., 2009). This is also confirmed by findings from cabbage, which showed significant impacts of water supply and cultivation time on its glucosinolate concentrations (Radovich et al., 2005). Plants grown without irrigation or under reduced irrigation regimes during a specific growth stage showed significantly higher amounts of glucosinolates compared those from well-irrigated treatments (Radovich et al., 2005). These findings confirm again the link between water supply, stress severity and the plants' growth stage. In contrast to the aforementioned results, decreasing levels of glucosinolates were observed in broccoli (*Brassica oleracea* var. *italica*) cultivated under moderate water reduction in contrast to full irrigated plants (Robbins et al., 2005). Though, the environmental factors during cultivation seem to play a significant role in determining the levels of glucosinolates or their derivatives in plant tissues of *Brassica oleracea* (Rosa et al., 1996). But, even if the concentration of phytochemicals such as glucosinolates can be modified by environmental conditions during cultivation, the genetic background is the major determining factor (Schreiner, 2005).

Spinach

Botanical classification, cultivation and commercial relevance

Spinach is a highly desirable leafy vegetable of the Chenopodioideae subfamily of the Amaranthaceae. The plant belongs to the annual long-day plants, flowering if day length is longer than 12 hours between May and August. Although there are differences between varieties, flowering is promoted by high temperatures and drought (Wonneberger, 2004). Therefore, spinach is usually grown under short-day conditions during spring and fall in northern regions, in order to avoid bolting (Preece & Read, 2005). Because of its short cultivation time, spinach is well suited as catch crop before and after cultivation of the main crop (Laber & Lattauschke, 2014). In general, plants form a taproot reaching up to 140 cm deep into the ground, while most of the root system is located in the top soil layer down to 30 cm depth (Laber & Lattauschke, 2014). The cultivation of spinach in open field requires deep soils and good water supply, thus,

cultivation without irrigation is almost impossible because of its sensitivity to water shortage and the high quality losses involved (Laber & Lattauschke, 2014). Spinach is mostly produced as ‘leaf spinach’, meaning that plants are being harvested with more or less proportion of their petioles. In case of ‘spinach heads’, the plants are being harvested by cutting them off just below soil surface and usually being sold as whole rosettes (Wonneberger, 2004). Approximately 80% of the spinach cultivated is used for the production of frozen goods and baby food (Laber & Lattauschke, 2014). The worldwide production of spinach has increased more than six times since 1990 (FAO, 2020), highlighting its commercial relevance in human nutrition and its importance to the human diet and nutrition as one of the most popular dark green leafy vegetables. According to Roberts & Moreau (2016), spinach is broadly classified into three types depending on its leaf structure: (i) smooth-leaf; (ii) savoy; and (iii) semi-savoy. While smooth-leaf spinach is preferably used for processing and salads, the savoy and semi-savoy are used for cooking (Roberts & Moreau, 2016).

Nutritional value

Spinach is considered one of the most nutrient-dense vegetables. Similar to other leafy vegetables, it is known for being rich in minerals and phytochemicals such as provitamin A (from β -carotene), Mg, folate, lutein and zeaxanthin (Alvino & Barbieri, 2015), dietary fiber (Souci et al., 2008), as well as K, Ca and Fe (Hedges & Lister, 2007). According to Wonneberger (2004), spinach is known as an extremely nutritious vegetable, being rich in minerals, especially Fe, why it is considered as very healthy food. In particular, phytochemicals such as carotenoids are of high nutritional importance. The carotenoids lutein and zeaxanthin have been strongly suggested to exert protecting properties against eye diseases such as age-related macular degeneration due to their specific accumulation in the macula lutea of the human retina (Johnson, 2014). While the effects of lutein and zeaxanthin are still under debate, all vitamins and numerous minerals have been fully accepted to be important for preventing deficiencies and for maintaining normal physiological function (Hedges & Lister, 2007). Moreover, spinach contains comparably high levels of phenolic compounds contributing to its high antioxidant capacity. For example, the flavonoid content of baby spinach was shown to be 13–23 mg/g DW and 0.6–1.5 g/kg FW, respectively (Bergquist et al., 2005), and 1.8–3.7 g/kg FW in different spinach

genotypes (Cho et al., 2008). The study conducted by Bergquist et al. (2005) revealed that most of the flavonoids found in baby spinach exhibits very high antioxidant capacity due to their position and degree of hydroxylation, such as for example the 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide as well as the different patuletin glycosides. However, in contrast to other vegetables, spinach contains very low levels of common flavonoids such as quercetin, kaempferol, myricetin, luteolin and apigenin (Bergquist et al., 2005), why spinach is classified as low-flavonoid containing vegetable at times (Hertog et al., 1992). Nevertheless, compared to other leafy vegetables, many health-promoting constituents in spinach ranked as medium-high or high (Alvino & Barbieri, 2015). According to Roberts & Moreau (2016), a 100 g serving of spinach meet or exceed the recommended dietary allowance of many health-promoting constituents such as vitamin K, vitamin A, folate, and vitamin C, and it contains substantially more minerals such as Mg, K and Fe than broccoli, cabbage and lettuce. The level of total carotenoids in spinach amounts to approx. 125 mg/kg FW (Bunea et al., 2008), with lutein in particular, but also β -carotene are most abundant. Thus, spinach as well as other green leafy vegetables are of a high nutritional relevance for human consumption. Finally, spinach has many functional properties, why it is considered as functional food with antioxidant, anti-inflammatory, antiproliferative, anti-obesity, hypoglycemic, and lipid-lowering activities (Roberts & Moreau, 2016). Besides the nutritional benefits for human consumption, spinach can also accumulate very high amounts of nitrate (Qiu et al., 2014). The accumulation is mainly promoted by low irradiation during cultivation (Proietti et al., 2004), while the nitrate concentration in spinach is also influenced by the type of fertilizer, as shown by Abubaker et al. (2010). Furthermore, low light conditions during plant growth promotes the accumulation of oxalic acid as well, being considered as 'anti-nutritional' factor limiting the bioavailability of Ca in the human body (Alvino & Barbieri, 2015). Nonetheless, a diet rich in vegetables has positive effects on human health by playing a role in the prevention of a number of chronic diseases.

Effect of water deficit and season on spinach cultivation and quality

As mentioned earlier, leafy vegetables such as spinach are relatively sensitive to drought stress, which can quickly result in high quality losses. Spinach grown under deficient water supply reacted by reduced biomass production due to less relative water content

of leaves, which recovered after re-watering, even though the plant growth remained affected (Esteban et al., 2014). However, it was shown that water shortage could increase the concentration of certain valuable substances in spinach and other leafy vegetables. For example, Koyama et al. (2012) have demonstrated the accumulation of ascorbic acid in spinach and lettuce due to drought stress imposition. Mild drought stress was also shown to increase fresh weight-based contents of chlorophyll, carotenoids, total phenols and flavonoids, but not if considering on dry weight basis (Xu & Leskovar, 2015). Besides such water-related effects, other environmental factors have also been shown to change the chemical composition of leafy vegetables like spinach. Assessing such impact, spinach grown under low irradiation showed increased levels of ascorbic acid (Lester et al., 2013), while low temperature led to an accumulation of carotenoids in spinach, but not in kale, which had reacted in a negative manner (Lefsrud et al., 2005). Both factors are inevitably connected to the cultivation time, which was shown to have an impact on the chemical composition of spinach (Howard & Pandjaitan, 2008). However, the environmental conditions of the growing season is known to change the accumulation of undesirable substances such as nitrate. For example, Santamaria et al. (2001) observed doubled amounts of NO_3^- in rocket grown under high temperatures, and also chicory grown under warm and wet conditions exhibited a significant accumulation of NO_3^- (Ćustić et al., 2003). As already mentioned before, the nutritional composition of spinach is determined by the plants' growth stage, too. Bergquist et al. (2005) observed significantly higher contents of flavonoids in young spinach leaves at early growth stage, while their concentration differed during leaf growth among sowing times. Interestingly, even if the flavonoids found at all growth stages and seasons were the same, the relative amount of individual flavonoids varied strongly (Bergquist et al., 2005). Thus, the annual fluctuations in temperature and radiation as well as the cultivation season are likely to affect the phenolic content of spinach.

Radish

Botanical classification, cultivation and commercial relevance

Radish is an annual vegetable species of the Brassicaceae (Crucifereae) family, which contains many vegetables of worldwide economic relevance. Radish is originally from

Europe and Asia (Gutierrez & Perez, 2004) and represents an important class of root vegetables being cultivated in many parts of the world. Some of the most common representatives and commercially most important crops of the Brassicaceae family are cultivars such as cabbage, turnip and rapeseed. Radish are usually grown in temperate climates and their taste is characterized by a certain degree of pungency (Gutierrez & Perez, 2004), being mediated by enzymatic break-down products of the aforementioned glucosinolates. The species yields tubers having highly variable shapes, sizes and colors, ranging from red to white and even black (Hedges & Lister, 2005). Radish is generally known as a highly desirable vegetable crop due to its short growth period (Wan & Kang, 2006) and its high nutritious value (Souci et al., 2008). The subspecies red radish (*Raphanus sativus* var. *sativus*) belongs to the round varieties, which is usually being consumed raw as salads. Their tubers consisting of the hypocotyl, while those of elongated varieties including hypocotyl and root (Wonneberger, 2004). Most of the common red radish varieties have round shape, though high temperatures usually result in elongated tubers. This might happen if cultivation takes place under glass or foil during wintertime. With focus on quality aspects, it has been shown that drought and heat as well as late harvest can lead to damages in texture in form of air-filled cavities (Wonneberger, 2004). In general, the cultivation of red radish requires loose soils evenly supplied with water. Altogether, the species is easily to grow and can be harvested 4–6 weeks after sowing. Even if red radish is marketed throughout the year, the focus of demand is during the spring month (Laber & Lattauschke, 2014).

Nutritional value

In general, radish is considered a vegetable containing good amounts of proteins, carbohydrates, fiber, vitamin C, minerals and antioxidants (Kaymak et al., 2010; Souci et al., 2008). Similar to other cruciferous vegetables, radish generally contains negligible amounts of fat and serves as a good source of carbohydrates (Manchali et al., 2012). A specificity of cruciferous vegetables such as radish are the glucosinolates, a group of sulfur-containing phytochemicals. The major glucosinolates found in red radish are aliphatic glucosinolates such as 4-methylthio-3-butenyl (glucoraphasatin) and 4-methyl-sulphinyl-3-butenyl (glucoraphanin) as well as indole-glucosinolates such as 3-indolyl-methyl (glucobrassicin). It is known that their breakdown products, the so-called isothiocyanates, are linked with positive properties such as potentially

anti-carcinogenic effects (Hirata et al., 2018). Similar to the aforementioned spinach, radish belongs to the nitrate-accumulating vegetable crops. According to Souci et al. (2008), an average of 220 mg/100 g FW were measured in red radish, however, the concentrations are generally higher in plants from greenhouse compared to those from open field cultivation (Herrmann, 2001). The phenolic substances found in red radish are limited to hydroxycinnamic acids, kaempferol-glycosides and anthocyanins, mainly located in the tubers exocarp. According to Herrmann (2001), radish contains hydroxycinnamic acids such as *p*-coumaric acid, caffeic acid, ferulic acid and sinapinic acid, occurring primarily in form of malic acid-esters. The anthocyanins, being responsible for the red coloration of the tubers, are characterized as pelargonidin-3-sophoroside-5-glucoside derivatives with cinnamic acids attached to the glycosyl moieties (Giusti et al., 1998), partly additionally acylated with malonic acid (Giusti & Wrolstad, 1996a). Even if their concentration is dependent on the cultivar and environmental conditions (Schreiner et al., 2002), anthocyanins extracted from red radish were shown to represent a promising natural alternative to the use of red azo dye FD&C Red No. 40 in food coloration (Giusti & Wrolstad, 1996b).

Effect of water deficit and season on radish cultivation and quality

Root based vegetables such as red radish might be particularly affected by water shortage, especially if stress situations occur during tuber or root initiation (Daryanto et al., 2016). Results from earlier studies indicated that reduced water supply significantly lowered radish yield, while N and NO₃⁻ levels of tubers tended to increase by decreasing irrigation frequency. However, N uptake decreased with decreasing water supply and the same was observed for P as a consequence of reduced P concentration and lower dry matter production (Hegde, 1987). Abiotic stress such as water deficiency usually leads to alterations in plant metabolism, and thus, changes the concentration of certain primary and secondary metabolites. On the other hand, drought can also increase soil rigidity, which consequently affects root or tuber growth indirectly. There is only little information about the effects of drought stress or deficient water supply on the quality of red radish, though inadequate water supply was shown to increase the levels of glucosinolates (GLS). For example, drought stress was shown to enhance the concentration of GLS in *Brassica* species such as *B. carinata* (Schreiner et al., 2009), *B. rapa* ssp. *rapifera* L. (Zhang et al., 2008) and *B. napus* L. (Jensen et al., 1996).

However, such increase was dependent on drought intensity, since minor water deficits did not lead to changes in the GLS levels (Jensen et al., 1996). In contrast, decreasing levels of mainly the indolyl-GLS were observed in broccoli if plants were grown under slightly reduced water supply (Robbins et al., 2005). Moreover, GLS seem to be dependent on the season and the climatic conditions involved. Zhang et al. (2008) proved a significant interaction of growing season and water supply on GLS in turnip roots (*B. rapa* ssp. *rapifera* L.). Red radish GLS, mainly determined by the alkenyl glucosinolate glucoraphasatin (>90%), seem to be less dependent on irradiation and temperature in contrast to those of broccoli and cauliflower (Schreiner, 2005). However, high irradiation combined with low daily mean temperature was shown to increase the level of alkyl-GLS such as glucoraphanin and glucoiberin in broccoli (Schonhof et al., 2007; Schonhof et al., 1999). Moreover, it was shown that total GLS in turnip roots increased significantly under high light intensities during summer (Zhang et al., 2008), which is known to be the up-regulator of the genes for amino acid synthesis (Ma et al., 2001), the precursors of glucosinolates. It was shown by Schreiner et al. (2002), that temperature and irradiation influenced the level of glucobrassicin in red radish, whereas those of alkenyl-GLS such as glucoraphanin and glucoraphasatin remained nearly unaffected. The climatic factors seemed to be restricted regarding their impact on GLS, presumably due to low exposure of radish tubers to direct irradiation (Schreiner et al., 2002). In contrast, glucoraphanin, which represents the main glucosinolate of broccoli, was strongly dependent on irradiation and temperature (Schonhof et al., 2007). Focus on phenolic compounds, red radish anthocyanins were shown to increase due to low temperatures, high irradiation and reduced N supply (Schreiner, 2005; Schreiner et al., 2002; Mazza & Miniati, 1993).

Cucumber

Botanical classification, cultivation and commercial relevance

Cucumber is a member of the Cucurbitaceae family, a genus of fruity vegetables, including other important species such as watermelon (*Citrullus lanatus*), sweet melon (*Cucumis melo* L.) as well as different varieties of the *Cucurbita* sp. such as pumpkin, squash and zucchini (Wonneberger, 2004). The plants have many similar morphological characteristics and most of them are adapted to warm, moist conditions, with some adapted to warm, arid climates. They are characterized by high sensitivity to frost, why

cultivation requires protection or needs to take place during annual warm cycles (Hodges & Lester, 2013). Cucumber represents one of the most popular species of this genus worldwide. It is extensively cultivated for its edible fruit, which is widely consumed fresh in salads or fermented as pickles (Abu-Reidah et al., 2012). The annual plant is considered as a widely distributed and very old vegetable crop, probably originated from India or Africa (Wonneberger, 2004). Most of the common varieties, including pickling cucumbers or gherkins are parthenocarpic, means that fruit development occurs without fertilization. Cucumbers develop rapidly and need shorter time from planting to harvest than most crops (Wehner & Guner, 2004). The fruits grow very fast and their visual appearance can be smooth, grooved or warty, while some fruits have spiky skin (Wonneberger, 2004). Pickling cucumbers are being divided in smooth-skinned and prickled varieties, while prickled varieties are often preferred because of their higher fruit firmness and better processing quality (Laber & Lattauschke, 2014). However, the preference also depends on regional habits. In general, members of the Cucurbits family are connected with high importance in human commerce and high economic value (Hodges & Lester, 2013). The production of gherkins usually takes place in open field cultivation, whereas cucumbers for fresh consumption are produced in protected greenhouse cultivation. However, if cucumbers are being cultivated in open field, they are subjected to direct sunlight, high temperature and wind, resulting in high crop evapotranspiration. Thus, open field-grown cultivars have comparably high water demand under semi-arid conditions in contrast to those grown under shaded greenhouse conditions (Rahil & Qanadillo, 2015). In principle, the water demand of cucumber plants is extremely high, because of their large leaf surface area. However, the cultivation of cucumbers in protected greenhouse cultivation is extremely energy and labor-intensive, and producer prices are low, why cultivation area declined (Laber & Lattauschke, 2014).

Nutritional value

In general, cucumber is not a nutrient-dense food (Hedges & Lister, 2005). The quality of cucumbers is usually classified based on shape, uniformity, defects and color (Schouten et al., 1997). Commercial cucumbers contain 96–97% of water and comparably low amounts of protein, fat and minerals (Souci et al., 2008; Herrmann, 2001). According to McFeeters et al. (1982), the major organic acid in pickling

cucumbers is malic acid, though citric acid was shown to become predominant during maturation and fruit enlargement. In terms of flavonoids, for example, cucumber ranked at the bottom compared to other vegetables such as spinach, cabbage or onions (Chu et al., 2000). However, Abu-Reidah et al. (2012) have identified 73 phenolic compounds in cucumber fruit extracts, highlighting their importance as source of bioactive antioxidants. The characteristic aroma of cucumber fruits, which emerges due to enzymatic reactions after tissue damage, mainly leads back to the (E,Z)-2,6-Nonadienal, (Z)-2-Nonenal and (E)-2-Nonenal resulting from lipoxygenase-catalyzed reactions (Herrmann, 2001). Carotenoids and chlorophylls are primarily located in the skin, but information about their detailed qualitative and quantitative composition is scarce. Herrmann (2001) mentioned certain amounts of lutein (0.470–0.670 mg/100 g FW) and β -carotene (0.130–0.222 mg/100 g FW) in cucumber, while their concentration can be higher in small and dark green-colored fruits. The internal flesh of immature cucumbers is dark green, whereas it become white or colorless at progressing maturity stages (Hodges & Lester, 2013).

Effect of water deficit and season on cucumber cultivation and quality

In general, cucumbers as well as other members of the Cucurbits family are extremely sensitive to drought, because of their sparse root system, which is predominantly located in the upper top soil layer (Rahil & Qanadillo, 2015). Based on the findings of Li et al. (2000), cucumbers seem to need more water than normal grain crops (Najarian et al., 2018). Nevertheless, the plant is one of the most popular greenhouse vegetables with high economic and nutritious value (Najarian et al., 2018). Even though cucumbers are extremely sensitive to any type of water deficit, previous studies addressing the impact of drought stress on cucumbers have primarily focused on physiological parameters such as yield and water use efficiency (Mao et al., 2003; Rahil & Qanadillo, 2015) as well as carbon assimilation and allocation (Janoudi & Widders, 1993). However, the chemical composition of fruits is often neglected, or analytical results are restricted to foliar composition (Akinçi & Lösel, 2010). It was observed that water supply and cucumber yield are in a linear relationship, independently on the plants' growth stage (Mao et al., 2003). This seems obvious, considering the cucumbers' sensitivity to any type of water deficit. In contrast, Rahil & Qanadillo (2015) observed highest yield if plants were grown under reduced water supply of 70% of the crops evapotranspiration (ET_c). The findings were traced back to the uniform water delivery and the more

efficient moisture availability in the root zone, which had probably decreased water leaching and enhanced plant nutrient uptake in contrast to full water supply of 100% ET_c . In addition, the plants' dry matter content and fruit number per plant were higher under 70% ET_c in contrast to full irrigation (Rahil & Qanadillo, 2015). Akinci & Lösel (2010) observed no significant effects of reduced water supply on the foliar sugar content in cucumbers, regardless of whether plants were affected by moderate or severe drought stress. On the other hand, water reduction was shown to enhance the flavonoid concentration in cucumber cotyledons, whereas the additional application of UV-B radiation led to a decrease in the flavonoid level in contrast to well-watered plants (Tevini et al., 1983). Beside the effects of water supply, the cultivation time, including global radiation, temperature and transpiration, was also shown to have an impact on the chemical composition of cucumber fruits (Gómez-López et al., 2006). In addition, the accumulation of dry matter in the different plant organs revealed that leaf photosynthetic capacity was adjusted to the plants' demand for assimilates, which again seem to be dependent on the fruit load (Gómez et al., 2003). As already mentioned before, dry matter partitioning depends on sink strength, and fruits are the major sinks in case of fruity vegetables such as cucumber. Furthermore, the sink strength is defined by its potential growth rate, which again is a function of both age and temperature. Finally, the number of fruits set per plant has a high impact on dry matter partitioning and fruit growth (Marcelis & Heuvelink, 1999).

Aims of the Doctoral Thesis

The objective of this study was to investigate the effects of moderately or even mildly reduced water supply on the chemical composition of three different types of field-grown vegetables. The vegetables of interest should differ in the harvested plant organs used for human consumption. Therefore, the leafy vegetable spinach, the root vegetable radish and the fruity vegetable cucumber were grown in open field cultivation and partly in a separate green-house trial during three consecutive years under well-watered and moderately or mildly reduced water supply. After harvest, the leaves, roots and fruits were subjected to comprehensive analyses to elucidate their chemical and nutritional composition. The study was motivated by on-going climate change-driven shifts in precipitation patterns and by growers reporting to have to deal with lower amounts of

total water supply during cultivation. As climatic conditions differ per season and year, the results from this triennial experiment should also reveal seasonal and year-to-year variabilities of the chemical composition of the vegetables' harvest organs. Therefore, the findings may contribute to answer the question to what extent low water reduction and the respective environmental conditions of season and year can influence the chemical compositions of the studied vegetable crops. These goals were set according to the interest of both producers, consumers and scientists, since detailed information about the effects of mild water shortage as expected by short- and mid-term consequences of climate change on a variety of chemical components in vegetables was extremely scarce. Most of the available reports concerning the effects of water reduction on vegetables had focused on the effects of water scarcity on plant organs others than those being harvested (Akinci & Lösel, 2010; Ashraf et al., 2013) or had examined morphological and physiological parameters such as growth (Afzal et al., 2014; Saeidi et al., 2017), yield (González et al., 2009), fruit length (Najarian et al., 2018) as well as photosynthesis (Afzal et al., 2014) and water use efficiency (Akram et al., 2015). Herein, a wide array of phytochemicals and constituents of the produce was targeted, including sugars, polyols, minerals, trace elements, organic acids, polyphenols, carotenoids, and glucosinolates. Therefore, this study offers detailed analytical information about the effects of seasonal variations as well as mild and moderate water deficits on the chemical and nutritional composition of spinach, radish and cucumber.

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Chapter 1: Chemical composition of field grown radish (*Raphanus sativus* L. var. *sativus*) as influenced by season and moderately reduced water supply

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

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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 & 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 & Frei, 2011). While the effects of considerable drought stress events on plant physiology have been investigated in detail (Taiz & Zeiger, 2010; Akinci & Lösel, 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 & 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 & 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-carcinogenic 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 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 Table 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 m², 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 (NO₃⁻-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/m² 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 (Table 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). Table 1 summarizes further detailed information on cultivation data and water supply.

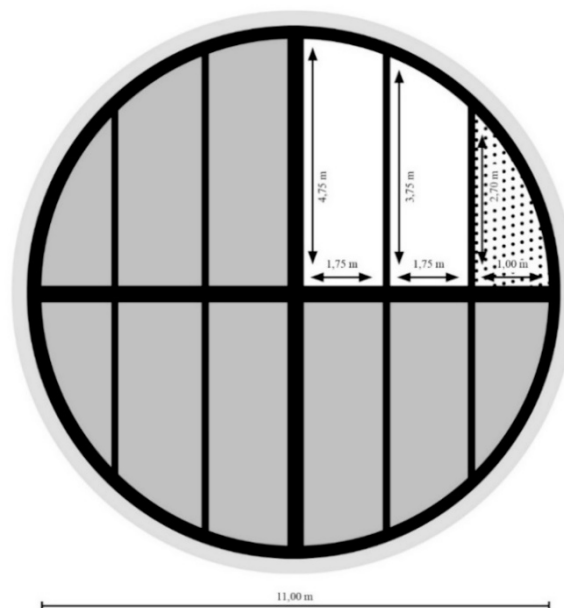


Figure 1: Circular structure of the open field experimental setup. Each quarter of the circle represents a plot divided into three subplots. Red radish was grown in one of the four plots. Subplots differed in the irrigation regimes. The outer subplot (marked by dot pattern) was excluded from the harvest.

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 (Table 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 Table 1. Based on detailed weather situations of daily temperature and air humidity profile (supporting information, Figures A.1 and A.4), the different growing periods were characterized 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, Figures A.2 and A.3), whereas the conditions in 2016 were much more constant.

Table 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 ($^{\circ}\text{C}$)	565.1	488.1	483.6	408.9
Daily mean air temperature ($^{\circ}\text{C}$)	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%

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 $-80\text{ }^{\circ}\text{C}$, 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 $4596 \times g$ to separate liquid and solid phases, the supernatants were collected, filtered, and stored at $-25\text{ }^{\circ}\text{C}$ 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 x 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

Titrate 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 x 4.6 mm and a Rezex Fast Fruit 100 x 7.8 mm (Phenomenex, Aschaffenburg, Germany). An isocratic elution profile with diluted aqueous phosphoric acid (1 mL H₃PO₄ (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 x 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, Titroline 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 H₂SO₄, 330 ml aqueous 30% H₂O₂ 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 x 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 & 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 & 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 & Dietrich, 2006). Injection volume was 4 μL and flow rate 250 $\mu\text{L}/\text{min}$ at 40 $^{\circ}\text{C}$. 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 & 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. (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):

$$y = \text{water supply} + \text{plot} \text{ (eq. 1)}$$

$$y = \text{water supply} + \text{plot:year} \text{ (eq. 2),}$$

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 & 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 & 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. 2) 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. 2). 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%) (Table 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. 2).

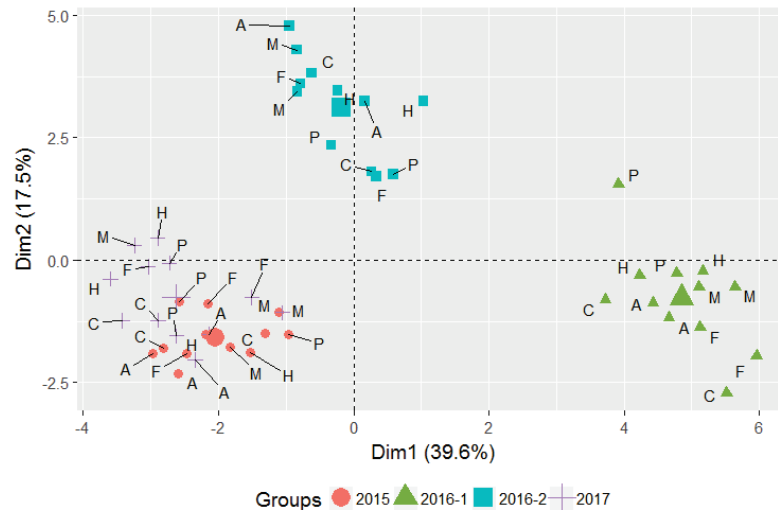


Figure 2: Principal Component Analysis of the original, non-adjusted dataset including all radish cultivation sets (2015 (red circles), 2016-1 (green triangles), 2016-2 (blue squares) and 2017 (violet crosses)). Score plot represents the individual samples of each cultivation set (separated by color)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

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

When comparing chemical parameters across the full dataset by 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. 3A). 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. 3B). 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 Table 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 (Table 2). When subjecting the fresh matter-related data to PCA, the distinction of both

treatments was substantially clearer (Figure 4A) than when using dry matter-related data (Figure 3A). 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. 4A–B). Univariate evaluation (Table 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 (Table 2), which was also shown for carrot tap roots subjected to drought stress just prior to harvest (Sørensen et al., 1997).

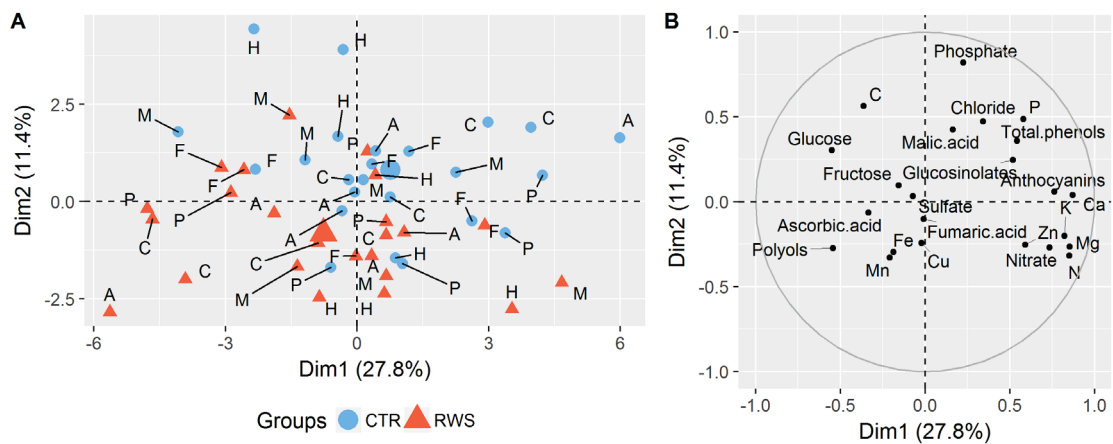


Figure 3: Multivariate evaluation by PCA (PC1+PC2) of the total radish dataset including all cultivation sets (2015, 2016-1, 2016-2 and 2017) based on dry matter-analyses. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according control CTR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

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., Table 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 Table 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 (Table 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 (Tables 2 and 3), except for the set 2017 grown under cold (daily mean air temp.: 12°C) and comparably well-watered (97% relative to control, Table 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 (Tables 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 (Table 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 $\leq 10\%$ 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.

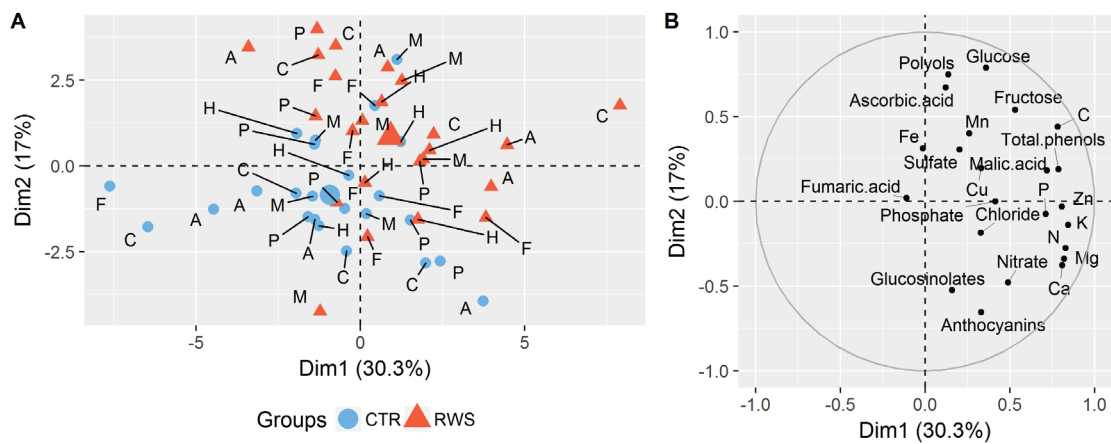


Figure 4: Multivariate evaluation by PCA (PC1+PC2) of the total radish dataset including all cultivation sets (2015, 2016-1, 2016-2 and 2017) based on fresh matter-analyses. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according control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

Anions

Nitrate (NO_3^-) contents were only insignificantly influenced by reduced water supply (Tables 2 and 3, Fig. 3A–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 Table 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 (Table 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 & 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 (Table 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 (Figure 4A–B).

Nitrogen

Mild reductions in water supply had no significant impact on dry matter-related total nitrogen (N) in radish (Table 2, Figs. 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 (Table 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. Gisléröd 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 & 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.

Table 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

Table 2. (continued)

	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
<i>Micronutrients</i>															
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

Table 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

Table 3. (continued)

	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
<i>Micronutrients</i>															
Iron [$\mu\text{g}/100\text{ g}$]	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 [$\mu\text{g}/100\text{ g}$]	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 [$\mu\text{g}/100\text{ g}$]	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 [$\mu\text{g}/100\text{ g}$]	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 [$\mu\text{mol}/\text{g}$]	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 [$\mu\text{mol}/\text{g}$]	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 [$\mu\text{mol}/\text{g}$]	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

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. 3A–B, Table 2). In contrast, significantly increased fresh matter-related contents were observed in the sets of 2015 and 2017 with reduced water supply (Table 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 & 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 (Table 2). Multivariate analyses by PCA support this result (Figures 3A–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 & 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 (Table 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 & Zeiger, 2010) including responses to abiotic stress like drought (Hu & 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 & 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 (Figures 3A–B). However, univariate evaluation demonstrated an increase in the Mg^{2+} content due to reduced water supply in 2016-1 ($p = 0.0946$) (Table 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 ($p = 0.0387$) (Table 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 (Table 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 & 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. 3B). 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 (Table 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 (Table 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 (Table 2), while fresh matter-related contents remained unchanged (Table 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 pelargonidin-3-*O*-(*p*-coumaroyl)-diglucoside-5-*O*-(malonyl)-glucoside and pelargonidin-3-*O*-(feruloyl)-diglucoside-5-*O*-(malonyl)-gluco-side, accounting for approx. 80% of the total anthocyanins (Wu & 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 (Table 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 (Figure 3A–B). Interestingly, both fresh and dry matter-related contents of anthocyanins were highest in 2016-2 followed by 2017 (Tables 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 & 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 (Tables 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 glucoraphanin (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 $\mu\text{mol/g DM}$) as compared to the other years (11.97–16.60 $\mu\text{mol/g DM}$). In 2016, precipitation was lowest and, thus, irrigation events were frequently released, but total water amounts were considerably 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.

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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.

Author Contributions

Conceptualization, H.D. and J.Z.; methodology, C.S., F.W. and H.D.; software, M.F. and C.S.; formal analysis, C.S. and M.F.; investigation, C.S.; resources, H.D., F.W., R.S. and J.Z.; data curation, C.S.; writing—original draft preparation, C.S.; writing—review and editing, C.S., J.Z., H.D., M.F, F.W. and R.S.; visualization, C.S.; supervision, R.S., H.D., M.F. and J.Z.; project administration, H.D. and J.Z.; funding acquisition, H.D. and J.Z. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

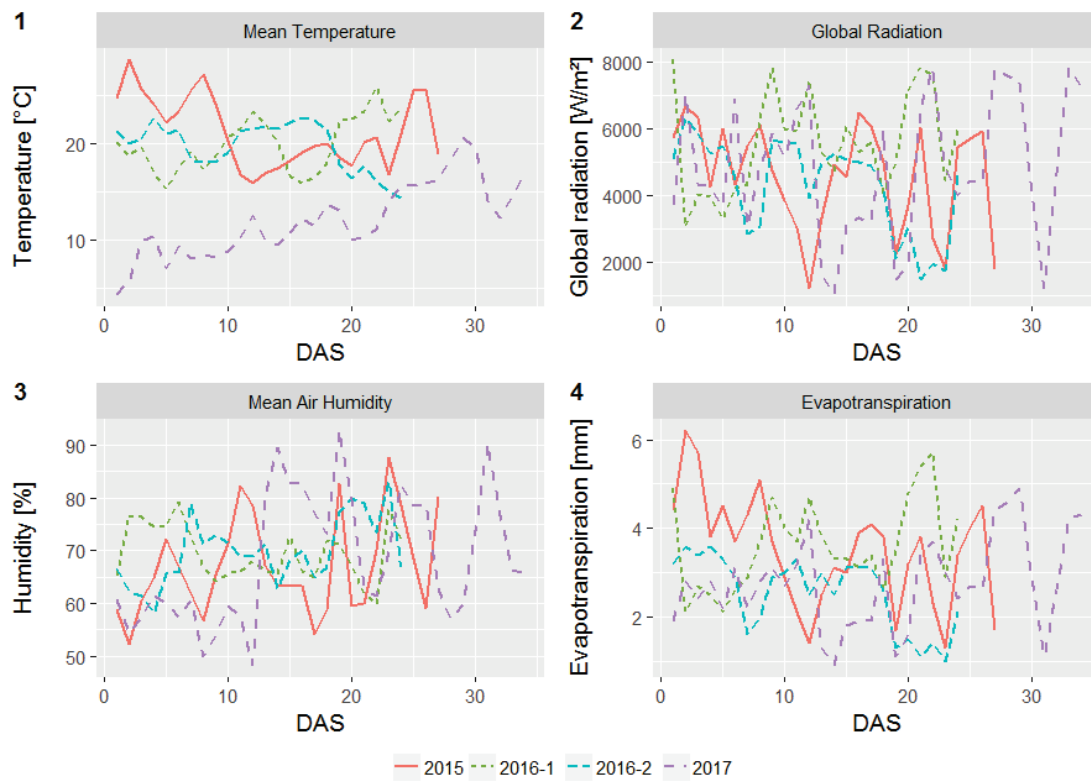


Fig. A.1–4 Daily mean weather conditions during the different cultivation periods 2015, 2016-1, 2016-2 and 2017: (1) Mean air temperature [°C], (2) Global radiation sum [W/m²], (3) Daily mean relative humidity [%] and (4) Daily mean evapotranspiration [mm]; DAS = Days After Sowing.

Chapter 2: Alterations in the chemical composition of spinach (*Spinacia oleracea* L.) as provoked by season and moderately limited water supply in open field cultivation

Abstract

The current use and distribution of agricultural water resources is highly prone to effects of global climate change due to shifting precipitation patterns. The production of vegetable crops in open field cultivation often requires demanding water applications, being impaired in regions where climate change will increasingly evoke water scarcity. To date, increasingly occurring precipitation-free periods are already leading to moderate water deficits during plant growth, e.g., in southern Europe. Among all vegetable crops, leafy vegetables such as spinach (*Spinacia oleracea* L.) are particularly vulnerable to limited water supply, because leaf expansion is highly dependent on water availability. Besides biomass production, water limitation might also affect the valuable nutritional composition of the produce. Therefore, we investigated the impact of moderately reduced water supply on the chemical composition of spinach, cultivated in the open field in three consecutive years. Two different water supply treatments, full and reduced irrigation, were used in a randomized block design consisting of three sets of six plots each. In the reduced water supply treatment, the total amount of supplied water, including both irrigation and natural precipitation, amounted to 90%, 94% and 96% in 2015, 2016 and 2017, respectively, of the full, optimal water supply treatment. Spinach grown under limited water supply showed significantly higher fresh biomass-based contents of polyols (e.g., inositol, glycerol), ascorbic acid, potassium, nitrogen, phosphorous, zinc and manganese, as well as total flavonoids and carotenoids. Increased dry biomass-based levels were found for total inositol, zinc and manganese, as well as decreased levels for malic acid, fumaric acid, phosphate and chloride. Furthermore, we report a high seasonal variation of several minor phytochemicals, such as single flavonoids. Spinacetin derivatives, spinatoside-glucoside as well as a rather unusual hexuronlated methylenedioxy flavonoid showed highest amounts when grown under relatively low irradiation in autumn. Levels of patuletin derivatives tended to increase under high irradiation conditions during spring. In summary, the chemical

composition of spinach was shown to be highly sensitive to moderately reduced water supply and seasonal variation, but the overall nutritional quality of fresh marketable spinach was only marginally affected when considering health-related constituents such as minerals, trace elements, flavonoids and carotenoids.

Keywords: vegetables; water deficit; climate change; polyols; minerals; flavonoids; carotenoids

SCHLERING, C.; ZINKERNAGEL, J.; DIETRICH, H.; FRISCH, M.; SCHWEIGGERT, R. (2020). Alterations in the Chemical Composition of Spinach (*Spinacia oleracea* L.) as provoked by season and moderately limited water supply in open field cultivation. *Horticulturae*, 6, 25, doi:10.3390/horticulturae6020025.

Introduction

The ongoing climate change as well as the associated side effects, such as rising temperatures and substantial shifts in precipitation patterns, may lead to less favorable conditions for the cultivation of the respective, currently grown crop plants. Future projections of precipitation indicate even more severe conditions for almost the entire European continent [1]. Changing climatic conditions do not only boost temperature and rainfall fluctuations, but also consequently influence soil evaporation and plant transpiration [2]. Adverse distributions of rainfall during the cultivation period are already likely to create short-term water deficiencies in horticultural crops, which lead to sub-optimal conditions for plant growth. These temporary stress-related conditions may induce complex interactions in plant metabolism, especially under heterogenic requirements in open-field cultivation, where a combination of different stress factors simultaneously occurs. The results in terms of physiological and biochemical responses can be very different as a function of growth stage, severity and duration of stress [3]. In addition, plant responses to stress are dependent on the tissue or organ affected by the stress [4]. The reaction of plants to drought consists of numerous coordinated processes to alleviate both cellular hyperosmolarity and ion disequilibria [5]. Plants respond to drought stress with physiological and biochemical changes, aiming at the

retention of water against a high external osmotic pressure and the maintenance of photosynthetic activity, while stomatal opening is reduced to counteract water loss [5]. While severe drought stress often results in clearly unmarketable crops, mild and moderate limitations in water supply induce more subtle changes and have earlier been considered to even enhance the formation of health-promoting antioxidant constituents in leafy vegetables [6]. However, this effect is highly plant-specific. For instance, experiments with moderately induced drought stress towards the end of the cultivation period followed by re-watering did not lead to significant increases in the concentrations of antioxidant compounds such as carotenoids and tocopherols in spinach (*Spinacia oleracea* L.), but did increase the levels of the aforementioned compounds in rocket [7]. Therefore, smart irrigation strategies are already used in horticultural systems to reduce the consumed irrigation water and even improve harvest quality [8]. However, severe water deficits evoked by fewer precipitation events or scarcity of irrigation water can be hard to manage and thereby cause short-term stress events during plant development. Leafy vegetables such as spinach are usually highly sensitive to water deficits, because transpiration is affected due to stomatal closure. Stomatal closure as well as leaf growth inhibition are among the earliest responses to drought, protecting the plants from extensive water loss [3]. Since the biological function of plant leaves, i.e., photosynthesis, requires their exposure to sunlight and air, they might be expected to most sensitively react to a number of stress factors. The sensitivity of the photosynthetic apparatus is the basis of chlorophyll fluorescence measurements for recognizing plant stress prior to other physiological and even macroscopically visible stress responses [9]. Among the latter, the total leaf area is determinative for biomass accumulation and crop yield and also represents a factor highly correlated with detrimental effects on crop growth under stressful conditions [10]. Mild environmental stress was shown to lead to a significant decrease in the yield of freshly harvested spinach due to diminution of the relative water content, which recovered to control values within three days after re-watering [7]. However, plant growth remained affected. Thus, attempts to enhance levels of health-promoting phytochemicals like antioxidants by manipulating environmental factors may be burdened by a drop in yield [7]. Water deficit inhibits plant growth by reducing water uptake into the expanding cells, and enzymatically alters the robustness and plastic properties of the cell wall [4]. A crop's need for water varies considerably among species, but water requirements for horticultural crops are

generally high [11]. Especially in the case of leafy vegetables, constant amounts of available water are crucial [11].

Spinach is a leafy vegetable belonging to the long-day plants, which flower, or bolt, if the light period is as long as or longer than a 'critical day length' [11]. In northern regions, spinach is usually grown under short-day conditions in spring or fall in order to avoid bolting [11] and unfavorable textural alterations like fibrousness and stringiness. In brief, bolting renders spinach unmarketable. Similar to other leafy vegetables like lettuce or cabbage, spinach can tolerate lower light levels than fruiting vegetable crops [11]. Spinach is viewed as a vegetable with high nutritional quality [12], providing many health-promoting antioxidant constituents like carotenoids, flavonoids and other phenolic compounds [13], as well as considerable amounts of minerals, trace elements and vitamins, like vitamin C [14]. As compared to other flavonoid-rich vegetables such as Swiss chard (2.700 mg/kg fresh matter (FM) [15]) or red lettuce (1.400 mg/kg FM [16]), spinach showed similarly high contents of flavonoids (1.000 mg/kg FM [17]). Spinach is also known to accumulate high levels of rather undesired substances such as nitrate (547–3.350 mg/kg FM) and oxalate (2.309–10.108 mg/kg FM) [18]. With respect to its high nutritional quality, we studied how moderately reduced water supply might influence the chemical composition and, thus, the product quality of field-grown spinach. By evaluating this during three different cultivation years, this study also demonstrated the seasonal variability of the above-mentioned nutritionally relevant substances in spinach.

Materials 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), (+)-catechin-hydrate, *myo*-inositol (>99,5%, HPLC), anhydrous glycerol ($\geq 99,5\%$) and *meso*-erythritol ($\geq 99\%$) from Sigma Aldrich (Steinheim, Germany). Quercetin-4'-O-glucoside (spiraeoside), (all-*E*)-lutein and (all-*E*)- β -carotene were obtained from Extrasynthese (Genay Cedex, France). L-aspartic acid (Ph. Eur., USP) was received from AppliChem (Darmstadt, Germany).

Plant material and field experimental design

Spinach cv. 'Silverwhale' seeds were purchased from Rijk Zwaan Welter (Welter, Deutschland) and were grown under open field conditions on a sandy loam at Geisenheim University, Germany (49°59' N, 7°58' E). The experimental field design has been reported earlier in detail by Schlering et al. [19]. In brief, one cultivation set per year was conducted in a plot installation between the years 2015 and 2017, resulting in three years, i.e., cultivation replicates, being grown as shown in Table 1. Each set consisted of six circular plots (marked by A, C, F, H, M and P) with an inner diameter of 11.9 m. Each plot was subdivided into four quarters (subplots) for the simultaneous cultivation of different vegetable crops with an annual crop rotation. Each subplot quarter was again divided into two further segments for the implementation of the different irrigation treatments following a randomized block design (cf. Schlering et al. [19]). The size of each segment was at least 6.5 m², respectively. A third segment located outwardly was not harvested to avoid boundary effects but cultivation and irrigation took place as described in the next section. The irrigation levels of the subplots described below remained unchanged during the full experimental period, irrespective of the annual crop rotation.

Cultivation and water supply

Spinach seeds were sown by a manual seed-drilling machine with row spacing of ca. 0.25 m and a sowing distance of ca. 0.013 m, corresponding to a sowing density of ca. 308 seeds per m². Uniform fertilization with calcium ammonium nitrate was carried out according to commercial standard specifications for the cultivation of standard-quality spinach for the fresh food market (135 kg/N ha⁻¹) based on mineralized N (NO₃⁻-N) in 0–30 cm soil depth. Crop protection was applied equally to all plots, whereby application depended on the growing set, described as follows: Chemical and biological insecticides were used depending on pest occurrence. NeemAzal®-T/S (Trifolio-M GmbH, Lahnau, Germany) was used against leafminer flies (*Agromyzidae*) and Fastac ME (BASF-SE, Limburgerhof, Germany) was applied against aphids (*Sternorrhyncha*). Goldor® Bait (BASF, Ludwigshafen, Germany) had been brought into the soil before sowing once per cultivation set against wireworms (*Elateridae*). Weed control was done manually.

Water supply by drip irrigation was generally activated when the soil suction tension fell below -20 kPa at a 10 cm depth, as controlled by a tensiometer with an electronic pressure sensor (Tensio-Technik, Bambach, Geisenheim and Deutschland). During the initial growth stages, i.e., until the appearance of the seedlings, the soil moistures of both the well-watered control (CTR) and the reduced water supply (RWS) treatments were kept evenly moist by irrigation. Depending on the year, this period of identical water supply lasted for 19 days (2015), 20 days (2016) and 17 days (2017). Then, the well-irrigated CTR segments were provided 6.2 L/m² per irrigation, whereas the RWS treatment was reduced to ca. 50% of that of the CTR treatment. Because of the natural precipitation, both the volume of irrigation water and also the ultimate total water volume varied strongly between years. As a result, the total water volume of the RWS was 90% (2015), 94% (2016) and 96% (2017) of that of the CTR treatments during the respective years (Table 1).

Harvest

A total of 40 plants per segment was harvested randomly when most of the leaves were unfolded, representing the commercially targeted maturation stage for fresh-marketed spinach. To avoid border effects, outer rows were omitted from harvest. Specifically, harvest dates were 51, 44 and 39 days after sowing years in 2015, 2016 and 2017, respectively (Table 1). After harvest, plants were cleaned twice manually with fresh tap water to remove adhered soil particles and roots were removed with a knife, before leaves were spun using a commercial salad spinner to remove remaining water. Subsequently, the fresh spinach leaves were frozen at -80 °C until further analyses.

Climatic data

Local climate data were supplied by the local weather station, which was located at 100 meters distance from the experimental field site in Geisenheim. Climatic parameters for each cultivation period are summarized in Table 1, while detailed weather conditions can be obtained from Figures S1–S4 in the Supplementary Materials. Based on global irradiation and total water amount (Table 1), the three different growing periods were characterized and assigned as follows: 2015 (less irradiation, dry), 2016 (more irradiation, moist) and 2017 (more irradiation, dry). More precisely, the cultivation periods 2016 and 2017 were characterized by high global irradiation and

evapotranspiration (Supplementary Figures S2 and S4), but relative air humidity was considerably lower during the vegetation period in 2016 (Supplementary Figure S3) and the temporal development of the temperature profiles were opposite between the cultivation periods. While the temperature constantly decreased in 2015, it increased substantially during the growth periods in 2016 and 2017. Altogether, temperature sum and mean air humidity did not differ strongly between years, whereas their profiles during plant growth varied strongly (Supplementary Figures S1–S4). In addition, the precipitation profiles were quite different between years (data not shown). While natural precipitation was relatively high during the first half of the growing period in 2015 and 2017, when additional irrigation was mainly initiated in the last two weeks of cultivation, the opposite was true for the cultivation set in 2016, when natural precipitation was very high in the second half of the cultivation period.

Table 1: Cultivation and climate data of the spinach years 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	2017
Sowing date (Year-month-day)	2015-09-07	2016-04-19	2017-04-20
Beginning of water supply differentiation (DAS)	23	23	26
Harvest date (DAS)	51	44	39
Temperature sum (°C)	626.0	593.2	549.8
Daily mean air temperature (°C)	12.0	13.2	13.8
Mean relative air humidity (%)	78.8	68.7	67.8
Global irradiation sum (MJ/m ²)	453.5	799.9	756.9
Daily mean global irradiation (MJ/m ²)	8.7	17.8	18.9
Wind speed sum (m/s) at height of 2 m	46.0	69.3	50.8
Evapotranspiration sum (mm) ^z	68.7	126.9	126.6
Precipitation sum (mm)	65	101	68
Number of differentiated irrigation events	4	5	2
Total irrigation (mm) ^y	39	95	45
Total water amount (mm) incl. precipitation	104	184	113
Total water amount of RWS (% of CTR)	90%	94%	96%

^z Reference evapotranspiration (ET_o) using grass and the FAO56 Penman-Monteith method. ^y Includes irrigation during the treatment period (6.2 L/m² x irrigation events) and the irrigation during initial growth stages.

Sample preparation

For all analyses, except for the determination of ascorbic acid, 150–200 g frozen spinach leaves of each sample were lyophilized (BETA 2-8 LDplus, Martin Christ Gefrier-trocknungsanlagen GmbH, Osterode am Harz, Germany) prior to grinding with a

laboratory mill (IKA M 20; IKA-Werke, Staufen, Germany). Dry biomass content was determined gravimetrically using fresh and 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 plant material was thoroughly homogenized with 500 mL ultrapure water at room temperature and rigorously mixing for ca. 10 s using a stainless-steel food blender (setting 2, Waring Blender, Waring Commercial, Torrington, CT 06790, USA). After transferring the extract including solids into an 800 mL beaker using another 150 mL of added ultrapure water, extraction 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 $4.596 \times g$ to separate liquid and solid phases, the supernatants were collected, filtered and stored at $-25\text{ }^{\circ}\text{C}$ until analyses. Extraction procedures for all other target analytes are given below.

Chemical analyses

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 spectrophotometrically using enzymatic kits (R-Biopharm, Darmstadt, Germany) and a Konelab 20 Xti analyzer (ThermoFisher, Dreieich, Germany).

Determination of polyols excluding the aforementioned sugars was carried out in duplicates as described by Schlering et al. [19]. Quantitation was carried out with linear external calibrations of *myo*-inositol, glycerol and erythritol.

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 spectrophotometrically using enzymatic kits (R-Biopharm, Darmstadt, Germany). Fumaric acid was determined by HPLC with UV detection and ascorbic acid by iodometric titration, as previously described [19].

Inorganic anions

Nitrate, sulfate and phosphate were determined by ion chromatography and chloride by potentiometric titration with an AgCl-electrode, as described previously [19].

Total carbon and nitrogen

Elemental analyses of spinach 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 lyophilized and powdered plant material at 950 °C. L-aspartic acid was used as a reference substance.

Minerals and trace elements

Minerals and trace elements were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (SPECTRO ARCOS, SPECTRO Analytical Instruments, Kleve, Germany) after Kjeldahl digestion with the Gerhardt Turbotherm rapid digestion unit (C. Gerhardt GmbH & Co. KG, Königswinter, Germany), as reported earlier [19].

Total phenols

An aliquot of 500 mg of lyophilized powdered plant material was extracted twice with 12 mL of 70% aqueous methanol under ultrasonication for 30 min. After centrifuging for 10 min at 12.857 x 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. A linear (+)-catechin calibration was used as described previously [20].

Flavonoids

Flavonoids were extracted from lyophilized powdered material as described for total phenols (see above) in duplicates. Extracts were filtered through a 0.2 µm membrane using a polytetrafluoroethylene (PTFE) syringe filter (Macherey-Nagel, Düren, Germany), before extracts were analyzed with reversed-phase HPLC equipped with a photodiode array detector (RP-HPLC/PDA) with negative electrospray ionization mass spectrometry (ESI-MS) on an Accela/LXQ system (ThermoFisher, Dreieich, Germany). Chromatographic separation was achieved on a 150 × 2 mm, 3 mm C18 (2) Luna column (Phenomenex, Aschaffenburg, Germany) protected with a guard cartridge of the same material. Injection volume was 4 µL and elution conditions were the following: flow rate 250 mL/min at 40 °C, solvent A was water/formic acid (95:5, v/v),

solvent B was methanol, 1 min isocratic conditions with 10% B, linear gradient from 10% to 55% B in 25 min, followed by washing with 100% B and re-equilibrating the column. MS scan range was set at m/z 250–1050 (negative mode). The MS settings were: ESI source voltage +4.5 kV, capillary voltage 32 V, capillary temperature 275 °C, collision energy for MSn experiments 30%. Flavonoids were identified by comparison of their retention times, UV/Vis and mass spectral data with those of authentic standards or published data [21,22]. Flavonoids were quantified at 360 nm using an external spiraeoside standard (Extrasynthese, Lyon, France) in a linear range of 5–100 mg/L.

Carotenoids

An aliquot of 75 mg of lyophilized powdered spinach was combined with ca. 0.4 g of NaHCO_3 in a glass tube prior to extraction with 4 mL of a mixture of n-hexane/acetone (2:3, v/v) using vortex stirring for 10 s. Then, the extracts were stored on ice in the dark for 10 min using an intermediate stirring step after 5 min. After centrifuging at $4.596 \times g$ for 10 min at 4 °C, the supernatant was collected in 25 mL amber glass volumetric flasks previously flushed with N_2 . The remaining solids were extracted further four times and the obtained extracts were combined and then transferred quantitatively to amber round-bottomed flasks prior to evaporation to dryness under reduced pressure at 25 °C (Rotavapor R-210, Büchi, Switzerland). The dried extract was re-dissolved in HPLC solvent A (see below), transferred to a 10 mL volumetric flask, made up to 10 mL, and filtered through a 0.2 μm membrane filter (Chromafil O-20/25 PTFE syringe filter; Macherey-Nagel, Düren, Germany) into an HPLC vial. Carotenoids contained in the extract were separated with a HPLC-PDA system consisting of a Dionex P 680 HPLC pump, a Dionex STH 585 column oven and Dionex PDA-100 Photodiode Array Detector (Dionex/Thermo Fisher Scientific, Germany), which was mounted with a YMC Carotenoid (C30)-column (4.6 \times 250 mm, 5 μm particle size, YMC, Kyoto, Japan) protected with a guard column of the same material. Column temperature was 20 °C. Solvent A was a quaternary mixture of methanol (MeOH), methyl *tert*-butyl ether (MTBE), water and a 1 M ammonium acetate solution (AAc) (MeOH/MTBE/ H_2O /AAc, 88:5:2:5, v/v/v/v) and solvent B a ternary mixture of MeOH/MTBE/AAc (20:78:2, v/v/v). A linear gradient from 0% to 85% B in 45 min followed by a linear gradient to 100% B in 5 min was applied, followed by an isocratic equilibration step with 100% B for 10 min. Flow rate was 1 mL/min. Total run time was 60 min. Injection volume was 20 μL . Compounds were identified by comparing their retention times and

UV/Vis absorption spectra to those of authentic reference compounds ((*all-E*)-lutein, (*all-E*)- β -carotene) and literature (violaxanthin, neoxanthin, (*Z*)-isomers of β -carotene, Britton (Britton, 1995)). Carotenoids were quantified at 450 nm. An external linear calibration of authentic (*all-E*)-lutein was used for quantitation of violaxanthin, neoxanthin and (*all-E*)-lutein, while authentic (*all-E*)- β -carotene calibrations were used for quantitating (*9Z*)- β -carotene and (*all-E*)- β -carotene.

Statistical analyses

Analytical results were calculated based on both dry and fresh biomass (i) to enable evaluations regardless of different water contents in the plant material by dry biomass-related data and (ii) to assess the nutritional values of the harvested edible plant material by fresh biomass-related data. Data were analyzed by fitting a linear mixed-effect model using the lmer-function within the lme4-package [24] of the statistical software R [25] in RStudio [26]. The evaluation of the single years was based on a model which aimed at correcting for random plot-effects (Equation (1)), while the total dataset was evaluated with respect to the interaction of plot and year (Equation (2)):

$$y \sim \text{H2O} + (1|\text{plot}) \quad (1)$$

$$y \sim \text{H2O} + (1|\text{plot:year}) \quad (2)$$

Comparisons of means derived from different treatments were considered significantly different if $p < 0.10$. Pairwise comparison of least-squares means was carried out with lsmeans-package [27] 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 lmer test-package [28]. The results for the treatment (CTR: control, RWS: reduced) were evaluated on the basis of adjusted data generated from the model mentioned above. Principal Component Analysis (PCA) was carried out by using the R-packages FactoMineR [29] and factoextra [30]. Within PCA analysis, individual samples were visualized in a score plot, while corresponding chemical components were represented in a loading plot inside a correlation circle presenting the relationship between the variables. To avoid an overweight of flavonoids and carotenoids by representation of individual components, only total amounts were considered for PCA.

Results and discussion

Evaluation of years and season

According to the PCA of data that had been corrected for plot effects (Equation (1)), the three years derived from three consecutive growing years (2015, 2016 and 2017) were clearly differentiated by their chemical composition, highlighting the strong influence of the year on the product quality. Similar results were already shown for radish grown within the same experimental field site [19]. The first two principal components (PCs) accounted for 63.7% of the total variance considering dry biomass-related data (Figure 1) and for 65.6% considering fresh biomass-related data (data not shown). Similar year-to-year variations have been reported earlier for other vegetable crops [31,32]. While the two sets from 2015 and 2016 were clearly distinguishable over the first principal component (Dim1, Figure 1), the set from 2017 was additionally separated over the second principal component (Dim 2). The year 2015 was characterized by comparably low sunlight exposure (global irradiation: 453.5 MJ/m²) as compared to 2016 (799.9 MJ/m²) and 2017 (756.9 MJ/m²). Consequently, evapotranspiration was higher in 2016 (126.9 mm) and 2017 (126.6 mm) in contrast to 2015 (68.7 mm). The years 2016 and 2017 were different when considering the total water amount the plants received. In 2016, total water supply was higher (184 mm) than 2017 (113 mm), with the latter being similar to that in 2015 (104 mm, Table 1).

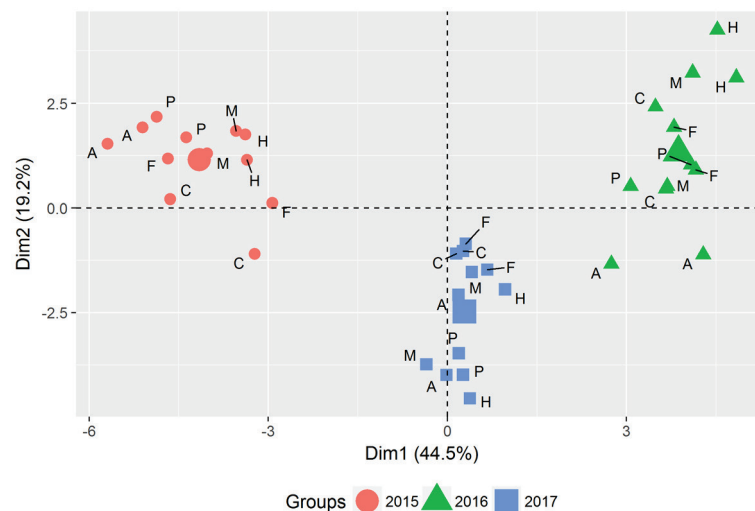


Figure 1: Principal Component Analysis (PCA) of the original, plot-adjusted dataset including all spinach years from 2015 (red circles), 2016 (green triangles) and 2017 (blue squares). Score plot represents the individual samples of each cultivation set (separated by color) and plot (marked by the letters A, C, F, H, M and P) based on dry biomass-related data.

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

The PCA of the entire dataset adjusted by the interaction of plot and year (Equation (2)) revealed that well-irrigated spinach samples were mainly clustered in sectors with negative PC2, while those grown under mildly reduced water supply were mainly located in sectors with positive PC2 (Figure 2A). Most of the individuals were separated by the second principal component PC2 (Dim2), which, however, accounted for only 14.1% of the total variance. The first two principal components (Dim1 + Dim2) accounted for 38.7% of the total variance in the dataset. As shown in the corresponding loading plot (Figure 2B), the most important variables with high contribution to Dim2 were dry-matter-based contents in trace elements like manganese (Mn), zinc (Zn), iron (Fe), total phenols, flavonoids, polyols, as well as nitrate, nitrogen (N), potassium (K) and carotenoids. Dim1 was mainly influenced by contents of sugars (glucose, fructose), organic acids (malic acid, fumaric acid) and anions (chloride, sulfate). Apart from these multivariate analyses, a univariate statistical evaluation shown in Table 2 was conducted to underpin the multivariate estimate. While CTR samples were characterized by higher contents of organic acids like malic and fumaric acid, as well as certain anions like chloride, phosphate and sulfate, the content of inositol and trace elements such as Zn and Mn as well as flavonoids was lower than in samples derived from RWS treatments (Table 2). However, the univariate results also pointed out that the levels of glucose, fructose, quantitatively abundant elements (K, N, P, Ca and Mg) and carotenoids remained widely unaffected by mild water reduction. Only in 2015, when the highest relevant water reduction was achieved, i.e., 10% less than the well-watered control, did some minerals and carotenoids show increased dry biomass-based contents in the RWS samples (Table 2). In our earlier study on red radish root tubers, the identical environmental conditions had not led to an increase in the corresponding levels [19]. In this study, leaves of spinach reacted to the identical water deficits by both the increase of polyols and the accumulation of certain trace elements such as Mn and Zn.

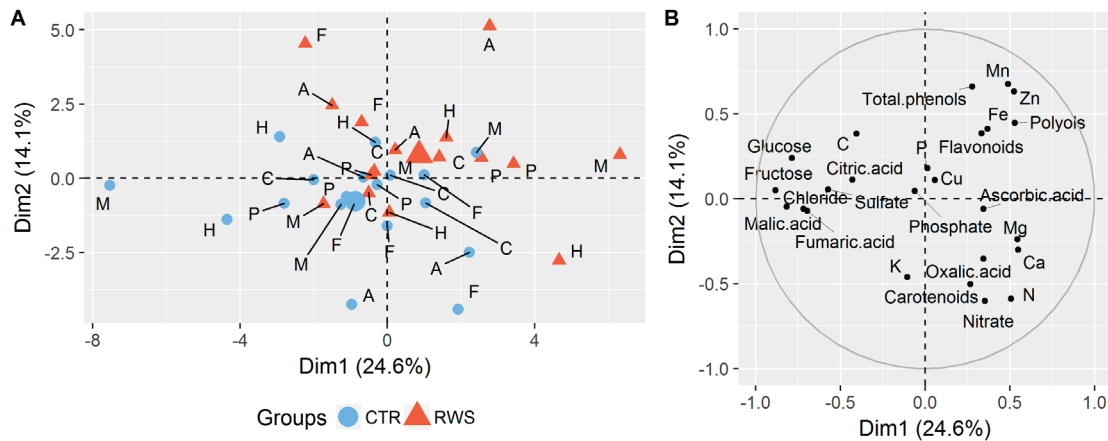


Figure 2. Multivariate evaluation by PCA (PC1 + PC2) of the total spinach dataset including all years (2015, 2016 and 2017) based on dry biomass-data. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according to the control (CTR, blue circles)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

The distinction of both water supply treatments by PCA with fresh biomass-related data was significantly clearer (Figure 3A) than that based on dry biomass-related data (Figure 2A). With very few exceptions, fresh biomass-based data allowed for distinguishing both groups clearly over the first principal component (Dim1), explaining 28.8% of the total variance. Upon addition of the second principal component (Dim2), a share of 46.7% of the total variance was explained. In contrast to red radish root tubers grown under control and reduced water supply conditions [19], the fresh biomass-based discrimination of spinach from well-watered versus reduced water treatments was not mainly based on primary metabolites like polyols, total carbon and ascorbic acid, but also on secondary metabolites such as total phenols, flavonoids and carotenoids, as well as selected minerals and trace elements (Figure 3). In brief, fresh biomass-related contents of most of the targeted chemical components, especially those of polyols, minerals, trace elements and secondary metabolites such as flavonoids and carotenoids, were increased in spinach samples grown under reduced water supply. The univariate evaluation shown in Table 3 confirmed these results. The fresh biomass-based contents of all of the studied carotenoids, i.e., violaxanthin, lutein and β -carotene, showed significant and rather uniform increases (Table 3, Supplementary Table S1). It is noteworthy that the ratio of individual carotenoids in chloroplast-containing plant tissues is rather constant due to their pivotal role in photosynthesis, commonly 20%–25% β -carotene, 40%–45% lutein, 10%–15% violaxanthin and 10%–15% neoxanthin [33]. Occasionally, the levels of xanthophyll cycle carotenoids, such as antheraxanthin,

were reported to be increased upon exposure of the plant to abiotic stress [34]. Findings similar to those of our study were found earlier for antioxidant compounds such as carotenoids and tocopherols in rocket (*E. sativa* Mill. var. Golden line) in the case of moderate drought stress followed by re-watering at the end of the cultivation period [7]. However, the same experiment did not result in higher levels of such antioxidants in spinach [7]. Altogether, fresh biomass-based contents of many other constituents in spinach showed a significant increase, possibly being related to higher dry biomass content of reduced watered plants (Table 2), which was also shown for cabbage (*Brassica oleracea* L.) subjected to drought stress just during head development [35].

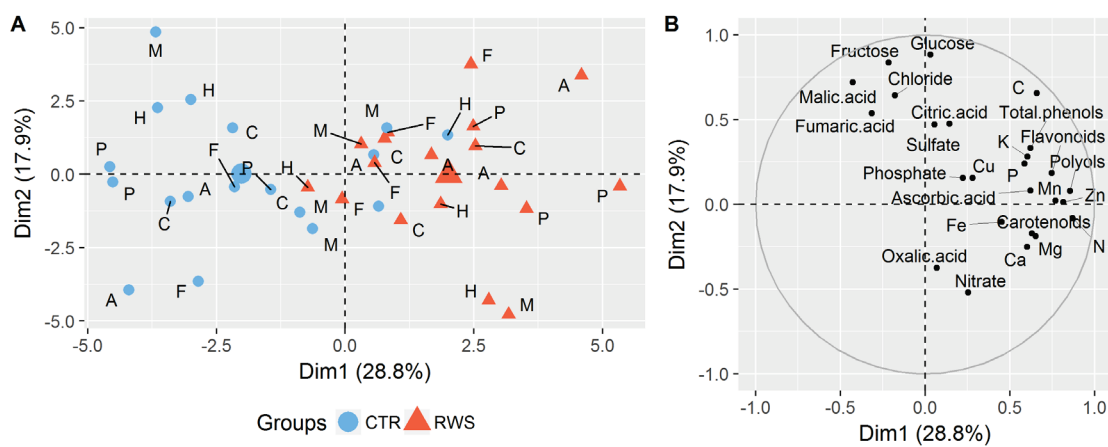


Figure 3: Multivariate evaluation by PCA (PC1 + PC2) of the total spinach dataset including all years (2015, 2016 and 2017) based on fresh biomass-data. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M and P) classified according to the control (CTR, blue circles) and reduced water supply (RWS, red triangles). The corresponding loading plot (B) shows the related variables, i.e., contents of constituents as determined by the chemical analyses.

Sugars and polyols

The content of sugars such as glucose and fructose was hardly affected by moderate water reduction, but was characterized by high variations between the years irrespective of the water supply treatment. The dry biomass-related data emphasized that glucose was highest in 2016 (62.70–72.92 mg/g dry matter (DM)) in contrast to other years (50.92–59.55 mg/g DM), while fructose levels were highest in 2015 (43.35–43.59 mg/g DM) compared to 2016 (30.94–37.58 mg/g DM) and 2017 (27.70–29.41 mg/g DM, Table 2). As shown in Table 1, the 2015 set was characterized by comparably low irradiation (453.5 MJ/m²) in contrast to 2016 (799.9 MJ/m²) and 2017 (756.9 MJ/m²), but the growing period was considerably longer in 2015 (51 days) in contrast to 2016 (44 days) and 2017 (39 days). These conditions, typically occurring during late growing seasons in autumn, resulted in the highest levels of fructose in 2015, while high light

intensities, which are known to increase soluble carbohydrates in spinach [36], led to the highest contents of glucose in 2016. While both tended to decrease with mild water reduction in 2016 ($p = 0.1521$ and $p = 0.0721$ respectively, Table 2), when global irradiation and evapotranspiration were high, values were unaffected or increased in 2015 and 2017 due to mild water reduction. These trends were found for both dry and fresh biomass-related data.

In general, contents of dry biomass were significantly higher in 2015 (8.54%–8.60%) compared to other years (5.40%–6.83%), which automatically leads to enhanced values in the fresh biomass-related results (Table 3). By analogy, the overall glucose and fructose levels were higher in 2015 (537–564 mg and 410–413 mg per 100 g FM, respectively) than in other sets (318–403 mg glucose and 171–208 mg fructose per 100g FM) (Table 3) without significant differences between the treatments.

Analogous to glucose and fructose contents, inconsistent effects of mild water reduction were found regarding the content of polyols in spinach dry biomass. Interestingly, the levels of polyols, particularly those of *myo*-inositol and glycerol, were lowest in 2016, although glucose levels were highest. These findings were in contrast to those found for radish, which showed congruently high levels of glucose and polyols [19]. Total polyols were generally lowest in 2016 (3.87 mg/g DM), being significantly increased to 4.09 mg/g DM upon reduced water supply ($p = 0.0302$) (Table 2). In contrast, levels in 2015 were higher (5.91 mg/g DM), but no changes in concentration were observed upon reduced water supply, although total water reduction was highest (–10%). These findings indicated that factors other than water supply had overruled a potential, marginal effect of the water supply. In our earlier study on red radish, a water reduction of 15%–20% led to significant increases of polyols [19]. Probably, reduced photosynthesis and the associated lowered growth under comparably “low light” conditions in 2015 (daily mean global irradiation: 8.7 MJ/m²) have not influenced the assimilation and allocation of photosynthetic products as much as in 2016 and 2017, when plant growth was accelerated by higher levels of daily irradiation (17.8 and 18.9 MJ/m², respectively). In this context, Quick et al. [37] has shown that the levels of soluble carbohydrates in leaves of two annual crops (*Lupinus albus* L. and *Helianthus annuus* L.) were maintained when exposed to water stress under field conditions, even though photosynthesis was strongly inhibited. Maintenance of soluble sugars probably occurs because partitioning was altered in water-stressed plants [37]. In brief, the accumulation

of polyols in spinach leaves seemed to be more sensitive to moderately reduced water supply than that in radish root tubers, which only showed increased levels after more severe reductions in water supply ($\geq 15\%$ lower than full water supply), as described in Schlering et al. [19].

Organic acids

Organic acids' contents in spinach were inconsistently influenced by moderate water reduction, depending on the cultivation period (year). While dry biomass-based contents of ascorbic acid significantly increased in 2015, those of oxalic and fumaric acid decreased (Table 2). Differences in malic acid contents were insignificant comparing the treatments in 2015, but they were significantly reduced by RWS in 2016. Significant differences for malic acid were also found considering the entire dataset. Cutler and Rains [38] observed significantly increased accumulations of malic acid in leaves of cotton (*Gossypium hirsutum* L.) exposed to water stress, explained by osmotic adjustment and the importance of malate in turgor regulation of stomatal guard cells [38,39]. However, in our study on spinach, rather inconsistent changes in malic acid levels were observed, presumably because mild water reduction applied in this experiment had not been severe enough to cause sufficient stress to alter the plants' metabolism.

The significant increase of dry biomass-related ascorbic acid levels in spinach grown in 2015 ($p = 0.0493$) was in agreement with findings of Koyama et al. [6], who demonstrated an augmentation of ascorbic acid by lower water supply in hydroponically grown leafy vegetables such as lettuce and spinach. In contrast to our study, Koyama et al. [6] did not observe significant alterations in the water content. In our study, by analogy to polyols, ascorbic acid levels were highest in the relatively “low light, dry” cultivation set in 2015, both in dry and fresh biomass, possibly being related to lower global irradiation, which has previously been shown to enhance ascorbic acid contents in spinach [40]. The comparably high content of dry biomass in 2015 contributed to the increased fresh biomass-based levels of ascorbic acid (80.49–85.30 mg/100 g FM) as compared to other years (23.62–46.02 mg/100 g FM).

Inorganic anions

The dry biomass-related contents of anions such as phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) were significantly decreased by RWS in 2015. By analogy, red radish root

tubers studied within the same field experiment exhibited significant decreases of PO_4^{3-} upon similar water reduction, i.e., to 80% of full water supply [19]. Phosphate uptake into the roots might have been diminished under RWS [41], because its mobility depends on soil moisture [10]. On the other hand, the mineralization of organic matter by microbial activity, responsible for the release of ions such as H_2PO_4 and HPO_4^{2-} [11], might have been negatively affected due to water reduction. A significant decrease of dry biomass-related levels of SO_4^{2-} was measurable in 2015 ($p = 0.0192$), when total water reduction was highest compared to other years, but the contents of SO_4^{2-} ($p = 0.0910$) and chloride (Cl^-) ($p = 0.0692$) were also reduced in 2016, when water reduction was lower (Table 2). When considering the overall dataset, a dry biomass-related decline of PO_4^{3-} ($p = 0.0594$) and Cl^- ($p = 0.0053$) was observed in plants that had received slightly reduced water amounts, but the content of SO_4^{2-} also declined ($p = 0.1151$). In contrast, Abdel Rahman et al. [42] observed an accumulation of Cl^- in grasses and legumes as a result of decreasing soil moisture.

In contrast to the above-mentioned anions, the influence of RWS on the dry biomass-related nitrate (NO_3^-) levels in spinach was insignificant. However, an unexpected increase of NO_3^- in fresh biomass-based levels upon limited water supply was observed in 2016 ($p = 0.0238$, Table 3). The opposite was found in previously reported studies of fresh biomass-related nitrate levels of lettuce (*Lactuca sativa*) [6] and carrots (*Daucus carota* L.) [43] exposed to drought stress. In principle, NO_3^- -flux from roots to leaves is believed to generally decrease during drought stress [44], being accompanied by a reduced expression of the nitrate reductase enzyme [45]. This did not apparently occur in spinach grown under RWS in our study, since dry biomass-related levels did not differ between water supply treatments. It is noteworthy that there was also a strong impact of the cultivation year. NO_3^- contents were substantially higher in the moist year 2016 (28.16–28.60 mg/g DM) compared to other comparably drier years 2015 and 2017 (7.73–12.98 mg/g DM), as shown in Table 2. Presumably due to frequent and high precipitation events towards the end of the cultivation period in 2016 (data not shown), a high absorption of NO_3^- might have led to higher NO_3^- levels. This hypothesis warrants further study. Nevertheless, in agreement with our study, a positive correlation was found between NO_3^- and water contents in spinach [46]. Ćustić et al. [47] also observed increased NO_3^- accumulation under warm and wet conditions in chicory (*Cichorium intybus*). It is known that NO_3^- accumulation varies with the season [47]

and is greatly affected by environmental factors [48]. Santamaria et al. [49] observed more than doubled NO_3^- levels in rocket, accompanied by a decrease in dry biomass, when temperature increased from 10 to 20 °C. An inverse correlation of fresh biomass-based NO_3^- and oxalate levels with those of dry biomass content was also confirmed by our results. Therefore, the markedly increased NO_3^- levels in 2016 might have occurred due to high temperature (Supplementary Figure S1) and a simultaneously high water supply (data not shown) along with decreasing irradiation (Supplementary Figure S2) by the end of the cultivation period. It is well known that low irradiation promotes NO_3^- accumulation in spinach [11,50].

Carbon

The dry biomass-related content of total carbon (C) remained unaffected by RWS in spinach, as shown in Table 2. Fresh biomass-related levels were significantly higher in less irrigated plants (2.48 mg/g FM) in contrast to well-irrigated samples (2.20 mg/g FM) only in 2017, when, simultaneously, the dry biomass content also significantly increased, despite the comparably low water reduction (by 4%). Nevertheless, multivariate analysis supports the contribution of C to the separation of both groups in the PCA plot on a fresh biomass basis (Figure 3).

Table 2. Influence of reduced water supply on the dry biomass (DM)-related levels of constituents of spinach leaves from different years (2015, 2016 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.

	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Leaf water potential												
Ψ (-MPa)	nd	nd	nd	0.14	0.18	0.1136	0.07	0.093	0.0842	nd	nd	nd
Dry biomass (%)	8.54	8.60	0.6186	5.40	5.48	0.6736	6.13	6.83	0.0032 **	6.69	6.97	0.0216 *
Sugars and Polyols (mg/g)												
Glucose	56.71	59.55	0.3342	72.92	62.70	0.1521	50.92	57.26	0.3902	60.18	59.84	0.9207
Fructose	43.35	43.59	0.9073	37.58	30.94	0.0721	27.70	29.41	0.6028	36.21	34.65	0.3965
Polyols total	5.91	5.91	0.9565	3.87	4.09	0.0302 *	4.96	4.91	0.4331	4.91	4.97	0.2929
Inositol	1.66	1.68	0.3461	0.99	1.06	0.0830	1.48	1.54	0.1647	1.38	1.42	0.0119 *
Glycerol	2.56	2.58	0.3724	1.67	1.70	0.2236	2.31	2.28	0.3984	2.18	2.19	0.7136
Polyol unknown	1.37	1.38	0.7343	0.71	0.79	0.0530	0.67	0.61	0.1224	0.91	0.93	0.5387
Erythritol	0.33	0.28	0.5110	0.51	0.55	0.2218	0.50	0.47	0.2676	0.44	0.43	0.6779
Organic acids (mg/g)												
Oxalic acid	49.89	44.51	0.0863	62.56	62.56	1.0000	53.70	52.46	0.8734	55.38	53.18	0.4438
Total acidity	16.73	18.73	0.1270	17.20	15.71	0.5452	16.87	18.26	0.2514	16.93	17.57	0.5028
Malic acid	12.81	12.63	0.7896	22.08	18.47	0.0103 *	14.08	13.31	0.6206	16.33	14.80	0.0389 *
Ascorbic acid	9.41	9.90	0.0493 *	4.42	4.84	0.4642	6.85	6.70	0.7087	6.89	7.15	0.2866
Citric acid	6.19	6.14	0.8849	3.18	3.15	0.8647	2.94	3.40	0.1219	4.11	4.23	0.4872
Fumaric acid	0.61	0.57	0.0761	2.26	1.69	0.1530	1.30	1.11	0.5026	1.39	1.12	0.0803
Anions (mg/g)												
Nitrate	8.57	7.73	0.2291	28.16	28.60	0.5373	12.98	12.53	0.8169	16.57	16.29	0.6854
Phosphate	13.65	11.08	0.0377 *	16.69	17.13	0.1913	13.12	12.44	0.2995	14.49	13.55	0.0594
Sulfate	3.44	2.64	0.0192 *	7.73	6.56	0.0910	3.30	3.88	0.1040	4.82	4.36	0.1151
Chloride	5.38	4.95	0.1268	7.28	5.83	0.0692	8.61	7.98	0.1430	7.09	6.25	0.0053 **

Table 2. Cont.

	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value	CTR	RWS	p-value
Elements (mg/g)												
Carbon	381.17	380.38	0.7578	360.43	356.70	0.3442	359.68	362.83	0.3491	367.09	366.64	0.8316
<i>Macronutrients</i>												
Potassium	78.27	81.38	0.0297 *	87.88	85.60	0.2540	90.68	89.89	0.7354	85.61	85.62	0.9932
Nitrogen	50.92	51.64	0.0682	38.98	38.07	0.7042	42.47	41.91	0.7088	44.12	43.87	0.7769
Phosphorous	5.59	5.93	0.0596	5.58	5.72	0.4096	5.22	5.00	0.1205	5.46	5.55	0.3650
Calcium	11.69	10.85	0.1553	14.78	14.94	0.8400	14.03	13.78	0.7268	13.50	13.19	0.4079
Magnesium	7.77	7.22	0.0196 *	6.20	6.29	0.7254	7.57	7.45	0.7784	7.18	6.99	0.2684
<i>Micronutrients (µg/g)</i>												
Iron	323.89	273.68	0.2516	300.40	406.83	0.0586	348.97	336.95	0.6028	324.42	339.15	0.5711
Zinc	73.63	80.85	0.0234 *	127.00	142.33	0.0245 *	101.87	111.03	0.0458 *	100.83	111.40	0.0001 ***
Manganese	60.93	58.30	0.1549	85.46	100.41	0.0039 **	76.31	81.83	0.0711	74.23	80.18	0.0138 *
Copper	15.21	15.75	0.8240	12.92	13.70	0.0112 *	13.70	12.64	0.2121	13.94	14.03	0.9141
Phenolic compounds (mg/g)												
Total phenols	10.31	10.09	0.4565	11.26	11.80	0.3630	11.42	11.38	0.9294	11.00	11.08	0.7317
Flavonoids	9.91	10.08	0.9490	9.91	10.56	0.1919	9.82	10.16	0.4886	10.08	9.88	10.27
Carotenoids (mg/g)												
Violaxanthin	1.13	1.18	0.1067	0.82	0.84	0.5267	1.78	1.71	0.5954	1.24	1.24	0.9793
Neoxanthin	0.38	0.39	0.1106	0.27	0.26	0.3837	0.65	0.64	0.8515	0.43	0.43	0.8136
Lutein	1.35	1.39	0.1431	0.94	0.90	0.6399	2.53	2.54	0.9492	1.61	1.61	0.9091
β-Carotene	1.00	1.04	0.0558	0.91	0.95	0.3429	2.05	2.09	0.7092	1.32	1.36	0.2836
9-cis-β-Carotene	0.11	0.12	0.0756	0.097	0.102	0.2031	0.248	0.250	0.9031	0.15	0.16	0.3772
Total carotenoids	3.98	4.11	0.0972	3.03	3.05	0.8677	7.25	7.22	0.9587	4.75	4.80	0.7542

Table 3. Influence of reduced water supply on the fresh biomass (FM)-related levels of constituents of spinach leaves from different years (2015, 2016 and 2017). Linear mixed model, t-test, *p*-values: < 0.1, < 0.05 (*), < 0.01 (**), < 0.001 (***); nd = not determined, CTR: Control treatment with full water supply, RWS: Reduced water supply treatment.

	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Leaf water potential Ψ (-MPa)	nd	nd	nd	0.14	0.18	0.1136	0.07	0.093	0.0842	nd	nd	nd
Dry biomass (%)	8.54	8.60	0.6186	5.40	5.48	0.6824	6.13	6.83	0.0032 **	6.69	6.97	0.0216 *
Sugars and Polyols (mg/100 g)												
Glucose	536.71	563.58	0.3345	402.68	346.22	0.1522	318.31	357.98	0.3903	345.00	372.50	0.4335
Fructose	410.34	412.52	0.9085	207.51	170.87	0.0721	173.19	183.83	0.6033	223.89	240.56	0.3937
Polyols total	49.83	49.99	0.8777	20.66	23.29	0.0065 **	32.81	34.80	0.0408 *	34.43	36.03	0.0048 **
Inositol	14.16	14.29	0.5232	5.26	6.02	0.0127 *	9.09	10.53	0.0170 *	9.50	10.28	0.0013 **
Glycerol	21.73	21.97	0.5287	8.91	9.67	0.0022 **	14.16	15.56	0.0115 *	14.93	15.73	0.0013 **
Polyol unknown	11.63	11.76	0.7103	3.78	4.48	0.0088 **	4.09	4.22	0.5782	6.50	6.82	0.0476 *
Erythritol	2.32	1.96	0.4704	2.72	3.12	0.0809	5.46	4.49	0.0264 *	3.50	3.19	0.2038
Organic acids (mg/100 g)												
Oxalic acid	472.07	421.23	0.0863	345.47	345.47	1.0000	335.73	327.99	0.8732	384.42	364.90	0.3067
Total acidity	158.32	177.21	0.1271	94.97	86.74	0.5455	105.46	114.17	0.2515	119.58	126.04	0.3662
Malic acid	121.21	119.54	0.7926	121.93	101.96	0.0103 *	88.05	83.21	0.6203	110.39	101.57	0.0528
Ascorbic acid	80.49	85.30	0.0568	23.62	27.50	0.2745	41.96	46.02	0.1822	48.69	52.94	0.0093 **
Citric acid	58.61	58.10	0.8880	17.57	17.42	0.8771	18.39	21.29	0.1219	31.52	32.27	0.5873
Fumaric acid	5.72	5.12	0.1202	11.91	9.48	0.2684	7.81	7.62	0.9169	8.48	7.41	0.2225
Anions (mg/100 g)												
Nitrate	72.08	65.80	0.2778	150.48	162.49	0.0238 *	79.17	84.78	0.6029	100.58	104.35	0.4025
Phosphate	115.97	95.26	0.0421 *	88.92	97.02	0.0027 **	80.25	84.77	0.3201	95.04	92.35	0.5248
Sulfate	29.12	22.64	0.0189 *	41.28	37.07	0.2234	20.18	26.39	0.0371 *	30.20	28.70	0.4426
Chloride	46.13	43.02	0.1800	39.00	32.69	0.1556	52.82	54.48	0.6515	45.98	43.40	0.1913

Table 3. Cont.

	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Elements (mg/100 g)												
Carbon	3.26	3.28	0.8151	1.94	1.95	0.9071	2.20	2.48	0.0105 *	2.47	2.57	0.0687
<i>Macronutrients</i>												
Potassium	662.16	694.89	0.0185 *	468.56	485.03	0.1523	555.12	613.09	0.0103 *	561.95	597.67	0.0002 ***
Nitrogen	407.22	410.14	0.7606	176.17	202.64	0.0065 **	238.58	262.42	0.0097 **	273.99	291.73	0.0013 **
Phosphorous	47.65	50.96	0.0984	29.77	32.39	0.0135 *	31.94	34.09	0.0297 *	36.45	39.15	0.0004 ***
Calcium	99.76	93.89	0.1860	79.13	84.88	0.2441	86.12	94.28	0.1152	88.34	91.02	0.3357
Magnesium	66.71	62.63	0.0117 *	33.16	35.67	0.1156	46.48	50.92	0.1760	48.78	49.74	0.4889
<i>Micronutrients (mg/100 g)</i>												
Iron	2.75	2.34	0.2768	1.60	2.31	0.0314 *	2.14	2.30	0.2856	2.16	2.31	0.4017
Zinc (µg/100 g)	632.02	699.59	0.0021 **	678.36	807.78	0.0030 **	625.07	760.62	0.0064 **	645.15	756.00	0.0001 ***
Manganese (µg/100 g)	519.79	501.32	0.2620	456.93	568.91	0.0009 ***	467.35	558.30	0.0112 *	481.35	542.84	0.0022 **
Copper (µg/100 g)	128.94	135.43	0.7665	68.96	77.63	0.0004 ***	84.02	86.30	0.6883	93.97	99.79	0.4075
Phenolic compounds (mg/100 g)												
Total phenols	88.00	86.23	0.4172	60.78	64.93	0.4388	70.09	77.85	0.1354	72.95	76.34	0.1695
Flavonoids	86.52	86.93	0.8607	54.45	59.17	0.3192	62.81	73.40	0.0495 *	67.93	73.17	0.0308 *
Carotenoids (mg/100 g)												
Violaxanthin	9.66	10.05	0.1835	5.13	5.99	0.0462 *	11.15	12.09	0.3774	8.65	9.38	0.0439 *
Neoxanthin	3.18	3.28	0.1826	1.70	1.86	0.1231	4.07	4.55	0.1484	2.98	3.23	0.0289 *
Lutein	11.52	11.87	0.2574	5.83	6.39	0.2920	15.91	18.00	0.1553	11.09	12.09	0.0474 *
β-Carotene	8.54	8.88	0.1166	5.68	6.73	0.0311 *	12.85	14.81	0.1022	9.02	10.14	0.0074 **
9-cis-β-Carotene	0.98	1.00	0.4962	0.60	0.71	0.0123 *	1.55	1.77	0.1128	1.04	1.16	0.0138 *
Total carotenoids	33.88	35.07	0.1866	18.94	21.68	0.0680	45.53	51.21	0.1728	32.78	35.99	0.0233 *

Nitrogen

Dry biomass-related total nitrogen (N) was not affected, but fresh biomass-related data was significantly influenced by RWS when considering the data across all three years (Tables 2, 3). For instance, dry biomass-related N levels remained unchanged in 2016 and 2017, irrespective of the water supply treatment ($p = 0.7042$ and 0.7088 , respectively), but they only marginally increased from 50.92 to 51.64 mg/g DM in 2015 ($p = 0.0682$). Upon reduced water supply, fresh biomass-related N levels significantly increased from 176.2 to 202.6 mg/100 g FM in 2016 ($p = 0.0065$) and from 238.58 to 262.42 mg/100 g FM in 2017 ($p = 0.0097$). A similar increase from 273.99 to 291.73 mg/100 g FM was found considering the whole dataset ($p = 0.0013$), but not when considering data of 2015 only (407.22 versus 410.14 mg/100 g FM for CTR and RWS respectively, Table 3). Multiple irrigation events in 2015 might have evoked drying-rewetting conditions, which are known to increase soil-available N and, therefore, might have alleviated the negative effect of drought on reduced N mineralization [51].

In agreement with our fresh biomass-related data, dry biomass-related total N levels were highest in 2015 (50.92–51.64 mg/g DM) as compared to the other years (38.07–42.47 mg/g DM). These findings might be explained by the fact that, in 2015, spinach was cultivated in autumn and therefore harvested after a longer growth period (51 days) as compared to 2016 and 2017 (44 and 39 days, respectively), as caused by lower irradiation in 2015. The lowest N levels were found in 2016 when total water amount as well as irradiation sum and temperature sum were highest compared to other years (Table 1) and precipitation was more frequent just before harvest (data not shown). Different results were found earlier for red radish with highest contents of nitrogen under moist conditions [19].

Potassium

Water supply limitation resulted in significantly increased levels of potassium (K^+) in spinach dry biomass in 2015 only (Table 2), when water reduction was most pronounced. According to fresh biomass-related PCA analyses (Figure 3), where most of the minerals were contributing to the separation of the treatments, significantly higher levels of K^+ were found in spinach grown under reduced water conditions in 2015 and 2017 (Table 3). While dry biomass content of reduced watered spinach was significantly higher in 2017, which might explain the increase in K^+ based on fresh biomass, the dry

biomass content did not differ in 2015 with reduced water supply. Anyway, the availability of K^+ from the soil was shown to decrease with declining soil water content due to decreasing mobility of K^+ [52]. Our results indicated that K^+ uptake, which is often reduced under drought conditions [53,54], was not significantly affected by moderate water reduction. The highest absolute K^+ levels in spinach were found in 2016 (85.60–87.88 mg/g DM) and 2017 (89.89–90.68 mg/g DM), when global irradiation and evapotranspiration rates were high and temperatures continuously increased during the cultivation period (Supplementary Figures S1, S2, S4).

Phosphorous

In accordance with other macronutrients, dry biomass-related levels of phosphorous (P) were higher in less irrigated than in well-watered spinach in 2015 ($p = 0.0596$), but not in other years (Table 2). Similar to N, fresh biomass-related P levels were significantly increased in all years as well as in the overall dataset ($p = 0.0004$, Table 3), which probably can be traced back to higher contents of dry biomass in samples obtained from the reduced watered plants. This result was also visible in the PCA on fresh biomass-based data, where P clearly contributed to the differentiation of both treatments. In contrast, red radish root tubers grown within the same experimental field site revealed a significant decrease of P based on dry biomass, while fresh biomass-related data showed no effect [19]. In general, the fresh biomass-based P levels differed strongly between the cultivation set of 2015 (47.65–50.96 mg/g DM) and those of 2016 and 2017 (29.77–34.09 mg/g DM). One possible explanation might be the prolonged cultivation time in 2015 (51 days) and the associated high content of dry biomass (8.54 %–8.60 %) in contrast to other years (5.40 %–6.83 %). The shortened cultivation times in 2016 (44 days) and 2017 (39 days) might have resulted in lower absolute nutrient uptake from the soil.

Calcium

Calcium (Ca^{2+}) content of spinach was independent of the water supply. Generally, lower Ca^{2+} levels were found in 2015 (10.85–11.69 mg/g DM) compared to the other years 2016 and 2017 (13.78–14.94 mg/g DM). This result is in contrast to those found for N, which displayed the highest levels in 2015. Since Ca^{2+} is a readily available element for plant uptake from the soil, high Ca^{2+} levels have earlier been suggested to

be associated with high concentrations in the soil rather than with potential relationships with uptake efficiency or transpiration velocity of the plant [41,55]. In agreement with our findings, Sánchez-Rodríguez et al. [56] did not find any difference in the Ca^{2+} accumulation of tomato leaves exposed to moderate drought stress, even though its uptake was significantly reduced. These findings are in further agreement with those from Hu and Schmidhalter [57], stating that Ca^{2+} accumulation was much less sensitive to drought than that of K and phosphate. Basically, Ca^{2+} has structural functions and acts as an important second messenger [58]. Since important plant functions are controlled by very small, but physiologically active pools of Ca^{2+} within the cytoplasm [59], the observed insignificant effects of moderate water reduction on the Ca^{2+} levels in spinach had been expected.

Magnesium

The dry biomass-based content of magnesium (Mg^{2+}) in spinach was significantly reduced by RWS in 2015, but not in 2016 and 2017. Thus, multivariate analysis by PCA did not show a strong contribution of Mg^{2+} to the differentiation of both groups (Figure 2). The same result was found for fresh biomass-based analyses (Table 3). In agreement with our findings, effects of moderate water reduction had no effects on Mg^{2+} levels in leaves of cherry tomatoes and were dependent on the cultivar [56]. In contrast, Pulupol et al. [60] found higher fresh biomass-based Mg^{2+} levels in tomato fruits grown under deficit irrigation, while dry biomass-related amounts were influenced insignificantly. Also, radish cultivated under RWS within the same experiment exhibited no significant changes in fresh biomass-related Mg^{2+} levels [19].

Micronutrients (Fe, Zn, Mn, Cu)

Significant effects on the dry biomass-related contents of iron (Fe) and copper (Cu) were not observed in our study, irrespective of water supply and cultivation year. However, dry biomass-based zinc (Zn) and manganese (Mn) contents were significantly increased by limited water supply (Table 2). PCA analyses supported the contribution of these micronutrients to variant differentiation (Figure 2). Similar findings were evident during analyses of fresh biomass-related data (Table 3, Figure 3). In contrast, moderate water reduction resulted in significantly decreased contents of Mn in radish root tubers grown within the same field experiment [19]. By analogy, water deficit-

conditions have been shown to lead to lower Mn levels in leaves of cherry tomatoes [56].

Phenolic compounds

In spinach dry biomass, total polyphenol content, including flavonoids, remained unchanged with RWS considering both the single datasets as well as the total dataset across all cultivation years. The levels of total flavonoids, as derived by summing up levels of individually quantitated flavonoids (Supplementary Tables S2 and S3), remained highly similar within a narrow range in all individual years (9.82–10.56 mg/g DM), while the amount of total polyphenol contents were marginally higher in 2016 (11.26–11.80 mg/g DM) and 2017 (11.38–11.42 mg/g DM) as compared to 2015 (10.09–10.31 mg/g DM).

In contrast to the quite stable dry biomass-related contents, fresh biomass-based contents displayed a much higher variability, particularly considering total flavonoids ranging from 54.45 to 86.93 mg/100 g FM. Fresh biomass-related levels of total flavonoids were significantly higher in reduced watered spinach than in well-watered spinach in 2017, which, however, was obviously based on significantly higher levels in dry biomass (Table 3). The levels found were in accordance with those of a previous study of Gil et al. [17], who found ca. 1000 mg/kg total flavonoids in fresh cut spinach. Because of generally higher contents of dry biomass in 2015, the fresh biomass-related levels of total flavonoids (86.52–86.93 mg/100 g FM) were substantially greater in 2015 as compared to those found in other years, reaching the lowest levels in 2016 (54.45–59.17 mg/100 g FM, Table 3). In accordance, strong seasonal variations in the levels of flavonoids such as quercetin and kaempferol were found in leafy vegetables such as lettuce (1.9–30 mg/kg quercetin) and endive (15–95 mg/kg kaempferol), based on fresh biomass-analyses [61]. Apart from fresh biomass-based variations, flavonoid levels in our study did not show any differences if compared on a dry biomass basis, even if there were strong differences in the global irradiation levels during the cultivation periods (Table 1, Supplementary Figures S1–S4). A rather low dependence or even independence of flavonoid contents from climatic factors such as temperature and global irradiation were reported previously in kale (*Brassica oleracea* var. *sabellica*) by Schmidt et al. [62].

Focusing on flavonoids, Bergquist et al. [22] observed the highest concentrations in baby spinach at early growth stages accompanied by high variations as associated with different sowing times. Furthermore, Hertog et al. [61] showed quite strong seasonal variations in the flavonoid levels of leafy vegetables such as lettuce and endive, which were 3 to 5 times higher in summer than in other seasons. In our study, the highest amounts of flavonoids in spinach fresh biomass were found in 2015 (86.52–86.93 mg/g FM), when cultivation took place in autumn with relatively low global irradiation in contrast to other years which had been sown at springtime (54.45–73.40 mg/ FM).

For individual flavonoids, RWS did not induce any clear effect in spinach. Univariate analyses indicated a dry biomass-related increase of some minor patuletin-derivatives in 2015 as well as an increase in the levels of a few more abundant flavonoids in 2016 upon moderate water limitation (Supplementary Table S3). For example, levels of specific patuletin- and spinatoside-derivatives (e.g., compounds 3, 5 and 11, Supplementary Table S2) were partly increased by RWS in 2015 and 2016. Among the other flavonoid-derivatives, the content of spinatoside, which represented one of the most abundant flavonoid-compounds in spinach according to a comparison of our mass spectral data to those reported previously by Aritomi et al. [63], tended to increase by RWS in 2016 ($p = 0.0677$). Apart from that, the study of Bergquist et al. [22] demonstrated that flavonoid profiles of baby spinach were highly similar during all growth stages, although the relative amounts of the individual flavonoids were prone to changes. Similar results were found in our study when comparing the different years, with hardly any difference in total flavonoids years, but individual flavonoids varied widely (Supplementary Table S3). The contents of individual flavonoids were almost the same when grown during early 2016 and 2017, while the proportions were markedly different when grown in late 2015. While an apparent methylenedioxyflavone-glucuronide (5,3',4'-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4'- β -D-glucuronide [21,22]) was the main flavonoid in 2016 and 2017 (30.0%–30.4%, Supplementary Table S4), the spinatoside accounted for the largest proportion in 2015 (20.2%–20.5%, Supplementary Table S4). A clear causal relationship between moderate limitations in water supply and changes in single or total flavonoids could not be established, which might indicate that the RWS effect on the levels of these compounds was rather low. Nevertheless, further studies in more controlled

environments and, possibly, with more severe reductions in water supply, should be encouraged.

Carotenoids

In general, (all-*E*)-lutein was the major carotenoid found in spinach, followed by β -carotene and violaxanthin, which is in accordance with what had been expected in photosynthetically active plant tissues [33]. Moderately reduced water supply did not show clear effects on the contents of total carotenoids in spinach when evaluating the dry biomass-related dataset (Table 2). There was a slight increase of β -carotene, (9*Z*)- β -carotene and total carotenoids in 2015, not reaching statistical significance. In contrast, fresh biomass-related levels of all carotenoids significantly increased with RWS in all three cultivation years (Table 3). In contrast to the lack of effect of water supply, a seasonal variation was observed. For instance, total carotenoid levels in spinach dry biomass were ca. two-fold higher in 2017 (7.22–7.25 mg/g DM) than in 2015 and 2016 (3.03–4.11 mg/g DM). The high level of global irradiation in 2017 (756.9 MJ/m²) had been expected to lead to comparably high levels of carotenoids, because increased radiation has been shown to enhance the accumulation of carotenoids in green vegetables [7]. This hypothesis would have explained the low carotenoid levels in 2015 with a global irradiation of 453.5 MJ/m², but not the low carotenoid levels in 2016 (3.03–3.05 mg/g DM) which were characterized by a high global irradiation (799.9 MJ/m²). Carotenoid synthesis and accumulation must have been impacted by other, yet unknown, factors, such as the occurrence of multiple short-time drought events during the plant growth in 2016. By analogy to the levels of flavonoids, a clear effect of moderate water limitation and season on the levels of carotenoids was not observed in our open field cultivation experiment.

Conclusions

In this study, the chemical composition of both dry and fresh biomass of spinach was shown to be strongly influenced by climatic conditions and/or water supply. The effects were highly dependent on the type of nutrient. Even moderately reduced water supply led to significant increases of dry biomass, which in turn often led to increased levels of numerous constituents and, thus, apparently enhanced the nutritional value of the vegetable product. For instance, fresh biomass-related levels of ascorbic acid,

potassium, nitrogen, phosphorous as well as total flavonoids and carotenoids increased upon limiting water supply. Our results indicated that changes in levels of characteristic flavonoids might depend on seasonal variations, although further study is needed in this regard. Considering the composition of the dry biomass itself, we demonstrated that even mild water supply reductions led to significant increases of inositol, zinc and manganese levels, while malic acid, phosphate and chloride levels decreased. It is likely that such climate-related reductions in water supply will occur more frequently in the future, as is already occurring more and more due to the presumably accelerating climate change. In summary, our results indicate that the nutritional composition of spinach is sensitive to even moderately reduced water supply, but the overall quality of fresh spinach did not suffer regarding the levels of health-promoting constituents such as minerals, trace elements, flavonoids and carotenoids.

Supplementary Materials

The following are available online at <http://www.mdpi.com/2311-7524/6/2/25/s1>: Figure S1–S4: Daily mean weather conditions during the different spinach cultivation periods of 2015, 2016 and 2017: (1) Mean air temperature (°C), (2) global irradiation sum (W/m²), (3) daily mean relative humidity (%) and (4) daily mean evapotranspiration (mm). DAS: Days after sowing. Figure S5: HPLC-DAD chromatogram of spinach flavonoids at 350 nm. Flavonoid peaks assigned by comparing retention times, UV/Vis-spectra and mass spectral data to those reported by literature as shown in Table S2. Table S1: Proportion of single carotenoids on the total amount of carotenoids in spinach fresh biomass from three years (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Table S2: Peak assignment to flavonoid-compounds as detected in spinach from years 2015, 2016 and 2017 by comparing retention time (RT), UV/Vis-spectra and negative ion *m/z* and important MS/MS-fragments to the literature. An exemplary chromatogram is shown in Figure S5. Table S3: Influence of reduced water supply on the dry biomass (DM)-related levels of single flavonoid compounds in spinach leaves from three years (2015, 2016 and 2017). Linear mixed model, t-test, *p*-values: < 0.1, < 0.05 (*), < 0.01 (**), < 0.001 (***); CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table S2. Table S4: Proportion of single flavonoids on the total amount of flavonoids in spinach dry biomass from three years (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table S2.

Author Contributions

Conceptualization, H.D. and J.Z.; methodology, C.S. and H.D.; software, M.F. and C.S.; formal analysis, C.S. and M.F.; investigation, C.S.; resources, H.D., R.S. and J.Z.; data curation, C.S.; writing—original draft preparation, C.S.; writing—review and editing, C.S., J.Z., H.D., M.F. and R.S.; visualization, C.S.; supervision, R.S., H.D., M.F. and J.Z.; project administration, H.D. and J.Z.; funding acquisition, H.D. and J.Z. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Materials

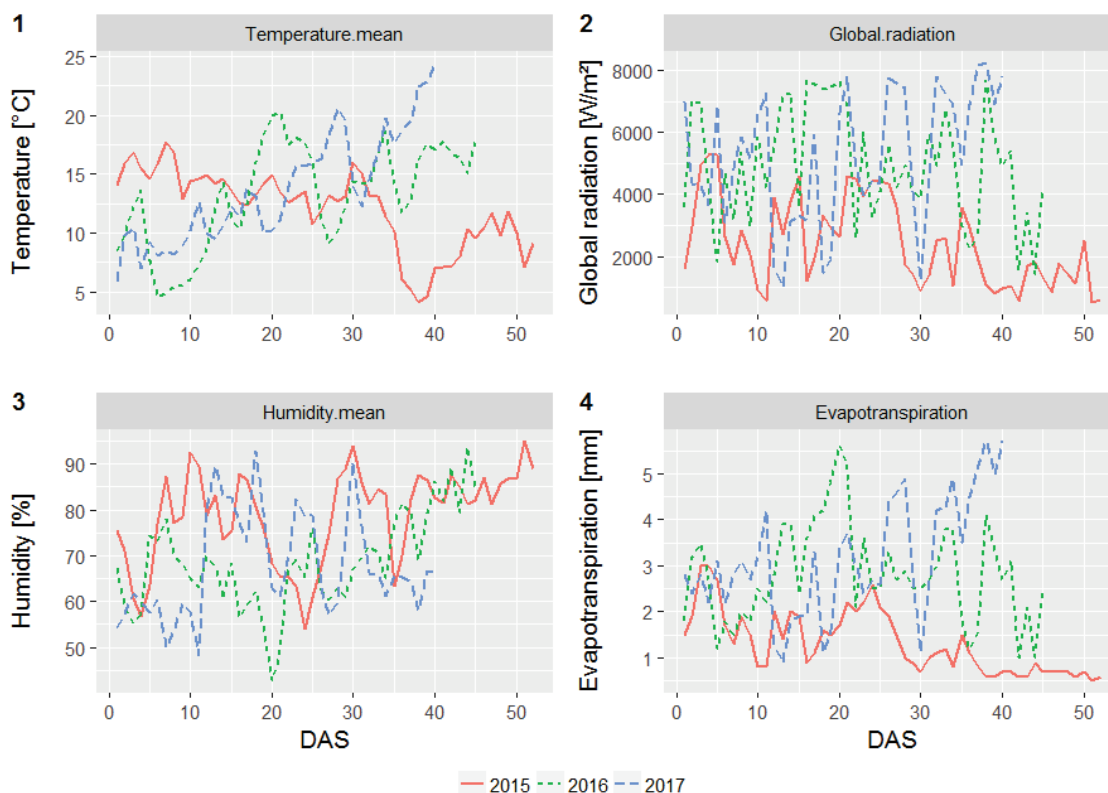


Figure S1-S4. Daily mean weather conditions during the different spinach cultivation periods (2015, 2016 and 2017): (1) Mean air temperature ($^{\circ}C$), (2) global irradiation sum (W/m^2), (3) daily mean relative humidity (%) and (4) daily mean evapotranspiration (mm). DAS: Days after sowing.

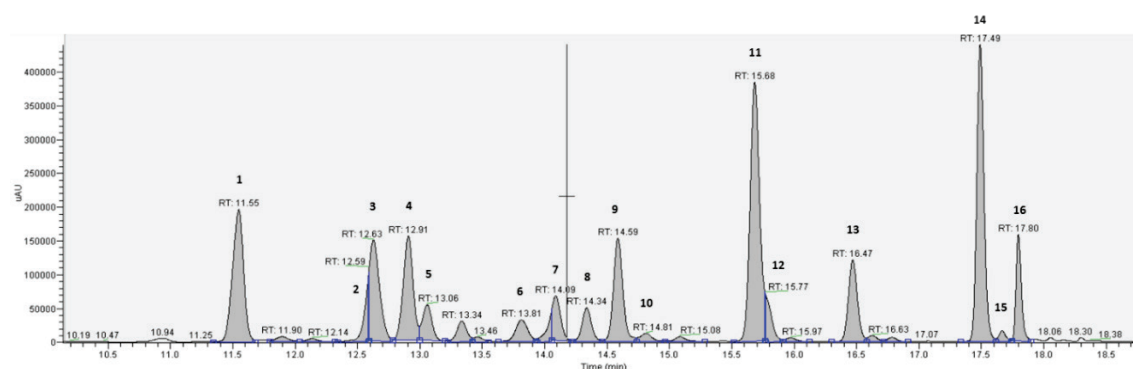


Figure S5. HPLC-DAD chromatogram of spinach flavonoids at 350 nm. Flavonoid peaks assigned by comparing retention times, UV/Vis-spectra and mass spectral data to those reported by literature, as shown in Table S2.

Table S1. Proportion of single carotenoids on the total amount of carotenoids in spinach fresh matter from different cultivation sets (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment.

	2015				2016				2017			
	CTR		RWS		CTR		RWS		CTR		RWS	
	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*
Violaxanthin	9.66	28.5 %	10.05	28.6 %	5.13	27.1 %	5.99	27.6 %	11.15	24.5 %	12.09	23.6 %
Neoxanthin	3.18	9.4 %	3.28	9.4 %	1.7	9.0 %	1.86	8.6 %	4.07	8.9 %	4.55	8.9 %
Lutein	11.52	34.0 %	11.87	33.8 %	5.83	30.8 %	6.39	29.5 %	15.91	34.9 %	18	35.1 %
β -Carotene	8.54	25.2 %	8.88	25.3 %	5.68	30.0 %	6.73	31.0 %	12.85	28.2 %	14.81	28.9 %
(9Z)- β -Carotene	0.98	2.9 %	1	2.9 %	0.6	3.2 %	0.71	3.3 %	1.55	3.4 %	1.77	3.5 %
Total carotenoids	33.88	100.0 %	35.08	100.0 %	18.94	100.0 %	21.68	100.0 %	45.53	100.0 %	51.22	100.0 %

*: abs.: absolute content in mg/100 g FW. rel.: relative content as percentage of total carotenoids.

Table S2. Peak assignment to flavonoid-compounds as detected in spinach from years 2015, 2016 and 2017 by comparing retention time (RT), UV/Vis-spectra and negative ion m/z and important MS/MS-fragments to the literature. An exemplary chromatogram is shown in Figure S5.

Peak	Identity	RT (min)	λ_{max} (nm)	[M-H] ⁻	Fragments	Reference
1	patuletin-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)-[β -D-apiofuranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	11,55	258, 351	787	655, 439, 331	[1]
2	patuletin-coumaroyl-pentosyl-hexoside	12,59	260, 347	933	787, 331	*
3	patuletin-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside	12,63	257, 351	655	493, 331	[1]
4	spinacetin-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)-[β -D-apiofuranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	12,91	257, 351	801	669, 345	[2]
5	patuletin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 \rightarrow 6)-[β -D-apiofuranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	13,06	258, 336	963	787, 655, 331	[2]
6	spinacetin-3-O- β -D-(2''- β -coumaroylglucopyranosyl-(1 \rightarrow 6)-[β -D-apiofuranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	13,81	260, 321	947	801, 345	[2]
7	spinacetin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 \rightarrow 6)-[β -D-apiofuranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	14,09	258, 332	977	801, 669, 345	[2]
8	patuletin-feruloyl-dihexoside	14,34	258, 335	831	655, 331	*
9	spinacetin-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside	14,59	257, 352	669	345	[1]
10	patuletin-coumaroyl-dihexoside	14,81	263, 347	801	655, 331	*
11	spinoside	15,68	270, 340	521	345	[1]
12	spinacetin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside	15,78	258, 336	845	669, 499, 345	[2]
13	jaceidin-4'-glucuronide	16,47	270, 340	535	359	[3]
14	5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'- β -D-glucuronide	17,49	277, 341	519	343	[3]
15	Dihydroxy-methoxy-methylenedioxyflavone-hexuronide	17,68	277, 336	503	327	*
16	5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylenedioxyflavone-4'- β -D-glucuronide	17,80	278, 341	533	357	[3]

* Tentatively identified

Table S3. Influence of reduced water supply on the dry biomass (DM)-related levels of single flavonoid compounds in spinach leaves from three years (2015, 2016 and 2017). Linear mixed model, t-test, *p*-values: < 0.1, < 0.05 (*), < 0.01 (**) and < 0.001 (***); CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table S2.

Flavonoid-compound (peak) (mg g ⁻¹ DM)	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Peak no. 1	1.10	1.12	0.5686	1.82	1.93	0.2204	1.81	1.86	0.5432	1.58	1.64	0.2135
Peak no. 2	0.14	0.16	0.0671	0.16	0.17	0.2031	0.15	0.16	0.3165	0.15	0.16	0.0093 **
Peak no. 3	0.74	0.78	0.0616	0.71	0.77	0.2628	0.71	0.74	0.4890	0.72	0.76	0.5199
Peak no. 4	0.85	0.86	0.6606	0.43	0.46	0.2987	0.43	0.44	0.6033	0.57	0.59	0.1996
Peak no. 5	0.31	0.31	0.7926	0.79	0.88	0.0805	0.78	0.84	0.1886	0.63	0.68	0.0218 *
Peak no. 6	0.20	0.20	0.4150	0.06	0.08	0.0540	0.06	0.07	0.2892	0.11	0.12	0.0220 *
Peak no. 7	0.35	0.36	0.6685	0.28	0.30	0.3663	0.28	0.29	0.6084	0.30	0.32	0.2332
Peak no. 8	0.24	0.25	0.3165	0.41	0.45	0.1237	0.41	0.43	0.2799	0.35	0.38	0.1084
Peak no. 9	0.70	0.70	0.9376	0.20	0.21	0.5862	0.20	0.21	0.8180	0.37	0.37	0.6579
Peak no. 10	0.07	0.07	1.0000	0.06	0.07	0.4302	0.06	0.07	0.3250	0.06	0.07	0.2256
Peak no. 11	2.03	2.04	0.9337	1.31	1.40	0.0677	1.30	1.35	0.3518	1.55	1.60	0.1251
Peak no. 12	0.20	0.20	0.7827	0.15	0.15	0.7089	0.14	0.15	0.8560	0.16	0.17	0.7550
Peak no. 13	0.66	0.67	0.6975	0.12	0.12	0.6109	0.11	0.11	1.0000	0.30	0.30	0.5886
Peak no. 14	1.73	1.76	0.6358	3.01	3.17	0.3166	2.99	3.05	0.6723	2.58	2.66	0.2231
Peak no. 15	0.06	0.06	0.6109	0.10	0.10	1.0000	0.10	0.10	1.0000	0.09	0.09	0.8045
Peak no. 16	0.53	0.54	0.5948	0.30	0.30	1.0000	0.29	0.29	0.9444	0.37	0.38	0.7462
Total flavonoids	9.91	10.08	0.9490	9.91	10.56	0.1919	9.82	10.16	0.4886	9.88	10.27	0.1500

Table S4. Proportion of single flavonoids on the total amount of flavonoids in spinach dry matter from different cultivation sets (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table A1.

	Spinach 2015				Spinach 2016				Spinach 2017			
	CTR		RWS		CTR		RWS		CTR		RWS	
	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*
Peak no. 1	1.1	11.1 %	1.12	11.1 %	1.82	18.4 %	1.93	18.3 %	1.81	18.4 %	1.86	18.3 %
Peak no. 2	0.14	1.4 %	0.16	1.6 %	0.16	1.6 %	0.17	1.6 %	0.15	1.5 %	0.16	1.6 %
Peak no. 3	0.74	7.5 %	0.78	7.7 %	0.71	7.2 %	0.77	7.3 %	0.71	7.2 %	0.74	7.3 %
Peak no. 4	0.85	8.6 %	0.86	8.5 %	0.43	4.3 %	0.46	4.4 %	0.43	4.4 %	0.44	4.3 %
Peak no. 5	0.31	3.1 %	0.31	3.1 %	0.79	8.0 %	0.88	8.3 %	0.78	7.9 %	0.84	8.3 %
Peak no. 6	0.2	2.0 %	0.2	2.0 %	0.06	0.6 %	0.08	0.8 %	0.06	0.6 %	0.07	0.7 %
Peak no. 7	0.35	3.5 %	0.36	3.6 %	0.28	2.8 %	0.3	2.8 %	0.28	2.9 %	0.29	2.9 %
Peak no. 8	0.24	2.4 %	0.25	2.5 %	0.41	4.1 %	0.45	4.3 %	0.41	4.2 %	0.43	4.2 %
Peak no. 9	0.7	7.1 %	0.7	6.9 %	0.2	2.0 %	0.21	2.0 %	0.2	2.0 %	0.21	2.1 %
Peak no. 10	0.07	0.7 %	0.07	0.7 %	0.06	0.6 %	0.07	0.7 %	0.06	0.6 %	0.07	0.7 %
Peak no. 11	2.03	20.5 %	2.04	20.2 %	1.31	13.2 %	1.4	13.3 %	1.3	13.2 %	1.35	13.3 %
Peak no. 12	0.2	2.0 %	0.2	2.0 %	0.15	1.5 %	0.15	1.4 %	0.14	1.4 %	0.15	1.5 %
Peak no. 13	0.66	6.7 %	0.67	6.6 %	0.12	1.2 %	0.12	1.1 %	0.11	1.1 %	0.11	1.1 %
Peak no. 14	1.73	17.5 %	1.76	17.5 %	3.01	30.4 %	3.17	30.0 %	2.99	30.4 %	3.05	30.0 %
Peak no. 15	0.06	0.6 %	0.06	0.6 %	0.1	1.0 %	0.1	0.9 %	0.1	1.0 %	0.1	1.0 %
Peak no. 16	0.53	5.3 %	0.54	5.4 %	0.3	3.0 %	0.3	2.8 %	0.29	3.0 %	0.29	2.9 %
Total flavonoids	9.91	100.0 %	10.08	100.0 %	9.91	100.0 %	10.56	100.0 %	9.82	100.0 %	10.16	100.0 %

*: abs.: absolute content in mg/100 g DW. rel.: relative content as percentage of total flavonoids.

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Chapter 3: Effects of moderately reduced water supply and picking time on the chemical composition of pickling cucumber (*Cucumis sativus* L.) in open field cultivation

Abstract

As climate change evokes changing precipitation patterns, the cultivation of vegetable crops in open fields might become more difficult in the future. Nowadays, many vegetable growers are already facing relatively long unprecedented precipitation-free periods. In many growing regions, irrigation is only available to a limited extent or not at all, and the cultivated plants will suffer from moderate water stress more often. Therefore, we examined the effects of moderately-reduced water supply on the chemical composition of pickling cucumber, cultivated in an open field and in a separate greenhouse trial. In the field trial, the reduced water supply treatment (RWS) provided 85–90% of the total water amount of the well-watered control treatment (CTR), applying a randomized block design with six replications comprising two consecutive weekly harvest periods. In fruits obtained by cultivation with reduced irrigation, levels of malic acid, calcium, and magnesium significantly increased, while those of phosphate, phosphorous, nitrogen, and iron decreased based on dry matter. Fresh matter-related results additionally revealed a decrease of *myo*-inositol and zinc, while sugars and total phenols remained unchanged. In the greenhouse experiment, the RWS obtained 60% of the irrigation amount of the CTR. Here, single cucumber compartments (exocarp, mesocarp, and endocarp) were examined. Chemical compositions changed in a similar, but more pronounced, manner as compared to the open field trial. The levels of individual, nutritionally relevant carotenoids in the peel of pickling cucumber, like lutein and β -carotene, were affected by RWS. Regarding the nutritional quality of fresh marketable cucumber fruits, malic acid, certain minerals and trace elements, as well as the carotenoids were shown to be sensitive to moderate water reduction.

Keywords: vegetables; water deficit; climate change; nutritional values; fruit compartments

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Introduction

The nutritional composition of vegetables grown in open fields depends highly on the water supply and local climate conditions. An adequate water supply is essential for the production of high-quality vegetables. However, natural precipitation patterns are changing and becoming increasingly difficult to predict due to climate change. Growers worldwide already struggle with the consequences of rising temperatures and fluctuating precipitation patterns, which might be temporarily absent during the cultivation period. These climate change-driven uncertainties are very likely to affect vegetable production in certain regions. For example, future projections indicate a decrease in the potential water availability during summer in the Mediterranean regions [1] that will affect the complex interactions in the plants' metabolism in any case, especially under highly heterogenic conditions in open field production. In addition, such variable growing conditions in open field cultivation severely complicate the derivation of suitable crop management strategies for farmers. As a result, unfavorable growing conditions occur more frequently and may have negative effects on the yield and nutritional quality of the vegetable products. In addition to those climate-related effects, the picking time in relation to the plants' growth stages has also been reported to have an effect on the chemical composition of fruity vegetables such as cucumber [2], which are being harvested over a certain period during the plants' growth. According to Gómez-López et al. [2], the highest fruit quality of pickling cucumber as defined in terms of firmness, skin color, and acidity as obtained during the first two weeks of the entire harvest period. However, ideal harvest times might change due to on-going environmental changes, leading to unfavorable climatic conditions at a higher frequency of occurrence in the future [3]. Therefore, we have examined the effect of moderately reduced water supply on the nutritional composition of pickling cucumber grown in open field cultivation and in a simultaneous greenhouse experiment under controlled conditions. The trials ran concomitantly to experiments on spinach and radish, which have been reported recently [4,5]. The crops were selected to represent

all harvest organs of vegetables: Spinach for leaves, radish for roots, and pickling cucumber for fruits. It is known that plant responses to abiotic stress can vary with the growth stage, severity, and duration of stress [6], but also between the tissue or organ affected by the stress [7]. Earlier studies have mainly focused on the impact of changing environmental conditions such as drought or changing precipitation patterns on the nutrient content of staple foods like cereals, legumes and root species [8,9]. In contrast, information about the effects of water scarcity on the nutritional quality of vegetables is less abundant. Particularly, fruity vegetables, such as indeterminate cultivars, like cucumber or tomato, appear to be sensitive to water deficits [10,11], because sensitive phenological stages, such as flowering or fruit set, occur continuously [11]. Similarly to other *Cucurbitaceae*, such as pumpkin and watermelon, cucumber have high water requirements in contrast to grain crops [12,13]. Cucumber plants have a sparse root system predominantly located in the upper top soil layer [14]. Therefore, cucumber fresh fruit yield is highly affected by total water supply at all growth stages [12]. Contrarily, the application of slight water deficits in horticultural systems, known as “regulated deficit irrigation”, may improve the product quality of reproductive organs such as fruits. Therefore, the technique has become widely used in horticultural practice [15]. Rahil and Qanadillo [14] have demonstrated that fresh cucumber yield was highest when using a scheduled irrigation system at 70% of the crops’ evapotranspiration in contrast to using fully-irrigated systems. Mild water deficits were also shown to increase the concentration of certain minerals in fresh matter of tomatoes produced under deficit irrigation [11].

In general, cucumbers are one of the most cultivated vegetables worldwide, thus having high economic values in worldwide human commerce [16]. With respect to the worldwide relevance of cucumber species as part of a vegetable-rich diet, we now sought to study how mild water reductions influence the chemical composition of cucumber fruits grown in an open field. Fruits were harvested in two consecutive weeks to demonstrate the variability of the nutritional quality of pickling cucumbers obtained at different harvest times. In addition, we studied the impact of moderate water deficits on the chemical composition of the cucumbers’ fruit compartments (exocarp, mesocarp, and endocarp) in a controlled greenhouse experiment.

Materials and methods

This chapter is subdivided into descriptions of the open field trial and that of the greenhouse trial conditions.

Chemicals

All reagents and solvents used were at least of analytical or HPLC quality unless specified differently. Folin-Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany); L-(+)-ascorbic acid from Carl Roth (Karlsruhe, Germany); D-(+)-catechin-hydrate, *myo*-inositol (>99.5%, HPLC), anhydrous glycerol ($\geq 99.5\%$) and *meso*-erythritol ($\geq 99\%$) from Sigma Aldrich (Steinheim, Germany). L-aspartic acid (Ph. Eur., USP) was received from AppliChem (Darmstadt, Germany).

Open Field Trial

Plant material and field experimental design

For the open-field trial, seeds of the cucumber F1 hybrid cv. 'Fuga F1' were used and purchased from Rijk Zwaan Welver (Wolver, Deutschland). Transplants were cultivated under greenhouse conditions for a total of nine days using a nitrate enhanced nutrient solution with 0.5% FERTY® 2 MEGA (Planta Düngemittel, Regenstauf, Germany). After initial emergence, transplants were planted in the experimental open field site on a sandy loam at Geisenheim University, Germany (49°59' N, 7°58' E). The experimental field design has been reported earlier by Schlering et al. [4]. In brief, the cultivation experiment was implemented within a plot installation comprising six circular plots (marked by A, C, F, H, M, and P) with an inner diameter of 11.9 m in July 2016. Each plot was subdivided into four quarters (subplots), while each of the subplots were again segmented into three parts, which were used for the implementation of the different irrigation treatments following a randomized block design (cf. Schlering et al. [4]). The size of each segment used for cultivation was at least 6.5 m². To avoid boundary effects, the third segment located outwardly was not involved into harvest, but cultivation and irrigation took place as described below. Three different crops (radish, spinach, cucumber) were simultaneously cultivated with an annual crop rotation. A graphical illustration of the experimental site was published earlier [4].

Cultivation and water supply

Cucumber transplants were planted manually at a distance of 0.3 m in one row per segment, resulting in a number of 16 plants in the outer segments and 18 plants in the inner segments of the circular experimental field plots. The plants' main shoots were equally adjusted to the same direction, while lateral shoots were removed manually after emergence. Uniform fertilization with calcium ammonium nitrate was carried out according to commercial standard specifications for the cultivation of pickling cucumber for the fresh food market (192 kg/N ha^{-1}) based on mineralized N (NO_3^- -N) in 0–30 cm soil depth. Crop protection was applied equally to all plots as described in the following, while chemical and biological insecticides were used depending on pest occurrence. Previcur N (Bayer CropScience Deutschland, Langenfeld, Germany) and Ortavia (Syngenta Agro, Maintal, Germany) were applied against downy mildew. Fastac ME (BASF-SE, Limburgerhof, Germany) was used against aphids (*Sternorrhyncha*) and Goldor® Bait (BASF, Ludwigshafen, Germany) was applied to the soil before sowing once per cultivation set against wireworms (*Elateridae*). Weed control was carried out manually.

The water supply was provided by irrigation and natural precipitation. Drip irrigation was activated when the soil moisture tension fell below -20 kPa in 15 cm depth, as controlled by a tensiometer with electronic pressure sensor (Tensio-Technik, Bambach, Geisenheim, Germany). Then, the well-irrigated segments (CTR) were provided with 100% water supply (3.0 L/m^2 per irrigation), whereas a reduced water supply (RWS) was realized by reducing the irrigation time to 60% of the CTR. Since the soil moisture was dominated by natural precipitation, the total amount of the supplied water, and, thus, the total water amount, varied between the two picking sets. In total, water supply of the RWS treatment was ultimately reduced by 10% compared to CTR, until the end of the first harvest period (11.07–17.07.2016, calendar week 28) and by 15% until the end of the second harvest period (18.07–24.07.2016, week 29), considering both irrigation and precipitation (Table 1). To ensure an initially uniform plant performance, the soil moisture of both CTR and RWS treatments was kept near field capacity until the 4th leaves on the main stems were unfolded. This aforementioned period of identical water supply lasted for 19 days after transplanting. Subsequently, the irrigation treatments were applied. More details on cultivation data and water supply are given in Table 1.

Harvest

Cucumber fruits were harvested twice a week during two consecutive harvest periods (week 28 and week 29) in 2016. All fruits with a length of 6–12 cm were harvested at once and were sorted by size. A total of 20 fruit of 9–10 cm fruit length per replicate were selected, cleaned manually with fresh water to get rid of adherent soil particles. Then, stems were removed with a knife and cucumbers were chopped into pieces before freezing at -80 °C until further analyses. The procedure was identical for each of the two harvest dates per week. Fruit of the same week were finally combined as a weekly sample.

Climatic data

Climate data were collected by the local weather station, which was located at 100 m distance to the experimental field site in Geisenheim. The climatic parameters for the cultivation period in 2016 are set out in Table 1, showing summed weather conditions for each harvest period from planting of the seedlings to the end of the picking period. Detailed weather conditions are described in Supplementary Figure S1. The growth conditions mean temperature, mean relative air humidity and mean global radiation were similar in both harvest periods. The growth period of the fruits averaged between 12–14 days after anthesis until fruits had reached a size of 9–10 cm. Until the end of the first harvest period (week 28), the cucumbers were irrigated 10 times and the reduction of the RWS amounted to 10%. Until the end of the second harvest period (week 29), the cucumbers were irrigated 15 times in total, resulting in a reduction of 15% until the end of the second harvest period (week 29) when considering both irrigation and precipitation.

Sample preparation

For all analyses, except for the determination of ascorbic acid, 300 g frozen cucumber of each weekly sample were lyophilized (BETA 2–8 LDplus, Martin Christ Gefrier-trocknungsanlagen, Osterode am Harz, Germany) prior to grinding with a laboratory mill (IKA M 20; IKA-Werke, Staufen, Germany). Dry matter content was determined gravimetrically using fresh and freeze-dried material.

D-Glucose, D-fructose, total titratable acidity, L-malic acid, citric acid, and fumaric acid, inorganic anions, such as nitrate, phosphate, sulfate, and chloride, as well as total phenols were determined from aqueous extracts prepared from the lyophilized powder.

For this purpose, an aliquot of approx. 12 g of lyophilized plant material was thoroughly homogenized with 500 mL ultrapure water at room temperature for ca. 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 including solids into an 800 mL beaker using another 150 mL added 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 4.596 x g to separate liquid and solid phases, the supernatants were collected, filtered, and stored at -25 °C until analyses.

Preparation procedures for all other target analytes are given below.

Table 1. Cultivation and climate data of the cucumber cultivation set in 2016 summed up for each picking period from sowing to the end of harvest. DAT: days after transplanting. Irrigation (mm): Total irrigation amount including irrigation during initial stage. Total water amount (%) RWS: water amount (%) of the reduced variants including watering after transplanting.

Picking time period	Week 28	Week 29
Date of transplanting (Year-month-day)	2016-06-02	2016-06-02
Beginning of water supply differentiation (DAT)	19	19
Beginning of harvest period after planting (DAT)	39	46
Temperature sum (°C)	775.7	922.9
Daily mean air temperature (°C)	18.9	19.2
Mean relative air humidity (%)	89.6	88.3
Global radiation sum (MJ/m ²)	777.3	931.9
Daily mean global radiation (MJ/m ²)	19.0	19.4
Wind speed sum (m/s) at height of 2 m	52.5	62.1
Evapotranspiration sum (mm)	133.3	161.8
Precipitation sum (mm)	84	84
Number of differentiated irrigation events	10	15
Total irrigation (mm) #	29	47
Total water amount (mm) of CTR incl. precipitation	114	131
Total water amount RWS (% of CTR)*	90%	85%

z: Reference evapotranspiration (ET_o) using grass and the FAO56 Penman-Monteith method, characterizing the respective cultivation period. #: Total irrigation amount of the CTR incl. irrigation events during initial growth. *: Total water amount of the reduced watered samples (RWS) up to the end of each picking period in contrast to the control (CTR).

Chemical analysis

All analytes studied in cucumber herein were determined as described in detail earlier [4]. In brief, D-Glucose and D-fructose were determined with enzymatic test kits (R-Biopharm, Darmstadt, Germany) using a Konelab 20 Xti analyzer (ThermoFisher, Dreieich, Germany). Polyols were analyzed according to Schlering et al. (2019) [4], except for using 250–300 mg of lyophilized powdered sample instead of 400 mg.

Titrateable 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 and L-citric acid were determined enzymatically on a Konelab 20 Xti analyzer (Thermo Fisher, Dreieich, Germany), using enzymatic kits (R-Biopharm, Darmstadt, Germany) for the determination of L-malic acid and the enzymatic-ultraviolet method (Boehringer Lebensmittelanalytik, Mannheim) for the determination of L-citric acid. Fumaric acid was determined on a Summit HPLC-UV system (Dionex, Idstein, Germany).

Inorganic anions such as nitrate, sulfate and phosphate were analyzed by ion chromatography on an ICS 2100-system, and chloride was analyzed by potentiometric titration as described earlier [4].

Total carbon and nitrogen determination were carried out in duplicate by the Dumas combustion method (Vario MAX CNS, Elementar Analysensysteme, Langenselbold, Germany). For minerals and trace element analyses, Kjeldahl digestion with the Gerhardt Turbotherm rapid digestion unit (C. Gerhardt GmbH and Co. KG, Königswinter, Germany) was applied following the procedure of Schlering et al. [4].

Total phenols were determined with the Folin-Ciocalteu reagent according to Schlering et al. [4].

Greenhouse Trial

Plant material and experimental cultivation setup

Cucumber cv. 'Fuga F1' seeds from Rijk Zwaan Welver (Wolver, Germany) were sown in multiport plates. After emergence of the cotyledons, each seedling was transferred into a single rectangular planting bowl containing 10 L potting substrate (SP T HF, Einheitserdewerke Patzer, Gebr. Patzer, Sinnatal-Altengronau, Germany), being placed on greenhouse tables. Subsequently, plants were cultivated under greenhouse conditions using a nutrient solution with FERTY® Basis 1 (Planta Düngemittel, Regenstauf, Germany), calcium nitrate and ammonium sulfate. The greenhouse experiment followed a randomized plot design, consisting of four plots (A–D) subdivided into two subplots each (Figure 1). One subplot was used for the fully watered (CTR) and one subplot for the reduced water supply treatment (RWS), yielding a total of four CTR and four RWS subplots. Each subplot contained three groups of three plants (=9 plants) per subplot with a distance of 2.3 m between the groups of three. The plants' main shoots

were directed horizontally to allow westward growth (Figure 1), while lateral shoots were removed manually after emergence. Fertilization was adapted to plant growth and conducted at 0.7 g N per m² per week from the first week, 1.4 g N/m² per week from the fourth week and 2.8 g N/m² per week from the sixth week. Crop protection was applied equally to all plots by using beneficial insects, while green lacewing larvae (*Chrysoperla carnea*) were used against aphids (*Sternorrhyncha*) and predatory mites (*Amblyseius cucumeris*) against thrips (*Frankliniella occidentalis*).

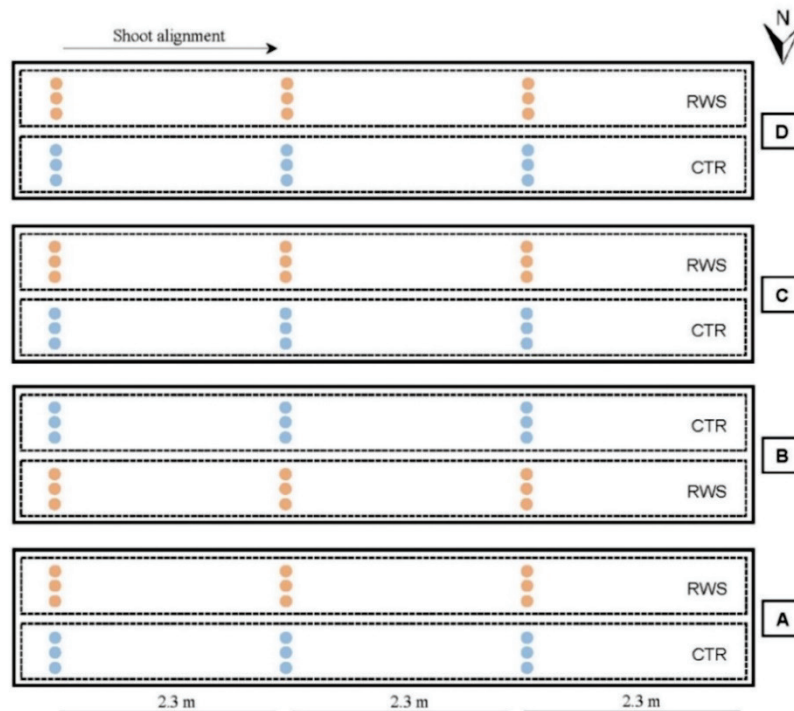


Figure 1. Structure of the experimental setup for the greenhouse cultivation of pickling cucumbers in 2015. Each plot (A–D) was divided into two subplots (CTR and RWS) for the application of two different irrigation regimes. Each subplot consisted of nine plants; fully watered plants are marked by blue circles, while reduced watered plants are marked by orange circles. CTR: control, full water supply (100%), RWS: reduced water supply (60%).

Water supply treatments

Water supply by drip irrigation was activated when the soil moisture tension fell below -40 hPa in 5 cm depth, as controlled by a tensiometer with electronic pressure sensor (Tensio-Technik, Bambach GbR, Geisenheim, Germany). The well-irrigated pots (CTR) were provided with 100% water supply (0.2 L/m² per irrigation per plant), whereas reduced water supply (RWS) was realized by reducing irrigation amount to 60% of the CTR. To ensure an initially uniform plant performance, the soil moisture of both CTR and RWS treatments was kept near field capacity until the 4th leaves on the

main stems were unfolded. i.e., ca. 14 days after sowing. Subsequently, differentiated RWS and CTR irrigation were activated.

Harvest

Cucumber fruits were harvested three times a week between 20.–26.07.2015, following the same procedure as described above for the open-field experiment. Instead of chopping the de-stemmed, harvested 20 fruits of 9–10 cm length, they were segmented into the compartments exocarp, mesocarp, and endocarp. The exocarp denotes the peel, while the mesocarp denotes the fleshy middle layer that makes up the bulk of the cucumber. The endocarp sample describes the inner part of the cucumber surrounding the seeds and, herein, included the seeds. All samples were frozen at $-80\text{ }^{\circ}\text{C}$ directly after segmentation until further analyses.

Sample preparation and chemical analysis

The sample preparation and chemical analysis for all target analytes except carotenoids were conducted as described before. The extraction procedure of carotenoids in the cucumbers' exocarp was carried out as described by Schlering et al. [5]. Separation, detection, and quantitation of single carotenoids in the exocarp extracts was carried out according to Schlering et al. [5], except for using a different YMC Carotenoid (C30)-column ($3.0 \times 150\text{ mm}$, $3\text{ }\mu\text{m}$ particle size, YMC, Kyoto, Japan).

Statistical analyses

Evaluation of analytical results was carried out for dry and fresh matter. While dry matter-related data enabled evaluations irrespective of varying water contents in the plant material, fresh matter-related data assessed the nutritional values of the harvested edible plant material. As described before [4], data were analyzed by fitting a linear mixed-effect model using the lmer-function within the lme4-package [17] of the statistical software R [18] in RStudio [19]. The evaluation of the single picking period (week) was based on a model that corrected for random plot-effects (Equation (1)), while the total dataset was evaluated with respect to the interaction of plot and week (Equation (2)):

$$y \sim \text{H2O} + (1|\text{plot})$$

$$y \sim \text{H2O} + (1|\text{plot:week})$$

where y represents the analyzed parameter, water supply (control, reduced) was a fixed factor and plot as well as the interaction plot:week were random factors. Comparisons of means derived from different treatments were considered significantly different if p -values < 0.10 . A pairwise comparison of least-squares means was carried out with `lsmeans`-package [20] to estimate the fixed effects of the treatment as well as random effects for the plot and week. Significances of random effects were calculated by the `lmerTest`-package [21]. The results for the treatment (CTR: control, RWS: 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` [22] and `factoextra` [23]. Within PCA analysis, individual samples were visualized in a score plot, while corresponding chemical components are represented in a loading plot inside a correlation circle presenting the relationship between the variables.

Results and Discussion

Evaluation of Picking Time

According to PCA analyses of the entire dataset, including both harvesting periods corrected for random plot effects (cf. Equation (1)), a clear-cut differentiation of the samples by the picking time (week) based on their chemical composition became obvious. The first two principal components (Dim1+Dim2) accounted for 50.5% and 65.6% of the total variance in case of dry matter (DM)- and fresh matter (FM)-related data (Figures 2A, B), respectively. Thus, the chemical composition of pickling cucumbers grown in open field was strongly dependent on the picking time, characterized by differing growing conditions. These findings are in agreement with an earlier study showing that fruit parameters, like flesh firmness, skin color, as well as selected substances, such as titratable acidity, depended on the maturity of the cucumber fruit [2]. Furthermore, irrespective of evaluating dry or fresh matter-related data (Figure 2A, B), the variability of the nutritional composition of the fruit was notably stronger within the first picking period (week 28) than that of fruits from week 29, especially when considering dry matter-related data (Figure 2A). Although the reasons for these findings remain widely unclear, the composition of the fruits of our genetically widely uniform

F1 hybrid plants might be asymptotically converging towards a uniform composition characteristic for the genotype's maturity.

Apart from that, dry matter contents were higher in the first picking set in week 28 (6.14–6.32%) compared to cucumber harvested in week 29 (5.29–5.37%). In this regard, Gómez et al. [24] investigated the distribution of dry matter accumulation into the different organs of cucumber plants, concluding that the leaf photosynthetic capacity was adjusted to the plants' demand for assimilates, which seemed to be strongly dependent on the fruit load. Possibly, the sink strength by the fruits was reduced after the first harvest period in week 28, thus potentially explaining the lower dry matter levels in week 29.

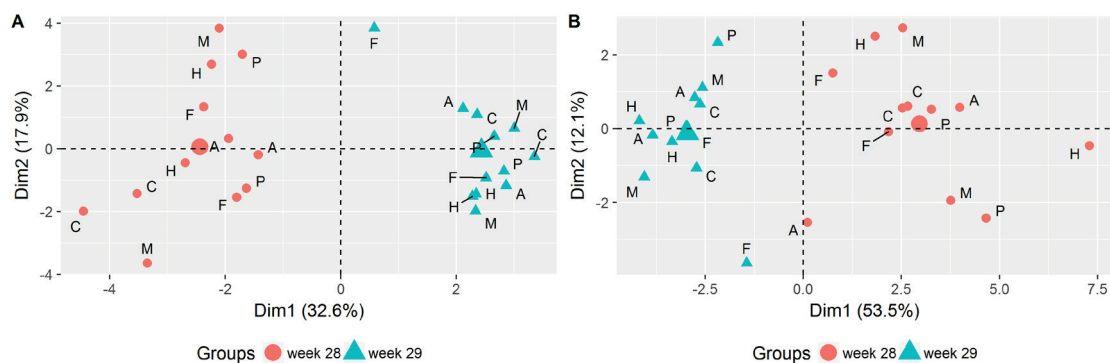


Figure 2. Principal component analysis (PCA) of the original, plot-adjusted dataset including both cucumber harvest sets from week 28 (red circles) and week 29 (green triangles). Score plots represent the individual samples of each picking set (separated by color) and plot (marked by the letters A, C, F, H, M, and P) based on dry matter-related data (A) and fresh matter-related data (B). The enlarged symbols (circle, triangle) represent group mean data points.

Effects of Reduced Water Supply on the Chemical Composition of Cucumber Biomass

Multivariate analyses of the entire dry matter-related dataset adjusted by the interaction of plot and week (cf. Equation (2)) revealed that well irrigated cucumber samples were mainly clustered in sectors with positive PC1 (Dim1), while those grown under moderately reduced water supply were mainly located in sectors with negative PC1 (Figure 3A). Most of the individuals were distinguished by the first principal component Dim1, which made up 28.1% of the total variance. The first two principal components (Dim1+Dim2) accounted for 41.0% of the total variance in the dry matter-based dataset. The corresponding loading plot (Figure 3B) pointed out a strong contribution of certain minerals, such as phosphorus (P), nitrogen (N), iron (Fe), zinc (Zn), and copper (Cu) to the differentiation of the different water supply treatments. In addition, dry matter-

related amounts of phosphate, chloride, malic acid, as well as glucose and fructose, have contributed strongly to the separation of both groups. The reason for the dissimilarity of samples from the reduced watered subplot of plot P to other RWS plots remains unknown, even when the data was corrected by the plot effect.

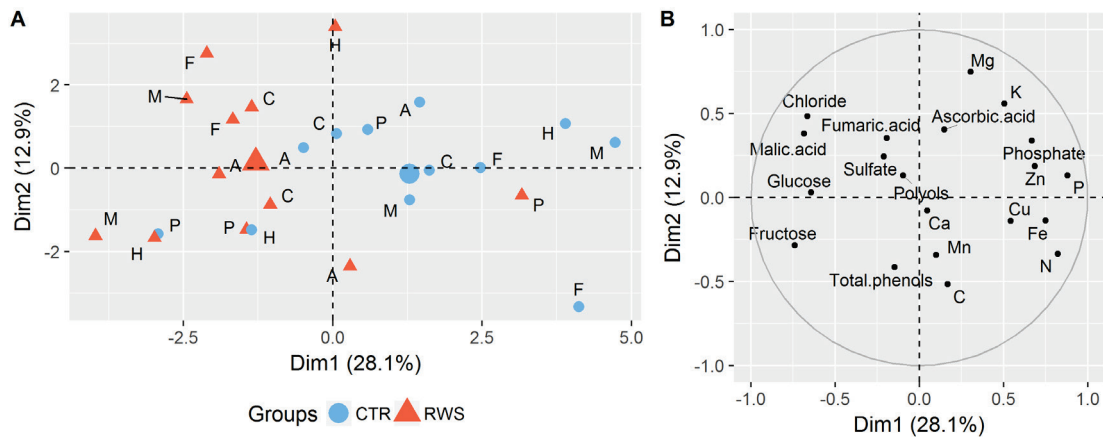


Figure 3. Multivariate evaluation by PCA (PC1+PC2) of the total cucumber dataset including both picking sets (week 28 and week 29) based on dry matter-data. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M, and P) classified according to the control CTR (blue circles) and reduced water supply RWS (red triangles). The corresponding loading plot (B) shows the related variables as determined by chemical analyses.

In addition, univariate statistical analyses were conducted to support the results obtained from PCA plots. As shown in Table 2, the RWS samples were characterized by significantly higher dry matter-related contents of malic acid, while those of phosphate, P, N, and Fe were significantly lower in relation to well-watered samples. Moreover, significantly higher levels of calcium (Ca) and magnesium (Mg) were observed in samples from RWS than in those of CTR harvested in week 29, while no effects were apparent during the first period (week 28) or when evaluating the total dataset. However, the univariate results also clarified that sugars and polyols, as well as most of trace elements and polyphenols, had been little affected by mildly-reduced water supply. These findings are partly contrary to our results observed for vegetables, such as spinach and radish grown within the same field experiment under similar conditions, where we observed a significant increase of polyols, such as inositol in both vegetables upon moderate water supply reduction [4,5]. These findings indicate that abiotic stress factors affect the compositional quality of vegetable crops very differently, depending on species and, putatively, also the plant organ. In agreement, Gargallo-Garriga et al. [25] have demonstrated very well the contrasting responses of shoots and roots to drought and seasonally changing conditions in two grass varieties (*Holcus lanatus* L.

and *Alopecurus pratensis* L.) by altering the allocation of biomass, but also by changing the metabolic activities of their organs.

As shown in Figure 4, the loading plot of fresh matter-based PCA analysis does not show a clear distinction between the treatments over a certain principal component as for that based on dry matter. However, samples were partly separated by the second principal component (Dim2), explaining 17.3% of the total variance (Figure 4A). By adding the first principal component (Dim1), a share of 51.9% of the total variance was explained. In contrast to the leafy spinach, where discrimination became clearer within fresh matter-based evaluation [5], the distinction between the treatments was not as clear in the case of cucumber fruits. In brief, the fresh matter-based chemical composition of the fruits was more alike than the dry matter-based one.

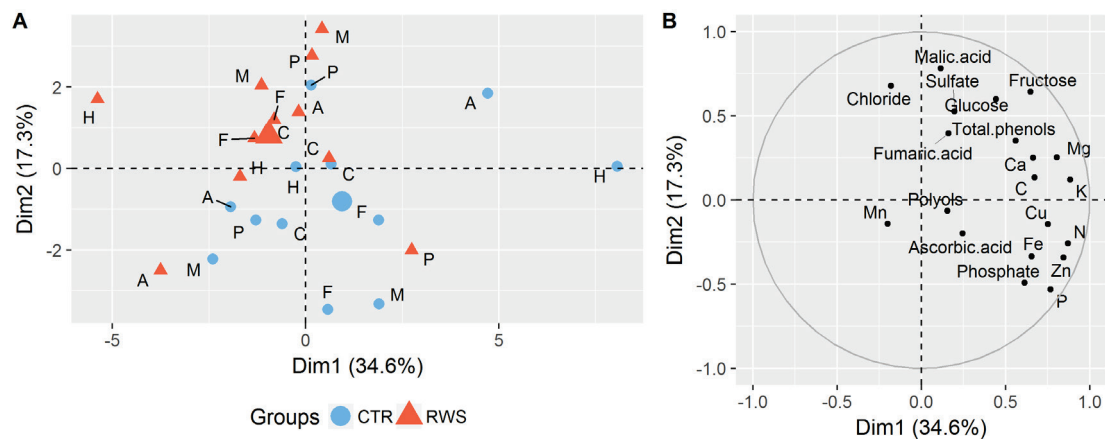


Figure 4. Multivariate evaluation by PCA (PC1+PC2) of the cucumber dataset including both harvesting sets (week 28 and week 29) based on fresh matter-data. Score plot (A) represents all individual plot samples (marked by the letters A, C, F, H, M, and P) classified according to the control CTR (blue circles) and reduced water supply RWS (red triangles). The corresponding loading plot (B) shows the related variables, i.e., contents of constituents as determined by the chemical analyses.

The most important variables contributing to the variants' distinction seem to be inorganic components such as N, P, Fe, Zn, chloride, sulfate, and phosphate, as well as primary organic plant metabolites like malic acid, glucose, and fructose (Figure 4B). These findings were only partially in agreement with those of our univariate evaluation. Except for malic acid contents, the contents of some chemical components, such as N, P, phosphate, Fe, and Zn, were significantly reduced under moderate water reduction when considering the overall dataset shown in Table 3. However, the contents of glucose and fructose remained unchanged. The observed increases of fresh matter-related Ca and Mg levels in cucumbers harvested in week 29 (Table 3) are similar to earlier observations of Pulupol et al. [11], who determined significantly higher contents

of those minerals in deficit-irrigated tomatoes based on fresh matter. Altogether, fresh matter-based contents of most of the studied constituents decreased when fruits were grown under a moderately-reduced water supply.

Sugars and Polyols

The effects on the contents of glucose and fructose by moderate water reduction in cucumber fruits and variations between the two picking periods were not significant. Dry matter-related contents of glucose were slightly higher in week 29 (197.5–200.2 mg/g DM) than in week 28 (179.0–181.0 mg/g DM), while fructose levels were marginally higher in week 28 (209.6–211.8 mg/g DM) than in week 29 (207.3–208.1 mg/g DM) as shown in Table 2. The findings are in accordance to the results of Rouphael et al. [15], who found effects of deficit irrigation and season to not be significant regarding the contents of total soluble solids in fruits of mini-watermelons during two growing seasons. Similarly, Akinci and Lösel [26] have not found significant changes in the content of glucose and fructose in leaves of drought stressed and unstressed cucumber cultivars.

The higher content of dry matter in week 28 leads inherently to enhanced values in the fresh matter-related results of fruits harvested in the first period (Table 3). By analogy, the overall glucose and fructose levels were higher in week 28 (1.11–1.13 g glucose and 1.30–1.33 g fructose per 100 g FW) than in week 29 (1.05–1.08 g glucose and 1.10–1.12 mg fructose per 100 g FW, Table 3). Unexpectedly, differences between the contents of dry matter, glucose, and fructose in fruit from CTR vs. RWS treatments were not significant. The same applied to dry matter-based levels of glucose and fructose in other vegetables, such as spinach and radish, exposed to moderate water reduction within the same field experiment [4,5]. However, irrespective of the absent effects of RWS on glucose and fructose, Akinci and Lösel [26] have shown that moderate drought stress already had affected certain growth parameters of cucumber seedlings such as shoot growth, dry and fresh weight as well as shoot/root ratio.

Similar to glucose and fructose contents, slight water reduction did not affect the dry matter-based content of polyols. The levels of individual polyols, particularly those of inositol, were marginally higher in fruits harvested during the first period (week 28, Table 2) than those of the second harvest (week 29, Table 2). Total polyols were also only slightly lower in week 29 (8.6–8.9 mg/g DM) compared to week 28 (9.2 mg/g DM). In general, differences in the dry matter-related levels of polyols were not

significant between water treatments, but the picking period was shown to influence polyols significantly (Table 2). In contrast, fresh matter-related levels of polyols, mainly governed by the most abundant inositol, were significantly lower in fruit of the RWS treatment than those of the CTR. In contrast, a mildly reduced water supply evoked significantly increased polyol levels in spinach [5] and radish [4] during our earlier experiment on the same experimental site. The absence of a water deficit-induced increase of fresh matter-related polyol levels in cucumbers was unexpected, because they are considered as relatively sensitive to water deficit because of their high water demand in contrast to grain crops [12,13]. Polyols can accumulate osmotically-significant levels without any negative effects on the plant's metabolism, rendering them suitable as compatible solutes in the osmotic adjustment of plants to drought or salt stress [27,28]. The reason for the absence of the increase in polyols in cucumbers of our experiment may be the relatively mildly reduced water supply. However, pickling cucumbers grown in the greenhouse experiment with 60% water supply of the well-watered control showed significant increases of polyols, such as inositol, in the fruits' skin (exocarp) and flesh (mesocarp) (Table 4).

Organic Acids

Regarding fruits harvested in week 29, dry matter-based levels of malic acid, the major organic acid in pickling cucumbers [29], were increased upon moderate water supply reduction (44.6 vs. 41.2 mg/g DM for RWS and CTR, respectively, Table 2). However, levels of fruits harvested in week 28 were only insignificantly different between the treatments. The aforementioned finding was also made when considering the entire dataset as shown in Table 2 ($p = 0.0049$). The significant contribution of malic acid to the differentiation of both treatments became also clear within the PCA (Figure 3). By analogy to our findings, Mitchell et al. [30] found increased levels of malic acid in tomatoes from deficiently watered plants, as compared to those from well-watered plants. However, we found an opposite trend earlier for radish root tubers grown under a moderately-reduced water supply within the same experimental field site [4]. It has to be noted that plant mechanisms involved in the accumulation of malic acid are very complex. Lobit et al. [31] have designed a model, assuming that the content of malic acid is determined by its transport and storage conditions. While malate acts as an important osmotic agent in leaves [32], its accumulation could also be a consequence of a slowed degradation metabolism [30]. In brief, it remains unclear why malic acid

had accumulated in cucumbers grown under moderately reduced water supply. However, when cucumbers were grown with a more reduced water supply, i.e., with 60% water amount of the full irrigation in the greenhouse experiment in 2015 (Table 4), malic acid levels were observed to be significantly increased in all of the cucumber compartments (exocarp, mesocarp, and endocarp). Similar results were reported by Mitchell et al. [30] for tomato fruits grown under moderate water deficits, concluding that the formation of organic acids acts as a buffer system to balance the charge of accumulated cations by maintaining the pH [33].

Fresh matter-related levels of malic acid were similar between the weeks, irrespective of the treatment. Nevertheless, a significant increase of malic acid was found in fresh matter of the reduced watered samples from week 29, despite having lower dry matter levels (5.29–5.37%) than samples from week 28 (6.14–6.32%, Table 3).

Differences in fresh- and dry matter-related ascorbic acid levels were not significant irrespective of the water supply treatment or the harvesting period (Tables 2 and 3).

Inorganic Anions

The dry matter-related contents of inorganic anions in fruits of pickling cucumber were mostly unaffected by reduced water supply (Table 2), except for those of phosphate (PO_4^{3-}), which were significantly decreased by moderate water reduction in week 29 ($p = 0.0911$) and when evaluating the entire dataset ($p = 0.0751$). These findings were supported by the multivariate evaluation by PCA (Figure 3). Considering the overall dataset, the PO_4^{3-} -contents were significantly lower in RWS (14.1 mg/g DM) in contrast to CTR (15.6 mg/g DM) as shown in Table 2. In general, plants use inorganic phosphate as primary source of phosphorous, although it is one of the less accessible elements due to its low solubility and poor mobility in the soil solution [34]. Therefore, plants have developed numerous morphological, biochemical, and molecular responses to acquire phosphate from the soil [35] and to actively and selectively accumulate phosphate in their cells [36]. The long-distance transport of phosphate from the root into above-ground organs by the symplast [36] may be potentially impaired by moderate water reduction. When water reduction was more pronounced as, e.g., in the greenhouse experiment, the levels of PO_4^{3-} were significantly increased in the cucumbers' skin and flesh (Table 4). In brief, these observations remain yet unexplained. With consideration to the picking period, PO_4^{3-} -contents were generally higher in week 29 (15.0–16.7 mg/g DM) in contrast to week 28 (13.2–14.6 mg/g DM).

Other inorganic anions such as nitrate (NO_3^-), sulfate (SO_4^{2-}) and chloride (Cl^-) were only marginally influenced by moderate reduced water supply when evaluating dry matter-related data (Table 2), even Cl^- was shown to contribute to the treatments' differentiation in the PCA (Figure 3). Levels of those anions were generally by trend higher in reduced watered samples in week 29 as compared to week 28, yet lacking statistical significance. The same independence from the water supply treatment was found for the fresh matter-related results (Table 3).

Carbon

As already shown for other vegetables such as spinach and radish [4,5], the content of carbon (C) in fruits of pickling cucumber was not influenced by moderate water reduction, neither on dry nor on fresh matter basis (Tables 2 and 3). Similarly, dry matter-related levels of C were independent from the picking period, while fresh matter-related data demonstrated higher levels of C in week 28 (2.41–2.48 g/100 g FM) in contrast to week 29 (2.10–2.13 g/100 g FM), in agreement with the higher contents of dry matter in week 28 as compared to week 29. However, cucumber grown under more severe water reduction in a controlled greenhouse environment showed significantly decreased contents of C in each compartment (Table 4), even though dry matter contents did not differ between the treatments or were increased in the case of the endocarp (4.86% DM for CTR vs. 5.00% DM for RWS).

Nitrogen

Evaluation of the entire dry matter-related dataset revealed a significant decrease ($p = 0.0648$) of total nitrogen (N) in cucumbers grown under a moderately-reduced water supply (Table 2). This was also notable within each picking period by trend, but without statistical significance. The same decrease was true if considering the fresh matter-based dataset (Table 3). Water deficit-induced, decreased N levels were even more pronounced in single compartments of cucumber fruits grown under controlled greenhouse conditions, when water reduction was 40% instead of 10–15% in the open field experiment (Table 4). In agreement, da Silva et al. [37] have earlier found decreased N levels in reduced-watered cucumbers, explaining the decreased by an impaired nitrogen mobility in dehydrated soil. While findings on decreased N levels upon limited irrigation appear to be quite consistent for cucumbers, increased nitrogen levels were observed in fresh matter of radish [4] and spinach [5]. In this context,

Gargallo-Garriga et al. [25] have demonstrated in a drought-stress study that shoots of two common C3-grasses exerted a decreased growth metabolism in case of drought, whereas roots increase it in a virtually mirrored response [25]. In general, the dry matter-related N levels were slightly higher in week 28 (26.6–27.4 mg/g DM) compared to those of week 29 (24.0–25.0 mg/g DM), paralleling dry matter contents. Thus, the differences in N levels generally became greater when considering fresh matter-related data (Table 3).

Potassium

The moderate limitation of water supply did not evoke significant changes in the concentration of potassium (K^+) in pickling cucumber, neither regarding dry matter nor fresh matter-related data (Tables 2 and 3). Similarly to those of N, dry matter-related levels of K^+ in cucumbers harvested in week 29 were only slightly lower than in those harvested in week 28 (Table 2), but significantly lower when considering the fresh matter-related dataset (Table 3).

Earlier findings on the effect of water deficit on K^+ levels in other vegetable crops are inconsistent. While we found unchanged K^+ levels in cucumbers due to irrigation treatments, significantly higher levels have been observed earlier in the fresh matter of radish [4] and spinach [5]. In contrast, Mitchell et al. [30] observed reduced K^+ levels in tomatoes when plants were exposed to water deficits. When water reduction was higher, i.e., by 40% in our greenhouse experiment, a significant increase of K^+ was found in the exocarp, mesocarp, and endocarp of pickling cucumbers from 49.0, 19.4, and 33.9 to 54.6, 23.9, and 35.4 mg/100 g DM, respectively. In parallel, the fruits compartments were characterized by a highly significant increase in malic acid levels from 19.2, 26.4, and 47.4 to 28.5, 39.9, and 53.5 mg/100 g DM as shown in the Table 4, respectively.

Table 2. Influence of moderate water reduction on the dry matter (DM)-related levels of constituents of cucumber fruits from two harvesting sets (week 28 and week 29) in 2016. 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.

	Cucumber Week 28			Cucumber Week 29			Cucumber Total			Effect of Harvest Period (Week)	Interaction of Water Supply (H ₂ O) * Harvest Period (Week)
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
Dry matter (% of FM)	6.32	6.14	0.2180	5.29	5.37	0.2371	5.81	5.75	0.5039	0.000 ***	0.000 ***
Sugars and polyols											
Glucose (mg/g)	179.0	181.0	0.7129	197.5	200.2	0.2706	188.2	190.6	0.3895	0.000 ***	0.000 ***
Fructose (mg/g)	209.6	211.8	0.3328	207.3	208.1	0.6342	208.5	210.0	0.2560	0.206	0.724
Polyols total (mg/g)	9.2	9.2	0.7729	8.9	8.6	0.1386	9.0	8.9	0.3768	0.007 **	0.857
Inositol (mg/g)	8.1	8.1	0.7879	7.9	7.5	0.1022	8.0	7.9	0.2820	0.015 *	0.965
Glycerol (mg/g)	0.8	0.9	0.6344	0.8	0.8	0.2606	0.8	0.9	0.2250	0.667	0.816
Erythritol (mg/g)	0.3	0.3	1.0000	0.2	0.2	0.7294	0.2	0.2	0.8735	0.000 ***	0.002 **
Organic acids											
Total acidity (mg/g)	16.7	16.1	0.7602	11.3	11.6	0.6594	14.0	13.9	0.8784	0.000 ***	0.002 **
Malic acid (mg/g)	36.3	37.8	0.2068	41.2	44.6	0.0095 **	38.7	41.2	0.0049 **	0.000 ***	0.000 ***
Ascorbic acid (mg/g)	1.2	1.2	0.5583	1.3	1.3	0.6535	1.2	1.2	0.9735	0.153	0.831
Citric acid (mg/g)	nd	nd	-	0.3	0.4	0.1099	nd	nd	-	-	-
Fumaric acid (mg/g)	7.2	6.4	0.7665	5.7	7.2	0.3429	6.5	6.8	0.8185	1.000	1.000
Anions											
Nitrate (mg/g)	nd	nd	-	0.5	0.5	0.6728	nd	nd	-	-	-
Phosphate (mg/g)	14.6	13.2	0.3732	16.7	15.0	0.0911	15.6	14.1	0.0751	0.112	0.612
Sulfate (mg/g)	5.3	5.3	0.8516	4.0	4.3	0.2664	4.6	4.8	0.2406	0.000 ***	0.001 **
Chloride (mg/g)	2.1	2.0	0.6668	2.5	2.7	0.2750	2.3	2.4	0.5686	0.002 **	0.052
Elements											
Carbon (mg/g)	392.4	392.1	0.7616	395.8	395.0	0.6843	394.1	393.3	0.4051	0.049 *	0.316
Macronutrients											
Potassium (mg/g)	40.39	39.61	0.1966	38.11	38.26	0.8241	39.25	38.93	0.4490	0.002 **	0.497
Nitrogen (mg/g)	27.44	26.59	0.2702	25.01	23.95	0.1639	26.22	25.27	0.0648	0.000 ***	0.001 **
Phosphorous (mg/g)	6.71	6.49	0.3852	6.84	6.47	0.0217 *	6.77	6.48	0.0365 *	1.000	1.000
Calcium (mg/g)	4.33	4.31	0.9028	4.35	4.49	0.0282 *	4.34	4.40	0.3417	0.609	1.000
Magnesium (mg/g)	2.48	2.45	0.3434	2.66	2.71	0.0694	2.57	2.58	0.6571	0.000 ***	0.196

Table 2. (continued)

	Cucumber Week 28			Cucumber Week 29			Cucumber Total			Effect of Harvest Period (Week)	Interaction of Water Supply (H ₂ O) * Harvest Period (Week)
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
<i>Micronutrients</i>											
Iron (µg/g)	72	64	0.2778	63	54	0.0479 *	68	58	0.0350 *	0.111	0.063
Zinc (µg/g)	34	33	0.4062	35	34	0.1611	34	33	0.1328	0.423	0.589
Manganese (µg/g)	17	17	0.5155	16	16	0.5891	16	16	0.3697	0.792	1.000
Copper (µg/g)	10	10	0.6336	11	11	0.5645	11	10	0.4407	0.327	0.793
Phenolic compounds											
Total phenols (mg/g)	2.8	2.9	0.4941	2.7	2.7	0.8606	2.7	2.8	0.5028	0.021 *	0.133

Table 3. Influence of moderate water reduction on the fresh matter (FM)-related levels of constituents of cucumber fruits from two harvesting sets (week 28 and week 29) in 2016. 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.

	Cucumber Week 28			Cucumber Week 29			Cucumber Total			Effect of Harvest Period (Week)	Interaction of Water Supply (H ₂ O) * Harvest Period (Week)
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
Dry matter (% of FM)	6.32	6.14	0.2180	5.29	5.37	0.2371	5.81	5.75	0.5039	0.000 ***	0.000 ***
Sugars and polyols											
Glucose (g/100 g)	1.13	1.11	0.6675	1.05	1.08	0.2438	1.09	1.09	0.8832	0.051	0.347
Fructose (g/100 g)	1.33	1.30	0.4618	1.10	1.12	0.3881	1.21	1.21	0.8238	0.000 ***	0.000 ***
Polyols total (mg/100 g)	57.9	56.6	0.2132	56.2	52.4	0.0607	57.0	54.7	0.0208 *	0.019 *	0.630
Inositol (mg/100 g)	51.0	49.8	0.2321	49.7	46.0	0.0395 *	50.4	48.1	0.0156 *	0.029 *	0.675
Glycerol (mg/100 g)	5.3	5.2	0.6916	5.1	5.1	0.9237	5.2	5.2	0.8980	0.947	1.000
Erythritol (mg/100 g)	1.6	1.5	0.3627	1.4	1.3	0.2192	1.5	1.4	0.1761	0.000 ***	0.000 ***
Organic acids											
Total acidity (mg/100 g)	105.3	99.0	0.5229	60.0	62.0	0.5784	82.7	80.5	0.6624	0.000 ***	0.000 ***
Malic acid (mg/100 g)	227.2	232.9	0.5577	218.6	239.1	0.0219 *	222.9	236.0	0.0436 *	1.000	0.429
Ascorbic acid (mg/100 g)	7.5	7.0	0.2268	6.9	7.2	0.5088	7.2	7.1	0.7140	1.000	1.000
Citric acid (mg/100 g)	nd	nd	-	1.6	1.9	0.1144	nd	nd	-	-	-
Fumaric acid (mg/100 g)	45.0	39.4	0.7437	30.3	38.5	0.3139	37.6	38.9	0.8840	1.000	1.000
Anions											
Nitrate (mg/100 g)	nd	nd	-	2.4	2.8	0.6398	nd	nd	-	-	-
Phosphate (mg/100 g)	91.1	81.2	0.3087	88.6	80.4	0.0897	89.9	80.8	0.0732	1.000	1.000
Sulfate (mg/100 g)	32.9	32.4	0.4909	21.1	23.3	0.2022	27.0	27.8	0.3540	0.000 ***	0.000 ***
Chloride (mg/100 g)	13.1	12.4	0.5563	13.2	14.5	0.2116	13.2	13.4	0.6985	0.489	1.000
Elements											
Carbon (g/100 g)	2.5	2.4	0.1987	2.1	2.1	0.3342	2.3	2.3	0.4776	0.000 ***	0.000 ***
Macronutrients											
Potassium (mg/100 g)	255.24	243.11	0.1372	201.69	205.23	0.1457	228.47	224.17	0.3231	0.000 ***	0.000 ***
Nitrogen (mg/100 g)	173.27	163.20	0.1218	132.37	128.60	0.4133	152.82	145.90	0.0829	0.000 ***	0.000 ***
Phosphorous (mg/100 g)	42.33	39.81	0.1845	36.23	34.70	0.0059 **	39.28	37.25	0.0296 *	0.000 ***	0.048 *
Calcium (mg/100 g)	27.35	26.39	0.2697	22.99	24.10	0.0646	25.17	25.25	0.8904	0.000 ***	0.052
Magnesium (mg/100 g)	15.66	14.98	0.1385	14.04	14.55	0.0095 **	14.85	14.77	0.7585	0.003 **	0.263

Table 3. (continued)

<i>Micronutrients</i>											
Iron (µg/100 g)	455	390	0.2138	334	290	0.0739	399	340	0.0404 *	0.001 **	0.002 **
Zinc (µg/100 g)	213	202	0.1443	183	182	0.1399	198	192	0.0848	0.000 ***	0.000 ***
Manganese (µg/100 g)	104	105	0.8338	84	87	0.4063	94	96	0.4567	0.001 **	0.008 **
Copper (µg/100 g)	65	61	0.3896	58	57	0.7708	61	59	0.3917	0.093	0.282
Phenolic compounds											
Total phenols (mg/100 g)	17.7	17.5	0.8832	14.1	14.3	0.6144	15.9	15.9	0.8957	0.000 ***	0.000 ***

Phosphorous

In accordance to phosphate, the content of phosphorous (P) in cucumber dry matter was significantly decreased by moderately reduced water supply considering the dry and fresh matter-related total dataset ($p = 0.0365$ and 0.0296 , respectively, Table 2) and the second harvest period in week 29 ($p = 0.0217$ and 0.0059 , respectively, Table 3). In the first harvest period in week 28, differences between P levels from CTR and RWS treatments were not significant (Tables 2 and 3). Earlier, a water deficit-induced decline in P was also found in radish root tubers grown within the same experimental field site [4]. Multivariate analyses of the total dataset by PCA support the findings that P contributed strongly to the differentiation of both water supply treatments in cucumber (Figure 3A, B). In contrast, Rouphael et al. [38] did not find significant differences in the content of dry matter-related P in mini-watermelons cultivated under different water regimes based on evapotranspiration rates. However, others have already reported lower concentration and reduced P uptake in leaves of cherry tomato plants when grown under water deficit [39]. Our study demonstrated that the observed decline in P became highly significant in week 29 ($p = 0.0059$), when considering fresh matter-based results (Table 3). However, the dry matter-related P levels did not differ between the picking periods, being 6.5–6.7 mg/g DM in week 28 and 6.5–6.8 mg/g DM in week 29. Fresh matter analyses revealed higher p -levels in week 28 than in week 29, parallel to the respective dry matter contents. In contrast to decreasing p -levels in cucumbers with 10–15% less water than the control in our open-field experiment, increasing and unchanged p -levels were observed in pickling cucumbers exposed to a water reduction by 40% in our greenhouse experiment. A significant increase was noted in the exocarp and mesocarp, while p -levels in the endocarp, which represents the inner part of the cucumber including the seeds, remained unchanged (Table 4).

Calcium

Mild water reduction led to a significantly higher dry matter-related content of calcium (Ca^{2+}) in cucumbers from week 29 ($p = 0.0282$), but did not evoke alterations in fruits being picked in week 28 ($p = 0.9028$) or when considering the overall dataset (Table 2). Similar effects were found for fresh matter-based results (Table 3). Contrary to the contents of minerals, such as N and P, whose levels were significantly decreased in reduced watered cucumbers, these findings are the opposite to those reported for radish

when reducing water supply by 15–20% [4]. Nevertheless, in our greenhouse experiment with reduced water supply by 40%, the increase in Ca^{2+} levels was consistently visible in the fruits' endocarp when water reduction was stronger (Table 4). It is known that Ca^{2+} accumulation is less sensitive to drought in contrast to other minerals such as K or P [40]. While univariate analyses were not significant over the total dataset, the PCA underline that Ca^{2+} did not contribute to the variants differentiation based on dry matter, as shown in Figure 3. In this context, Mitchell et al. [30] observed a dry matter-based decrease by 28% in the total ion accumulation in cucumber fruits grown under moderate water deficit, including Ca^{2+} .

Magnesium

Similarly to Ca^{2+} , dry matter-based contents of magnesium (Mg^{2+}) increased upon moderate water reduction by trend only in fruits grown in week 29 ($p = 0.0694$). This difference became significant when evaluating on fresh matter basis ($p = 0.0095$). However, the increase was not observed when considering the results of week 28 or the total dataset. Thus, the contribution of Mg^{2+} to the differentiation between CTR and RWS within the PCA was low regarding dry matter evaluation and slightly stronger considering the fresh matter-based loading plot (Figure 4B). In general, the effects of moderately reduced water supply on the content of Mg^{2+} in fruits of pickling cucumber followed a pattern similar to that of Ca^{2+} . In agreement with our findings, Pulupol et al. [11] found higher contents of Mg^{2+} on fresh weight basis in tomato fruits grown under deficit irrigation, while dry matter-related concentrations were unaffected. Nevertheless, as a result of our greenhouse experiment with a water reduction by 40%, an increased level of Mg^{2+} provoked by water reduction was found in the mesocarp of pickling cucumber, but not in other fruit compartments (Table 4).

Table 4. Influence of reduced water supply on the dry matter-related levels of certain constituents of pickling cucumber fruit compartments from a greenhouse experiment in summer 2015. Linear mixed model, *t*-test, *p*-values: <0.05 (*), <0.01 (**), <0.001 (***); nd = not determined, CTR: Control treatment with full water supply, RWS: Reduced water supply treatment (60% of the water amount of the control).

	Exocarp			Mesocarp			Endocarp		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Dry matter (% of FM)	6.73	6.78	0.5601	4.86	4.89	0.7417	4.86	5.00	0.2588
Sugars and polyols									
Glucose (mg/g)	28.5	30.0	0.5997	271.4	275.6	0.2227	252.3	260.7	0.0092 **
Fructose (mg/g)	35.9	36.2	0.9084	288.1	288.3	0.9307	270.3	274.8	0.0201 *
Polyols total (mg/g)	4.9	5.4	0.0383 *	7.0	7.5	0.0288 *	9.0	9.2	0.2718
Inositol (mg/g)	2.9	3.4	0.0245 *	6.4	6.9	0.0354 *	7.9	8.2	0.2135
Glycerol (mg/g)	1.3	1.3	0.8460	0.5	0.6	0.0805	1.0	1.0	0.4950
Erythritol (µg/g)	770	678	0.1298	90	73	0.0689	30	35	0.6042
Organic acids									
Total acidity (mg/g)	24.6	15.4	0.0005 ***	13.4	14.5	0.7520	26.4	24.5	0.6365
Malic acid (mg/g)	19.2	28.5	0.0007 ***	26.4	39.9	0.0002 ***	47.4	53.5	0.0027 **
Citric acid (mg/g)	3.9	3.3	0.1001	2.3	3.2	0.1350	0.2	1.1	0.0009 ***
Fumaric acid (mg/g)	3.5	4.1	0.0495 *	3.9	0.8	0.0018 **	4.5	2.9	0.4826
Anions									
Nitrate (mg/g)	2.9	2.1	0.1577	1.1	0.9	0.3363	nd	nd	0.3762
Phosphate (mg/g)	15.0	18.6	0.0058 **	7.4	10.1	0.0299 *	19.7	18.6	0.6379
Sulfate (mg/g)	12.1	14.5	0.0061 **	4.7	4.7	0.9687	4.7	9.3	0.0446 *
Chloride (mg/g)	9.7	9.2	0.0226 *	3.3	3.8	0.0410 *	6.1	5.7	0.4458
Elements									
Carbon (mg/g)	407.7	394.5	0.0547	387.2	381.5	0.0020 **	389.8	387.1	0.0035 **
Macronutrients									
Potassium (mg/g)	48.98	54.60	0.0016 **	19.40	23.89	0.0002 ***	33.92	35.39	0.0764
Nitrogen (mg/g)	43.84	36.23	0.0052 **	20.23	15.43	0.0037 **	30.46	26.98	0.0011 **
Phosphorous (mg/g)	7.79	8.99	0.0146 *	3.44	4.05	0.0027 **	6.71	6.78	0.1434
Calcium (mg/g)	7.66	8.00	0.1574	4.86	4.88	0.8896	1.58	1.76	0.0210 *
Magnesium (mg/g)	2.86	3.01	0.2182	2.46	2.57	0.0390 *	2.13	2.16	0.4860
Micronutrients									
Iron (µg/g)	86	87	0.9618	43	51	0.3444	47	40	0.3233
Zinc (µg/g)	43	36	0.0039 **	26	20	0.0056 **	40	35	0.0047 **
Manganese (µg/g)	46	51	0.2572	16	18	0.1112	18	21	0.0175 *
Copper (µg/g)	16	12	0.0004 ***	6	5	0.1965	10	7	0.0166 *
Phenolic compounds									
Total phenols (mg/g)	4.0	3.7	0.0219 *	3.2	3.8	0.0572	6.1	5.7	0.4458
Carotenoids									
Violaxanthin (µg/g)	16	13	0.0577	nd	nd	-	nd	nd	-
Neoxanthin (µg/g)	75	56	0.0215 *	nd	nd	-	nd	nd	-
Lutein (µg/g)	380	274	0.0105 *	nd	nd	-	nd	nd	-
β-Carotene (µg/g)	166	135	0.0110 *	nd	nd	-	nd	nd	-
Total carotenoids (µg/g)	637	477	0.0111 *	nd	nd	-	nd	nd	-

Micronutrients (Fe, Zn, Mn, Cu)

Moderately-reduced water supply led to a significant reduction of Fe in cucumber dry matter in week 29 ($p = 0.0479$), also being significant when considering the total dataset ($p = 0.0350$), but not in week 28 ($p = 0.2778$, Table 2). Multivariate analysis supports the contribution of Fe to the groups' differentiation within the PCA plots (Figure 3). Similar results were found for the fresh matter-based evaluation by both, univariate and multivariate analysis (Table 3, Figure 4). However, in our greenhouse experiment the

effect of water reduction on Fe was found to be not significant in all fruit compartments (Table 4). Thus, our findings on Fe are quite inconsistent and further study is warranted. Differences in dry matter-related zinc (Zn) and copper (Cu) levels were not significant when comparing well-watered samples to those grown under moderately reduced water supply (Table 2). Fresh matter-related evaluation yielded similar results (Table 3). In the greenhouse experiment with the more pronounced water reduction by 40%, a significant decrease of Zn and Cu was observed upon water supply limitation, except for Cu in the mesocarp of cucumber harvested in week 29, where the observed decrease was not sufficient to reach statistical significance (Table 4). Nevertheless, in agreement with our findings, low soil moisture has been reported to induce deficiencies in micronutrients such as Zn [40]. A general trend was noted when considering fresh matter-based data. Concentrations of all investigated micronutrients (Fe, Zn, Mn, Cu) in fruits were higher in week 28 than in week 29, paralleling the development of the cucumbers dry matter content (Table 3).

Phenolic Compounds

No effect of moderately reduced water supply on the content of total phenols was found in cucumber fruits, neither in dry nor in fresh matter. While the contents of total phenols were similar between the harvesting periods based on dry matter, the cucumbers harvested in week 28 had higher total phenolic contents (17.54–17.65 mg/100 g FM) than those from week 29 (14.11–14.33 mg/100 g FM), again paralleling the development of cucumber dry matter, which was higher in week 28 than in week 29. In our greenhouse experiment with a higher reduction of the water supply, significantly lower contents of total phenolics were found in the skin (exocarp), while significantly higher contents were found in the flesh (mesocarp) of pickling cucumber grown under reduced water supply (Table 4).

Although cucumber is not a nutrient-dense food [41], it is worth mentioning that cucumber fruits are an abundant source of phenolic compounds, comprising at least 73 phenolic compounds as analyzed by HPLC-ESI-Q-TOF-MS [42]. The potential contribution of cucumbers and the phenolics therein to human health merit further investigation.

Carotenoids

The contents of individual and total carotenoids in the exocarp of the greenhouse grown cucumber fruits (Table 3) were significantly lower when grown under RWS than under CTR conditions. In particular, the content of lutein, which was shown to be the most abundant carotenoid in the peel of pickling cucumber (274–380 $\mu\text{g/g DM}$, Table 4), decreased significantly upon water reduction of 40% ($p = 0.0105$). Similar findings were already reported by Ripoll et al. [43] for tomato fruits, whose carotenoids were negatively influenced by moderate water reduction by 60%, particularly if the water deficit occurred during maturity, even though the effect size was highly dependent on the genotype. Lutein represents a nutritionally-relevant constituent of cucumber peels, because it has been shown to accumulate in the retina and brain of humans and may help preventing or at least delaying the onset of chronic diseases, such as age-related macular degeneration [44,45].

Conclusions

Reduced water supply, as predicted by future climate scenarios, was shown to influence the concentrations of nutritionally-relevant constituents of pickling cucumbers grown in open field and greenhouse cultivation. The impact was strongly dependent on the respective type of nutrient. Regarding the majority of our target analytes, a decrease in the fresh matter-related concentrations was observed upon inducing slight water deficits. Exclusively, the content of malic acid, calcium, and magnesium seemed to increase by trend, whereas the fresh matter-related contents of polyols, phosphate, phosphorous, nitrogen, iron, and zinc were significantly lower in fruits grown under moderate water reduction when considering the overall dataset. Generally, the changes and trends we observed were more pronounced when increasing the severity of the applied water deficit, i.e., from 10–15% of the CTR treatment in our open-field trial to 40% of the CTR in the greenhouse experiment. For example, moderate water reduction in the open field did not alter the dry matter-based contents of sugars and polyols, while fruits grown under higher water reduction in our greenhouse experiment revealed increased levels of polyols in the exocarp and mesocarp as well as increased levels of glucose and fructose in the endocarp. Furthermore, our results indicated that differences between CTR and RWS treatments became more noticeable with the progressing

harvest period. In brief, the overall results indicate that the chemical composition of pickling cucumber was significantly influenced by mild to moderate reductions in water supply. Despite experimental differences making the direct comparison of our greenhouse and open field trials intricate, compositional changes were generally stronger in fruits grown under controlled greenhouse conditions at a water supply moderately reduced by 40% than in fruits grown under open field conditions at a water supply mildly reduced by 10–15%. Given the water scarcity, which is likely to become more frequent in the future, the overall quality of the cucumber crop could suffer if the right agricultural measures are not taken to deal with water shortages. However, the overall quality of vegetables is influenced not only by water deficit, but also by various abiotic and biotic factors, particularly when grown in open fields. Thus, cultivation methods and harvest times will most likely need to be adapted to future conditions to keep quality characteristics. Moreover, breeding of resistant varieties and the optimization of storage conditions might become increasingly important. Being out of scope of this work, further study may be needed to evaluate the effects of mild and moderate water reduction on the crop yield and the shelf life of pickling cucumber in detail.

Supplementary Materials

The following are available online at www.mdpi.com/xxx/s1, Figure S1: Daily mean weather conditions during the cultivation period of pickling cucumber in open field cultivation in 2016: (A) Mean air temperature (°C), (B) global radiation sum (W/m²), (C) daily mean relative humidity (%), and (D) daily mean evapotranspiration (mm); DAT = days after transplanting.

Author Contributions

Conceptualization: H.D. and J.Z.; methodology: C.S. and H.D.; software: M.F. and C.S.; formal analysis: C.S. and M.F.; investigation: C.S.; resources: H.D., R.S., and J.Z.; data curation: C.S.; writing—original draft preparation: C.S.; writing—review and editing: C.S., J.Z., H.D., M.F., and R.S.; visualization: C.S.; supervision: R.S., H.D., M.F., and J.Z.; project administration: H.D. and J.Z.; funding acquisition: H.D. and J.Z. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

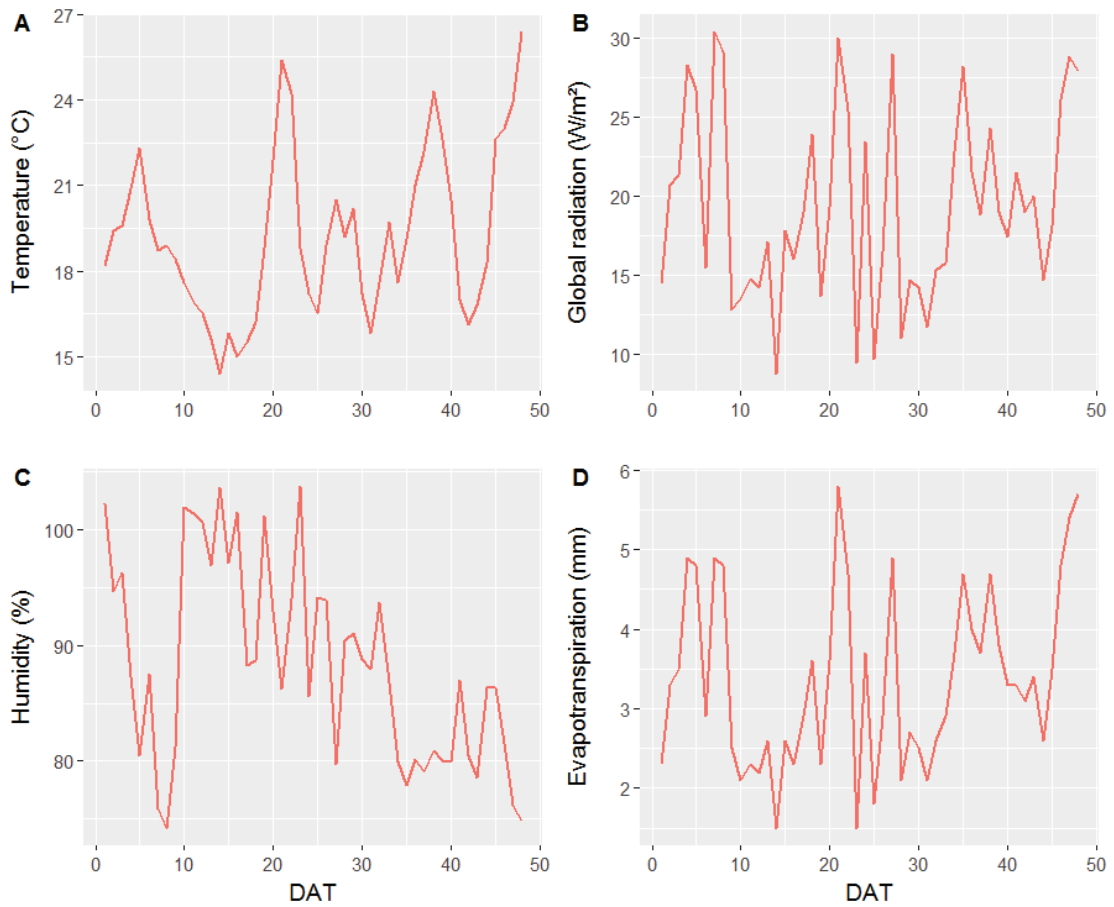
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Supplementary Materials



Figures S1: Daily mean weather conditions during the cultivation period of pickling cucumber in open field cultivation in 2016: (A) Mean air temperature (°C), (B) Global radiation sum (W/m²), (C) Daily mean relative humidity (%) and (D) Daily mean evapotranspiration (mm); DAT = Days After Transplanting.

General Discussion

Drought stress is considered one of the most common and dramatic environmental stress factors in many cultivation areas, and its impact is expected to even increase due to the on-going climate change (Toscano et al., 2019). The resulting effects of a specific drought event on the chemical composition of field-grown vegetables are intricate to assess, because they are highly plant and tissue dependent, and drought can occur in a number of ways. The extent, to which a water deficit situation may lead to physiologically and physio-chemically measurable alterations, depends on the plants' phenological stage, the intensity and duration of the stress event and the interacting effects with other environmental factors such as temperature, irradiation and air humidity. Therefore, the prediction of the plants' physiological reaction to moderate or even mild water reduction, which is indeed able to change the chemical composition of their harvest organs, is considerably more difficult than predicting and quantitating the effects of severe drought events. This work intended to approach this challenging research topic by studying three different vegetable crops with different consumable plant organs to be harvested (root, leaf, fruit) as grown under moderately reduced water supply in open field cultivation. Spinach, radish and cucumber were used as representatives to assess the potential effects of moderately or mildly reduced water supply on the nutritional quality of leaf-, root- and fruit-based vegetables.

The results of our triennial field experiment presented within this work have shown that sensitivity to mild water reduction substantially differs between the vegetable crops, based on analyses of the respective plant organs harvested for consumption. Moderate or even mild water reduction was shown to have the potential to decrease the concentration of dry matter-based malic acid and phosphate in spinach and radish, while malic acid increased in cucumbers due to moderate water reduction. However, cucumbers grown under deficient water supply were characterized by a concomitantly decrease in phosphate, N, P and Fe. Moreover, moderate water reduction led to higher levels of dry matter and polyols in spinach and radish, but not in cucumber. While levels of inositol, Zn and Mn were increased in spinach due to water reduction, radish tubers showed higher contents of polyols and Cu. At the same time, levels of P, Mn, total phenols and glucosinolates decreased. The results from both varieties may suggest that polyol metabolism is more susceptible in radish tubers than in spinach leaves. However,

because the extent of water reduction was lower in the spinach cultivation sets due to higher natural precipitation, such statement cannot be drawn from the results.

It should be noted that moderate water reduction has increased certain trace elements such as Zn and Mn in spinach, while it decreased the levels of Mn in radish based on dry matter evaluation. In addition, radish tubers revealed lower concentrations of P, total phenols and glucosinolates. Even though glucosinolates have been shown to increase due to drought stress in other *Brassica* species (Schreiner et al., 2009; Zhang et al., 2008; Jensen et al., 1996), it was also shown that less severe drought situations do not induce significant changes in the glucosinolate concentration (Jensen et al., 1996) or even reduce their concentration (Robbins et al., 2005), which is in full agreement with the results presented herein for radish grown under moderately reduced water supply. In contrast to spinach and radish, cucumber fruits did not show higher levels of polyols if plants had been grown under water restriction, despite water restrictions being more severe than in spinach cultivation sets. On the contrary, it became apparent from the open field trial that cucumbers grown under reduced water supply had lower concentrations of phosphate, N, P and Fe based on dry matter analyses. If cucumber had been grown under more severe water reduction of -40% in contrast to well-watered plots in the greenhouse, the analytical results of their fruit compartments revealed much more pronounced effects. For example, in the greenhouse trial, malic acid was significantly increased in each of the cucumbers' compartments, while C, N and Zn were significantly decreased. On the contrary, whole fruits from the open field trial showed merely slight or no decreases of such analytes based on dry matter. Furthermore, greenhouse-grown cucumber exhibited lower concentrations of total phenols and carotenoids in the exocarp, while glucose, fructose, citric acid, K, Ca and Mn were higher in the endocarp in deficient watered plants. Nevertheless, some findings were unexpectedly contrary to those from the open field experiment. For example, phosphorous, which was decreased in cucumber under moderate water reduction in the open field trial, increased in the exocarp and mesocarp of deficiently watered greenhouse-grown cucumber. Furthermore, total polyols increased due to water restriction in the cucumbers' exocarp and mesocarp, while the endocarp showed increased levels of glucose and fructose due to reduced water supply (cf. chapter 3). Ultimately, the results show how differently the effect of reduced water supply affects the individual compartments, not just the different plant organs.

In contrast, the effect of moderate or mild water reduction is different when evaluating the results based on fresh matter, which represents the nutritional composition of the harvest product. Especially fresh spinach from water-deficient cultivation sets revealed significantly higher concentrations of polyols, ascorbic acid, C, K, N, P, Zn and Mn as well as higher levels of flavonoids and carotenoids (cf. chapter 2). Malic acid was the only compound, which was still decreased in fresh matter from reduced watered spinach. Of course, spinach yield was reduced to some extent with losses of -14.7% in 2016, but was increased by +4.2% in 2017, based on random samples of 40 plants per plot. An increase of certain product quality-relevant compounds was also observed in radish, when evaluating fresh matter-based results. Radish tubers from less watered plots were characterized by higher levels of glucose, polyols, ascorbic acid, C, K, Zn and Cu, while none of the investigated parameters were significantly decreased (cf. chapter 1). For sure, total radish yield was partly reduced by moderate water reduction by -8.7% (2016-1) and -3.2% (2017), but was increased by +5.1% in 2016-2 based on random samples of 110 plants per plot. Nevertheless, apart from moderate losses in yield quantities, moderate or even mild water reduction seem to be able to enhance the concentration of some quality-related compounds in field-grown spinach and radish. In contrast, cucumber fruits from deficient water supply treatments revealed mainly significantly decreased levels of certain compounds such as polyols, phosphate, N, P, Fe and Zn, while malic acid remained increased. These findings might suggest, that sink-strength of the fruits was decreased under moderate water reduction in cucumber. On the other hand, differences in the main carbohydrates were insignificant, contradicting the assumption of sink limitation.

Despite generally negative effects of reduced water supply on plant physiology as well as on fresh biomass production, it was shown that drought stress can also allow enhancing the quality of selected vegetables crops, as already summarized by Toscano et al. (2019). Many of the aforementioned studies this work referred to had shown that the chemical composition of vegetables can be readily manipulated by moderate drought stress, without reducing total dry matter accumulation such as shown for tomatoes (Mitchell et al., 1991). Boyer (1970) have shown earlier that stress, which is mild enough to not affect photosynthesis, can reduce the development of the leaf surface area of, e.g., sunflower (*Helianthus annuus* L.) and thus, reduces transpiration.

With focus on the vegetables investigated in this work, a slight reduction of the total water amount during the growth period of spinach, radish and cucumber did generally not evoke a significant enhancement of desired compounds such as ascorbic acid, polyphenols or glucosinolates based on dry matter. However, low water scarcity was shown to increase selected health-promoting constituents in the fresh matter of radish and spinach, while cucumber did not benefit at all. For example, radish tubers from less watered plots showed higher levels of dry matter, sugars, ascorbic acid as well as selected minerals and trace elements (chapter 1). Similarly, fresh spinach was also characterized by increased levels of dry matter, ascorbic acid, minerals and trace elements, flavonoids and carotenoids (chapter 2). In contrast, fresh cucumber fruit did not show any increase based on fresh matter, with exception of malic acid, which increased significantly due to water shortage (cf. chapter 3). In brief, mild water reduction did not lead automatically to higher accumulation or synthesis of certain quality-related compounds in vegetables, but the results herein showed that quality improvement due to higher contents of minerals, polyphenols or carotenoids in the vegetables fresh matter was possible, probably because of higher contents of dry matter in the harvest products. Nevertheless, such increases of selected chemical components will always be dependent on multiple factors beyond waters supply, including environmental factors such as temperature, radiation, nutrient availability as well as plant-specific conditions such as variety, harvest organ and genetic preconditions. From a human nutrition perspective, the application of reduced water supply may represent the possibility to enhance the concentration of certain health-promoting bioactive compounds such as carotenoids, flavonoids and glucosinolates in certain vegetable crops (Toscano et al., 2019; Schreiner et al., 2009; Koyama et al., 2012; Ripoll et al., 2016). However, the impact of the cultivation period, harvest date and year on the chemical composition of the investigated vegetables seemed to be much clearer in contrast to those of mild water reduction. For instance, the nutritional and visual quality of spinach leaves was shown to be better when harvesting a few days earlier than what is commercial practice, because flavonoids and ascorbic acid levels were higher (Bergquist et al., 2005; Bergquist et al., 2006). Moreover, leafy vegetables such as lettuce reacted to deficient water supply by activating the biosynthesis of specific bioactive substances like ascorbic acid and polyphenols (Koyama et al., 2012). Unfortunately, if water supply is reduced during spinach cultivation with the aim of

enhancing specific antioxidants, it may most likely be at the expense of production yield (Esteban et al., 2014).

However, the reactions associated with drought stress differ greatly from plant species and other environmental conditions. Considering literature and the results of this work, it becomes clear that the interaction between plants and their environment can profoundly affect the nutritional quality of their harvest products (Proietti et al., 2009). Against this background, the findings for spinach and radish figured out a strong effect of the cultivation season and/or year, whose environmental conditions significantly influenced the product quality. Spinach and radish samples from the different cultivation sets were strongly differentiated within the PCA analyses based on their chemical composition (cf. chapters 1 and 2), which was led back to strong differences in global radiation and accompanied changes in evapotranspiration. It was clearly shown that the chemical composition of spinach and radish was strongly dependent on the season with all its individual climatic conditions involved. These findings highlighted once again the impact of year and/or season on the chemical composition of the product. Similar year-to-year variations have been reported earlier for leafy (Mercadante & Rodriguez-Amaya, 1991) and selected cruciferous vegetables (Schreiner, 2005). In addition, Ripoll et al. (2016) proved that the chemical response of tomato fruits to drought was based on a specific developmental window. It was shown that the plants' reaction to drought was different depending on the developmental stage, at which the stress occurred. Moreover, the effect of drought stress was shown to be highly dependent on genotype and cultivation year, as shown for polyphenol profiled in potato tubers (Andre et al., 2009). Nevertheless, drought stress exposure often affects biomass production and thus, affects the pursued yield in a negative way. There is a clear trade-off for the cultivation of vegetable crops aiming to enhance such quality-related plant compounds by drought stress, meaning a reduced harvest quantity. Finally, the respective harvest organ and the environmental conditions of the cultivation period determines to what extent an imposed stress affects its nutritional composition.

Focusing on future drought prediction, climate change does not merely impact precipitation patterns by promoting more frequent drought or water deficit-related situations in certain cultivation areas, but also warming will be more noticeable, especially during autumn and winter season, when radiation is degraded (Räisänen et al., 2004). This will automatically influence the chemical composition of those

vegetables grown during winter time in Central Europe, such for example in kale, which was shown to alter the proportions of important flavonols such as quercetin, kaempferol and isorhamnetin in response to decreasing temperature and radiation intensity (Schmidt et al., 2010). If drought stress occurs simultaneously with other abiotic factors such as high light intensity or heat, photoinhibition and concomitantly decreasing mesophyll photosynthesis might happen (Chaves, 1991). Finally, the impact a given type of stress has on plant performance is, according to Chaves (1991), the result of the interaction between the plant as a controlled system according to its genetic information, and the type of stress, imposing limitations of variable intensity depending on its severity and duration. For example, Tevini et al. (1983) already stated that cucumber cotyledons were more sensitive to UV-B radiation than those of radish, which was explained by lower levels of flavonoids in cucumber compared to radish, whose cotyledons exhibited 3- to 4-fold increased flavonoid levels upon UV-B radiation. In contrast, protein content was enhanced in water stressed cucumber cotyledons grown under UV-B radiation in contrast to well-watered plants, why cucumber was believed to be one of the most UV-B sensitive crop species (Tevini et al., 1983). With regard to cucumber quality, fruit firmness is largely determined by genotype and is known to play an important role in assessing the quality of pickling cucumber (Suojala-Ahlfors, 2005). Against this background, such parameters should be taken into account when investigating the cultivation conditions of gherkins under reduced water supply conditions in the future.

Finally, the results from this work and literature lead to the conclusion that making a general statement regarding the effects of drought or even moderate water reduction on the chemical composition of selected vegetables is difficult, if not impossible. Different plant species react in different ways and the outcomes depend on the harvest organ to be analyzed. Furthermore, other environmental conditions during plant growth impose further difficulties. In addition, the findings from different publications are often presented either on dry or on fresh matter basis, which makes it difficult to compare the results. Fresh matter-based results are suitable to value the nutritional composition of the harvested produce, but they are not suitable for drawing a conclusion of water reduction on the plants' physiologically aspects. This fact often aggravates to conclude the results of a multitude of findings and to discuss them in the context of our own

findings obtained out of this work. Therefore, reporting both fresh- and dry matter-related results should be encouraged.

Since climate change is mainly driven by increasing greenhouse gas emissions, it is also important to consider the impact of higher atmospheric CO₂ concentrations when evaluating the quality of field-grown vegetables under future conditions. It was shown, for example, that elevated CO₂ suppresses the nutritional quality of leafy vegetables such as lettuce and spinach. The accumulation of both minerals and trace elements was significantly lower in plants grown under elevated CO₂ in growth chambers (Giri et al., 2016; Jain et al., 2007). Against this background, Dong et al. (2018) also found decreased concentrations of dietary fiber, crude protein and amino acids in cucumber fruits grown under elevated CO₂. It was shown that low N availability accompanied by elevated CO₂ concentrations led to increased levels of P, K, Ca and Mg, while microelements Fe and Zn decreased. Adding the potential effects of reduced water supply, the chemical composition, and thus, the quality of future grown vegetables will most likely change noticeably under future climate conditions and the extent is still difficult to foresee. However, methods and solutions are being sought to address the cultivation of vegetable crops even under such altered conditions including drought stress situations. For example, Xu & Leskovar (2015) presented that the application of seaweed extract (*Ascophyllum nodosum*) on drought-stressed spinach plants improved growth, physiology and nutritional values. It was shown that both relative water content as well as leaf area were increased under drought stress, if plants were treated with the seaweed extract. These findings give hope as they suggested that seaweed extract could help maintaining cell turgor pressure and expansion (Xu & Leskovar, 2015), which was already shown to improve drought tolerance in orange trees (Spann & Little, 2011).

However, the effects of prospective water scarcity events on vegetable crops are multifaceted regarding quality-related parameters and economic yield, especially under future projection of the on-going climate change. They further depend on variety and genetic preconditions, individual water demand, environmental conditions as well as on water availability, and economic value. Nowadays, vegetables play an important role in a balanced diet and consumers are oriented to low-energy foods with high levels of bioactive and health-promoting compounds, whose concentration can be modulated by their imposition to abiotic stresses, as summarized, for example, by Toscano et al. (2019). Although crop biomass production is linear to crop transpiration, or water use,

the relationship between the economic yield of, e.g., fruits or tubers and water use is far more complex, whereas economic yield is not equated with total biomass (Blum, 2011). There is no doubt that vegetable crops are generally sensitive to environmental stresses, why limited soil moisture and high temperatures represent the major causes of low yield as well as several often unfavorable changes in the physiological and biochemical processes. In addition, climate change has an impact on the occurrence of biotic stress factors such as insects and pathogens. For example, pests and their associated pathogens are expected to move into new regions, as the geographical distribution of pests shift due to climate change, exposing different crop varieties to new viruses and phytopathogens being transferred by insect pests (Andrew & Hill, 2017; Coakley et al., 1999). While insects are being affected mainly by temperature, plant pathogens are highly responsive to humidity and rainfall, along with temperature (Hatfield et al., 2011; Coakley et al., 1999).

These downsides will be further aggravated by climate change (Ayyogari et al., 2014). As a result, the production of vegetable crops will be negatively affected to a large extent by climate change in many regions worldwide due to higher temperatures and accelerating crop development, reduced water availability, increasing salinity, higher CO₂-concentration or by changes in relative humidity, air pollutants and UV-radiation (Ayyogari et al., 2014). According to Pereira (2016), the complexity of a plants' response to abiotic stress factors provides a multitude of morpho-physiological, biochemical, gene expression, and other molecular responses. It is indisputable that abiotic stress will remain a challenge to horticulture, agriculture and the natural environment (Pereira, 2016). In the future, further experiments should focus on developments of specific adaption strategies for drought-sensitive crops such as vegetables being cultivated in natural environments.

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Summary

In this doctoral thesis, the effects of moderately reduced water supply and seasonal variations was studied regarding the chemical and nutritionally relevant composition of the edible harvest organs of three different vegetable crops, i.e. cucumber fruit (*Cucumis sativus* L.), radish roots (*Raphanus sativus* L. var. *sativus*), and spinach leaves (*Spinacia oleracea* L.). The study was conducted to demonstrate putative plant-physiological alterations in their chemical composition that may be evoked by moderate water deficits during cultivation under open field conditions. The vegetable crops were selected according to differing harvest organs (leaves, roots and fruit), being cultivated during three consecutive years under open field conditions and two different water supply treatments within a randomized block design with multiple sets of six plots each. Depending on the vegetable, the provided water amount of the moderately reduced water supply treatment varied between 80–97% of the well-watered control treatment. Chapter 1 dealt with the effects of moderate water reduction of 3–20% on radish. Including one cultivation set per year and an additional set in 2016, i.e. a total of four separate sets, a dry matter-based increase of *myo*-inositol was observed, whereas the levels of selected minerals and trace elements like iron and manganese as well as those of phenolic compounds and glucosinolates decreased or remained unchanged upon moderately reduced water supply. Only insignificant effects of reduced water supply were seen on the levels of acylated anthocyanins, which represent a highly important commercial quality trait due to their importance for the roots' or hypocotyls' color. In contrast, the levels of fresh matter-related analytes were mainly enhanced by reduced water supply due to higher contents of dry matter. Thus, the overall quality of radish tubers seemed to be only marginally affected by moderate water limitation.

Similar results were observed for spinach as presented in Chapter 2. Nevertheless, reducing the total water amount by only 4–10% during cultivation led to various significant alterations in the chemical composition of the leaves. Similar to radish, higher dry matter-based levels of *myo*-inositol were found in spinach grown under low water reduction. Moreover, increased contents of zinc and manganese were found, while the levels of malic acid, fumaric acid, phosphate and chloride decreased upon slight water reduction in spinach dry matter. In contrast, spinach fresh matter was expectedly characterized by significantly higher contents of polyols, ascorbic acid, potassium, nitrogen, phosphorous, zinc and manganese, as well as total flavonoids and

carotenoids, including nutritionally highly relevant compounds like the provitamin A-active β -carotene and the macular pigment lutein. Noteworthy, strong seasonal variations have been observed for numerous minor phytochemicals, such as for example for a series of specific flavonoids, whose contents seemed to be dependent on the respective irradiation conditions of the cultivation set.

In contrast to radish and spinach, cucumber from open field cultivation reacted differently to slightly reduced water supply, as summarized in Chapter 3. When cucumber fruits obtained from plants received 85–90% of the total water amount of the control treatment, they exhibited significantly higher levels of malic acid, calcium, and magnesium, while those of phosphate, phosphorous, nitrogen, and iron decreased based on dry matter. Fruit dry matter-content did not significantly change due to moderate water restriction, and, as a result, the concentration of most analytes tended to decrease in the cucumber fresh matter when grown under reduced water supply. The chemical analyses of single cucumber fruit compartments (exocarp, mesocarp and endocarp) from fruits grown under 60% of the full supply in a separate greenhouse experiment confirmed that malic acid, certain minerals and trace elements, as well as carotenoids like the most abundant lutein were relatively sensitive to moderate water reduction.

Altogether, a moderately reduced water supply was shown to significantly alter the chemical composition of the aforementioned vegetable crops. Considering the broad range of analytes from, e.g., minerals and anions to polyols and acids to micronutrients like provitamin A, it became clear that water supply reduction as studied herein did not lead to generalizable effects such as an undesired decrease or a desired enhancement of valuable compounds. For instance, the levels of selected health-promoting constituents in the fresh matter of radish and spinach were increased, while those of the same compounds contained in cucumber did not benefit at all. Generally, the overall quality of vegetables was clearly shown to be influenced not only by water supply, but also by the interaction with other abiotic factors, especially when cultivation took place in open field. Further study in the future should target interactions of moderately reduced water supply situations with changes in other abiotic factors, such as elevated CO₂ concentrations.

Zusammenfassung

Die vorliegende Dissertation befasst sich mit den Auswirkungen einer mäßig reduzierten Wasserversorgung sowie dem Einfluss saisonaler Klimabedingungen auf die chemische und ernährungsphysiologisch relevante Inhaltsstoffzusammensetzung von Ernteorganen unterschiedlicher Gemüsekulturen wie Spinat-Blätter (*Spinacia oleracea* L.), Radieschen-Wurzeln (*Raphanus sativus* L. var. *sativus*) und Gurken-Früchte (*Cucumis sativus* L.). Ziel der Studie war es, pflanzenphysiologisch bedingte Veränderungen in der Inhaltsstoffzusammensetzung der Ernteorgane aufzuzeigen, die durch ein leicht oder mäßig reduziertes Wasserangebot während der Kultivierung im Freiland hervorgerufen werden. Die untersuchten Gemüsekulturen wurden im Hinblick auf ihre Ernteorgane (Blatt, Wurzel und Frucht) ausgewählt und in drei aufeinanderfolgenden Jahren mit jeweils zwei unterschiedlichen Bewässerungsstufen innerhalb einer randomisierten Blockanlage im Freiland angebaut. Dabei wurden mehrere Anbausätze mit jeweils sechs Wiederholungen durchgeführt und die Ernteorgane auf ihre chemische Zusammensetzung hin analysiert. Je nach Gemüsekultur lag die Gesamtwassermenge der moderat reduzierten Variante zwischen 80–97% der der Kontrollvariante. In Kapitel 1 werden die Auswirkungen einer moderaten Wasserreduktion von 3–20% auf die Inhaltsstoffe von Radieschen dargestellt. Auf der Grundlage von insgesamt vier separaten Anbausätzen, einem Anbausatz pro Jahr sowie einem zusätzlichen Anbausatz in 2016, konnte ein signifikanter Anstieg von *myo*-Inositol in Radieschen beobachtet werden, wohingegen die Gehalte ausgewählter Mineralstoffe und Spurenelemente wie Eisen und Mangan, sowie von phenolischen Verbindungen und Glucosinolaten, durch ein moderat reduziertes Wasserangebot abnahmen oder unverändert blieben. Zudem wurden nur geringe Effekte einer moderaten Reduktion der Gesamtwassermenge auf die Gehalte acylierter Anthocyane festgestellt, die aufgrund ihrer Bedeutung für die Farbe der Radieschen-Wurzel, bzw. des Hypokotyls, ein wichtiges kommerzielles Qualitätsmerkmal darstellen. Im Gegensatz dazu zeigten sich die Gehalte einer Vielzahl von Analyten auf Basis der Frischmasse erhöht, was hauptsächlich auf einen höheren Trockenmassegehalt in den reduziert bewässerten Varianten zurückzuführen ist. Dementsprechend scheint die Gesamtqualität von Radieschen durch eine derart moderate Wasserlimitierung nur geringfügig beeinträchtigt zu werden.

Ähnliche Ergebnisse wurden in Bezug auf Spinat beobachtet, wie in Kapitel 2 dargestellt ist. Eine Reduzierung der Gesamtwassermenge um lediglich 4–10% führte zu signifikanten Veränderungen in der chemischen Zusammensetzung der Blätter. Vergleichbar mit den Ergebnissen zu Radieschen wurden auch in reduziert bewässertem Spinat signifikant höhere *myo*-Inositol-Gehalte nachgewiesen. Die Gehalte an Zink und Mangan waren ebenfalls bei geringen Wasserdefiziten erhöht, während die Gehalte an Äpfelsäure, Fumarsäure, Phosphat und Chlorid in Spinat bezogen auf die Trockenmasse abnahmen. Dagegen zeigte sich in der Frischmasse von reduziert bewässerten Spinatblättern erwartungsgemäß erhöhte Gehalte an Polyolen, Ascorbinsäure, Kalium, Stickstoff, Phosphor, Zink und Mangan sowie erhöhte Gehalte an Flavonoiden und Carotinoiden, darunter auch ernährungsphysiologisch hoch relevante Verbindungen wie Provitamin A-aktives β -Carotin und das Makulapigment Lutein. Nennenswert sind die starken saisonalen Schwankungen zahlreicher sekundärer Pflanzeninhaltsstoffe wie z. B. spezifischer Flavonoide, deren Gehalte von den jeweiligen Strahlungsbedingungen der Anbauzeit abhängig zu sein scheinen.

Im Gegensatz zu Radieschen und Spinat reagierten Gurken im Freilandanbau deutlich unterschiedlich auf eine leicht reduzierte Wasserversorgung, wie in Kapitel 3 beschrieben ist. Früchte von Pflanzen, die 85–90% der Gesamtwassermenge der Kontrollbehandlung erhielten, wiesen im Gegensatz zur Kontrolle signifikant höhere Gehalte an Äpfelsäure, Calcium und Magnesium auf, während die Gehalte an Phosphat, Phosphor, Stickstoff und Eisen auf Basis der Trockenmasse reduziert waren. Der Anteil der Trockenmasse in Gurkenfrüchten hat sich durch eine moderate Reduzierung der Gesamtwassermenge nicht verändert, weshalb die meisten Analyten in der Frischmasse der Früchte von reduziert bewässerten Pflanzen tendenziell abnahmen. In einem separaten Gewächshausversuch, in welchem die Gesamtwassermenge der defizitär bewässerten Gurkenpflanzen um 40% gegenüber der Kontrollvariante verringert wurde, konnte an Hand von analytischen Daten zu den einzelnen Frucht-Kompartimenten (Exokarp, Mesokarp und Endokarp) gezeigt werden, dass Äpfelsäure, ausgewählte Mineralstoffe und Spurenelemente sowie Carotinoide, wie z. B. das am häufigsten vorkommende Lutein, relativ empfindlich auf eine moderate Wasserreduktion reagierten.

Insgesamt konnte gezeigt werden, dass eine mäßig reduzierte Wasserversorgung die chemische Zusammensetzung der genannten Gemüsekulturen deutlich verändert. In

Anbetracht des breiten Spektrums an Analyten, von u. a. Mineralstoffen und Anionen über Polyole und Säuren bis hin zu Mikronährstoffen wie Provitamin A, wurde deutlich, dass eine mäßige Reduktion in der Gesamtwassermenge nicht zu generellen Effekten wie z. B. einer unerwünschten Abnahme oder einer erwünschten Zunahme von wertgebenden Inhaltsstoffen führt. So wurden beispielsweise die Gehalte ausgewählter gesundheitsfördernder Inhaltsstoffe in der Frischmasse von Radieschen und Spinat durch eine moderate Wasserreduktion erhöht, während die Gehalte der gleichen Verbindungen in Gurken nicht zunahm. Grundsätzlich zeigte sich, dass die Gesamtqualität von Gemüse nicht nur durch die angebotene Gesamtwassermenge, sondern auch durch dessen Interaktion mit weiteren abiotischen Faktoren beeinflusst wird, was insbesondere im Freilandanbau eine große Rolle spielt. Zukünftige Untersuchungen sollten daher auf die Wechselwirkung einer moderat reduzierten Wasserversorgung mit den Veränderungen anderer abiotischer Faktoren, wie z. B. einer erhöhten CO₂-Konzentration, abzielen.

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Statutory Declaration

I declare that I have prepared the submitted thesis independently and without unauthorized third-party help and that no other than the in the thesis listed facilities have been used. All text passages that are quoted literally or analogously from other published papers and all information that are based on verbal statements are identified as such.

I have observed the principles of good scientific practice as defined in the statutes of the Hochschule Geisenheim University and the Justus Liebig University Giessen for safeguarding good scientific practice when carrying out the analyses of my research mentioned in the thesis.

Geisenheim, March 2021

Christine Schlering