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Accumulation of carbohydrates and pungent principles in characteristic seed and set grown onion varieties (*Allium cepa* L.)

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Summary

The profile of carbohydrates of onions (*Allium cepa* L.), mainly consisting of fructooligosaccharides (FOS), has a strong impact on digestibility, processability and storability. This study focused on the accumulation of FOS and pungent principles in onions during bulb maturation. Different onion varieties were grown from both, seeds and sets. Total FOS concentrations in onions of cv. 'Sturon' were higher when grown from sets than from seeds throughout the entire maturation period, reaching final levels of 75.7 ± 2.2 and 61.8 ± 11.8 g/L FOS, respectively. Higher levels in set grown onions might be due to their earlier emergence, thus resulting in an extended photosynthetically active period (+12% total sunshine hours). However, seed grown, so-called dehydrator onions (cv. 'Stardust') had significantly higher FOS contents than set grown cv. 'Sturon' onions at all sampling points (final FOS level: 129.3 ± 16.6 g/L), indicating cultivar-dependant accumulation. Furthermore, dehydrator onions accumulated FOS with highest molecular weight and a unique FOS distribution, allowing clear discrimination of such dehydrator cultivars. Besides carbohydrates, pungency as indicated by pyruvic acid levels was shown to be determined by sulphurous fertilization and its timing.

Keywords: Onion, *Allium cepa* L., set onion, seed onion, cultivation method, FOS accumulation, fructooligosaccharides, FOS, alk(en)yl cysteine sulfoxides, ACSO, irritable bowel syndrome

Introduction

Soluble carbohydrates account for approx. 80% of onion (*Allium cepa* L.) dry matter. Carbohydrate profiles are astonishingly variable among different cultivars. While in some cultivars D-fructose and D-glucose prevail, others are characterized by predominant levels of fructooligosaccharides (FOS) (Vågen and Slimestad, 2008; Pöhnl et al., 2018). The latter are enabling commercial long-term storability of onions bulbs, during which their metabolism fuels the respiratory activity of the bulbs (Jaime et al., 2001; Petropoulos et al., 2017). This metabolic degradation may be slowed down by chilled storage and atmospheres with reduced oxygen (Ernst et al., 2003; Benkeblia and Shiomi, 2006). Sprouting and onion decay were shown to be delayed when bulbs were rich in FOS at the beginning of onion storage (Jaime et al., 2001). Furthermore, the ratio of non-reducing sugars including FOS and reducing sugars mainly comprising glucose and fructose in onions is known to be highly important regarding the production of dried products, because particularly fructose is highly reactive in the non-enzymatic browning (Maillard) reaction due to the predominance of its open-chained form in aqueous media (Dills, 1993; Lievonen et al., 2002). Cultivars high in FOS and low in fructose are, due to their lower Maillard reaction potential, favoured for the production of dehydrated onions as well as for the production of onion juices because of their lighter colour (Mitra et al., 2012; Symrise AG, 2016). Contrarily, cultivars low in both FOS and fructose might be favourable for consumption by patients suffering from the irritable bowel syndrome. The consumption of so-called fermentable oligo-, di and monosaccharides and polyols (FODMAPs), which includes fructose and FOS, has been shown earlier to aggravate the symptoms of irritable bowel syndrome (Gibson and Shepherd, 2005). Consequently, detailed knowledge about the biosynthesis and accumulation of carbohydrates, in particular of FOS, during onion bulb maturation is most relevant for consumers and the food industry.

During biosynthesis of FOS in onions catalysed by the enzyme sucrose:sucrose fructosyltransferase (EC 2.4.1.99), fructose is transferred from one donor sucrose molecule to a second acceptor sucrose molecule. Thus, two initial sucrose molecules are converted to 1-kestose, releasing one glucose molecule. 1-kestose represents a most important precursor for the synthesis of higher polymerized FOS, serving as a building block throughout all subsequent polymerization steps of FOS synthesis (Henry and Darbyshire, 1980; Shiomi et al., 1997). 1^F-fructosyltransferase (EC 2.4.1.100) catalyses the prolongation of oligomeric fructose molecules at the C1 position, under the stepwise consumption of one 1-kestose molecule by transferring one fructose molecule from the donor 1-kestose to an additional acceptor FOS molecule. Additionally, onions contain the enzyme 6^G-fructosyltransferase (EC 2.4.1.243), catalysing the prolongation of the (oligo-)fructosyl chain at the C6 position of the glucose molecule requiring one donor molecule of 1-kestose per prolonged fructosyl unit. Consequently, a wide range of linear FOS isomers with identical molecular weight has been found in onions (Shiomi et al., 2005; Pöhnl et al., 2017). The accumulation of FOS has been reported to be modulated by the expression of the three aforementioned enzymes, whose activities have been shown to be higher in FOS rich cultivars (Shiomi et al., 1997).

Recently, both, cultivar selection and cultivation method (growing from seed *vs*. transplantation of sets) have been shown to be highly relevant when aiming at increased FOS levels in the obtained bulbs. For instance, set grown onions were shown to yield significantly higher FOS concentrations than identical cultivars grown from seeds (Pöhnl et al., 2018). Similarly, Bufler et al. (2003) reported FOS levels in bulbs of onions grown from transplants initially raised in the greenhouse to be higher than those of seed grown onions (Bufler et al., 2003). Nevertheless, highlighting the importance of cultivar selection, the highest FOS concentrations were found in seed grown, so-called dehydrator type onions (Ernst et al., 1998; Pöhnl et al., 2017). While FOS concentrations have been investigated post-harvest, detailed knowledge about their accumulation during bulb development and maturation is lacking to date. Therefore, we sought to study the accumulation of FOS and further soluble carbohydrates in seed grown common onions, their set grown counterparts, and in dehydrator type onions. We hypothesise the observed discrepancy between FOS contents of seed and set grown onions of the same variety

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to be driven by specific environmental factors, i.e. by different sun-exposure and different growth-durations. Due to their importance for the pungency and, thus, the overall value of onions (Resemann et al., 2004), the accumulation of alk(en)yl cysteine sulfoxides (ACSOs) during bulb growth should also be studied. Likewise, we aimed at providing evidence whether ACSO accumulation is dependent on genetic or environmental factors, including the study of applied sulphur fertilizations. In brief, our study aimed at providing new insights into the accumulation of nutritionally and technologically relevant components of onions.

Materials and methods

Onion seeds and onion sets

Onion seeds and sets (cv. 'Sturon') were obtained from Mitteldeutsches Zwiebelkontor (Calbe, Germany) and onions seeds (cv. 'Stardust') were from Bejo (Warmenhuizen, Netherlands). All sets and seeds were planted on April 22, 2015, in fields of the Gardening School of the University of Hohenheim (Stuttgart-Plieningen, Germany; 48°42'51.8"N 9°11'14.3"E). All onions were grown under conditions, treatments and planting parameters identical to those described in our earlier study (Pöhnl et al., 2018). Briefly, weekly sampling was started when first signs of bulb development were observed (set grown: 06/25/2015; seed grown: 07/14/2015) and continued until leaves had fallen and regular harvest of onions. As a result, the study included a total of 6 sampling time points for set grown onions and 7 for seed grown onions. On each sampling date, samples of five maturing onion bulbs were collected from 3 parcels per variant, i.e. 15 bulbs per sampling date and variant. Due to the earlier soil emergence and induction of bulb development in set grown onions, sampling dates differed between the two variants as described in the results and discussion section. Fertilization was applied at two dates. First fertilization was carried out on April 21, 2015, before sowing and transplanting sets into soil, to yield a final concentration of 50 kg nitrogen ha^{-1} and 30 kg sulphur ha^{-1} applying calcium ammonium nitrate (27% [N_2], 6% [CaO], BayWa, Munich, Germany) and superphosphate (18% phosphate [P_2O_5]; 27.5% sulphur trioxide [SO_3]; 29% calcium monoxide [CaO], Donau Chemie, Vienna, Austria). A second fertilization was conducted when the induction of bulb development became visible, i.e. on July 7 for set grown onions and July 14 for seed grown onions. The concentrations applied were 80 kg ha^{-1} N (calcium ammonium nitrate; 27% nitrogen [N_2]; 6% CaO; 4% magnesium monoxide [MgO]) and 50 kg ha^{-1} S (kieserite; 50% SO_3; 25% MgO, K + S Kali, Kassel, Germany).

Dry matter and total soluble solids

Onion dry matter was determined gravimetrically by the weight loss of 5 onions from each parcel during freeze-drying, i.e., 15 bulbs per sampling date and variant. Total soluble solids were analogously determined using a RX-5000 digital refractometer (Atago, Tokyo, Japan) in freshly squeezed onion juice as described earlier (Pöhnl et al., 2018).

Quantitation of carbohydrates and pyruvic acid

Carbohydrates were analysed by high performance anion exchange chromatography coupled on-line to a pulsed amperometric detector (HPAEC-PAD) according to our previously published and validated method (Pöhnl et al., 2017). Briefly, authentic standards of D-glucose monohydrate, D-fructose and sucrose were purchased from Merck (Darmstadt, Germany). Authentic FOS standards with a degree of polymerization (DP) from 3 to 5, i.e. 1-kestose, 1,1-kestotetraose (nystose) and 1,1,1-kestopentaose [$(1)_3$-kestopentaose, syn. 1^F-fructosylnystose], were obtained from Wako Chemicals (Osaka, Japan). Higher oligomerized (DP 6–9) authentic FOS standards; 1,1,1,1-kestohexaose [$(1)_4$-kestohexaose, purity 90%], 1,1,1,1,1-kestoheptaose [$(1)_5$-kestoheptaose, purity 85.2%), 1,1,1,1,1,1-kestooctaose [$(1)_6$-kestooctaose, purity 80.0%), and 1,1,1,1,1,1,1-kestononaose [$(1)_7$-kestononaose, purity 76.9%) were provided by Carbosynth (Compton, United Kingdom). Pungency was determined by the 2,4-dinitrophenylhydrazine assay (Sigma Aldrich, St. Louis, MO, USA). As described by Pöhnl et al. (2018), the assay was based on the findings of Schwimmer and Weston (1961) with modifications by Anthon and Barrett (2003). For analyses of both, carbohydrates and pungency, freshly squeezed juices from onion bulbs were used as described earlier. Such juices have been found to be most suitable for the analysis of the aforementioned soluble carbohydrates and pungent principles (Anthon and Barrett, 2003; Pöhnl et al., 2017).

Climate data

Climate data was collected and provided by the Institute of Physics and Meteorology of the University of Hohenheim (Stuttgart, Germany). The meteorological station was in close distance to the field (< 2 km) and daily recording total sunshine hours and total amount of precipitation.

Statistical analyses

Statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, NC). Means and standard deviations were calculated across data derived from 3 individual parcels per cultivar and sampling date (n = 3). Normal distribution was verified with a Shapiro-Wilk test. Subsequently, Duncan's test was applied to identify significant differences between mean data of different variants Sturon (grown from seeds), Sturon (grown from sets), and Stardust (grown from seeds) at individual sampling points, and differences between mean data of different sampling points within each variant. All differences were significant ($p < 0.05$), if not stated otherwise.

Results and discussion

Cultivation

Identical growing conditions for all samples were ascertained by sowing seed grown and transplanting set grown onions on April 22, 2015. All three parcels were block-wise randomly distributed on our fields and included in a previously published larger field experiment (Pöhnl et al., 2018). Soil emergence of set grown onions was observed 13 days (May 5) after transplanting, while seed grown onions emerged 48 days after sowing (June 9), corresponding to a 35 days delay of seed grown onions compared to set grown onions. Expectedly, set gown onions reached maturity faster and were harvested 29 days earlier (July 27) than the seed grown ones (August 25). Thus, set and seed grown onions had a total vegetation period of 83 and 77 days between soil emergence and harvest, respectively.

Accumulated sunshine hours and precipitation during onion growth

Due to the different soil emergence dates, accumulated total sunshine hours between soil emergence and harvest were recorded. Total sunshine amounted 753 and 674 hours for set onions and seed grown onions, respectively, resulting in a 12% longer sunlight exposure of set grown onions. Noteworthy, total day lengths from soil emergence until harvest added up to 1318 and 1199 hours for set and seed grown onions, respectively, resulting in 10% longer exposure for set grown onions. Furthermore, considering the entire vegetation period from sowing or transplanting until harvest, rainfall was 30% less for set grown onions (152.3 L/m²) compared to seed grown onions (220.2 L/m²). Between sowing and emergence through soil of seed and set grown onions, total rainfall amounted to 68.3 and 41.4 L/m².

Development of total bulb weights

As illustrated by Fig. 1a, total bulb weight increased throughout the whole vegetation period, reaching final average bulb weights of 115.4 ± 3.4, 95.0 ± 26.7, and 73.6 ± 23.9 g for set grown (cv. 'Sturon'), seed grown common onions (cv. 'Sturon'), and seed grown dehydrator onions (cv. 'Stardust'), respectively. While bulb growth of seed grown onions was clearly delayed until the second sampling point (Fig. 1a), immediate onset of bulb growth in set grown onions was noticeable at the second sampling point. As a result, bulb weights of set grown 'Sturon' onions were 21% higher at harvest than those of seed grown 'Sturon' onions. Higher bulb weights of set grown onions were due to the undelayed weight gain in the first two weeks of growth (+ 47.1 g/bulb in set onions), being delayed and thus lower in seed grown onions (+ 27.4 g/bulb in common seed onions; +25.9 g/bulb in dehydrator onions). Upon transplanting, set grown onions had initial weights of 2-5 g per plantlet being high in stored carbohydrates, thus promoting growth and development. Their growth was just continued after transplanting, while onion seeds (2-5 mg grain weight) had to undergo previous germination and growth activation. In agreement, BUFLER et al. (2003) found bulbs obtained from onions, transplanted from greenhouse preparatory culture, to be higher in weight and size compared to those from sown onions of the same cultivar. However, during further growth, bulb weight of all variants increased substantially. Comparing common seed grown onions to seed grown dehydrator onions, bulb weight at harvest of common onions was 29% higher (Fig. 1a). Possibly, the dehydrator seed onions invested a higher proportion of energy for the biosynthesis of higher oligomeric FOS than common seed onions, and the energy input for FOS biosynthesis might have been run at the expense of bulb growth. The results are in agreement with those reported during our previously published detailed study focusing on different onion cultivars (PÖHNL et al., 2018).

Dry matter (DM) and total soluble solids (TSS)

As shown in Fig. 1b, initial dry matter concentrations of the juices obtained from dehydrator onions (cv. 'Stardust'; 13.8 ± 1.2% DM) were 22 and 35% higher than those from common seed grown onions (cv. 'Sturon'; 11.3 ± 0.2%) and common set grown onions (cv. 'Sturon'; 10.3 ± 2.0%), respectively. During growth, dry matter concentrations in samples of set grown onions showed the most pronounced increase reaching up to 54% of its initial value, with a final yield of 15.8 ± 0.5% dry matter. The dry matter gain of seed grown common onions was lower, i.e., finally reaching 13.6 ± 1.0%, corresponding to only 120.5% of its initial value. In contrast, dry matter content in seed grown dehydrator onions augmented by 36.8%, reaching maximum final dry matter contents of 18.9 ± 0.1%.

By analogy, initial total soluble solids (TSS) concentrations were highest in samples of the dehydrator onions cv. 'Stardust' (10.6 ± 0.5 °Brix), surpassing those of common seed and set grown onions (8.5 ± 0.1 and 9.8 ± 0.9 °Brix, resp.). The relative increase in TSS during growth was similar in common seed and set grown onions (Fig. 1c). Nevertheless, higher final TSS concentrations were found in set grown variants, i.e., 12.7 ± 0.1 °Brix, while seed grown onions exhibited 10.7 ± 0.9 °Brix. However, TSS in the dehydrator cv. 'Stardust' strikingly rose from 10.6 ± 0.5 to 16.5 ± 0.5 °Brix (Fig. 1c). Both DM and TSS in dehydrator onions were among the highest values for onions reported in literature (SINCLAIR et al., 1995; PÖHNL et al., 2017; PÖHNL et al., 2018).

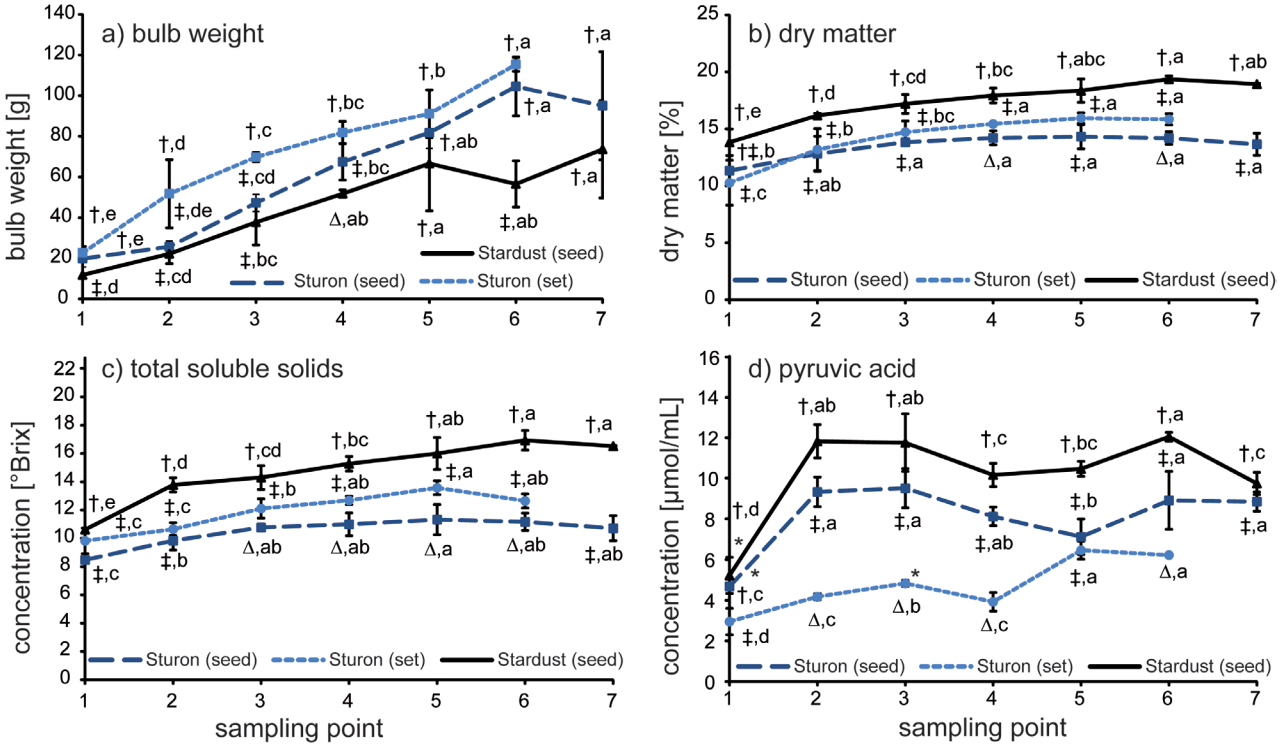

Fig. 1: Bulb weight (a), dry matter (b), total soluble solids (c) and pyruvic acid (d) concentrations in onion samples of different stages of maturity and of different cultivars, i.e. 'Sturon' (grown from sets), 'Sturon' (grown from seeds) and 'Stardust' (grown from seeds). Timing of the second fertilization is indicated by asterisks (*). Sampling point 1 represents the time point when first signs of bulb development were observed. Subsequent time points represent weekly sampling intervals, continued until leaves had fallen, i.e. until regular harvest of onions. On each sampling date, samples of five maturing onion bulbs were collected from 3 parcels per variant, i.e. 15 bulbs per sampling date and variant. Symbols †, ‡ and Δ indicate significant differences of means ($p < 0.05$) between individual variants Sturon (grown from seeds), Sturon (grown from sets) and Stardust (grown from seeds) at different sampling points. Different letters (a, b, c, d, e) indicate significant differences of means ($p < 0.05$) within individual variants, sampled at different stages of maturation.

Carbohydrate accumulation

Mono- and disaccharides

Glucose concentrations in common onions grown from sets remained on a constant level between 14.4 and 16.7 g/L (Fig. 2a) throughout the whole vegetation period. Although glucose concentrations of common onions grown from seeds were comparable to those grown from sets (initial concentration: 19.0 ± 2.6 g/L), they peaked during the first two sampling weeks to reach ca. 23.1 ± 0.5 g/L. Subsequently, their contents declined to values slightly below the initial levels (14.7 ± 0.8, Fig. 2a). The identical final concentrations of glucose in seed and set grown common onions (14.7 ± 0.8 and 15.2 ± 2.3 g/L, Fig. 2a) might indicate maturity regardless of the cultivation method. In clear contrast, glucose concentrations in dehydrator onions (cv. 'Stardust') dropped from initially 18.9 ± 3.3 g/L to only 4.5 ± 1.0 g/L after 6 weeks. Consequently, the final concentration was more than 3-fold lower in dehydrator onions than in common onions, regardless of the cultivation method (Fig. 2a), indicating a clear distinction from common onion cultivars.

Concentrations of fructose decreased steadily during growth in all tested variants. Initial concentrations were highest in common seed grown onions (17.9 ± 1.3 g/L), followed by seed grown dehydrator onions (13.9 ± 2.3 g/L) and common set grown onions (8.2 ± 0.2 g/L). During growth, fructose concentration in set grown onions dropped by 43.0% to reach 4.7 g/L. The decline was even more pronounced in seed grown onions, amounting to 71.1 and 67.4% for fructose in common and dehydrator type seed grown onions, respectively. Finally reached concentrations were comparably low for all variants (4.5 - 5.2 g/L, Fig. 2b). However, the observed differences between seed and set grown onions are most likely related to the more advanced growth stage of the set grown onions at the start of the sampling period. This would be supported by the observed, apparently undelayed initial boost in growth. Both, glucose and fructose levels of both common seed and set grown onions reached a fairly constant plateau at similarly high levels of fructose (4.7 ± 1.2 and 5.2 ± 0.5 g/L, resp.) and glucose (15.3 ± 2.3 and 14.7 ± 0.8 g/L, resp.) during the last stages of growth (Figs. 2a and 2b). Dehydrator onions might be more effective in reducing osmotic pressure by reducing the amount of monosaccharides (glucose + fructose) to a minimum (final concentration: 9.0 g/L) as compared to common seed and set grown onions (final concentrations: 20.0 and 19.9 g/L, respectively). Simultaneously, dehydrator type 'Stardust' onions accumulated higher polymerized FOS (DP > 10), indicating a genotype-dependant differentiation (for detailed description see section 3.5.2). These observations were similar to those reported earlier (PÖHNL et al., 2018), stating similar glucose and fructose concentrations in bulbs of the same cultivars grown under different conditions (seed *vs*. set). In contrast to our results, SHIOMI et al. (1997) reported constant concentrations of fructose during bulb development, however without having observed high initial concentrations as described in our study.

Sucrose levels during ontogenesis were more variable than for those of glucose and fructose, ranging from 4.0 - 14.1 g/L during growth of all studied cultivars, respectively (Fig. 2c). Sucrose concentrations in set grown onions increased from initially 6.9 ± 0.6 g/L to 13.5 ± 1.3 g/L at harvest. Common seed grown onions showed a similar increment from 4.0 ± 1.0 g/L to 13.0 ± 1.0 g/L at harvest. Contrarily, sucrose concentrations of seed grown dehydrator onions (cv. 'Stardust') were initially high in sucrose (6.5 ± 1.7 g/L), dropping by 47% to 3.0 ± 0.3 g/L at harvest. On average, sucrose concentrations were 10.9 g/L in seed grown, 10.7 g/L in set grown and 4.2 g/L in seed grown dehydrator onions (cv. 'Stardust') during our study. Sucrose is an intermediate product, fuelling the synthesis of FOS

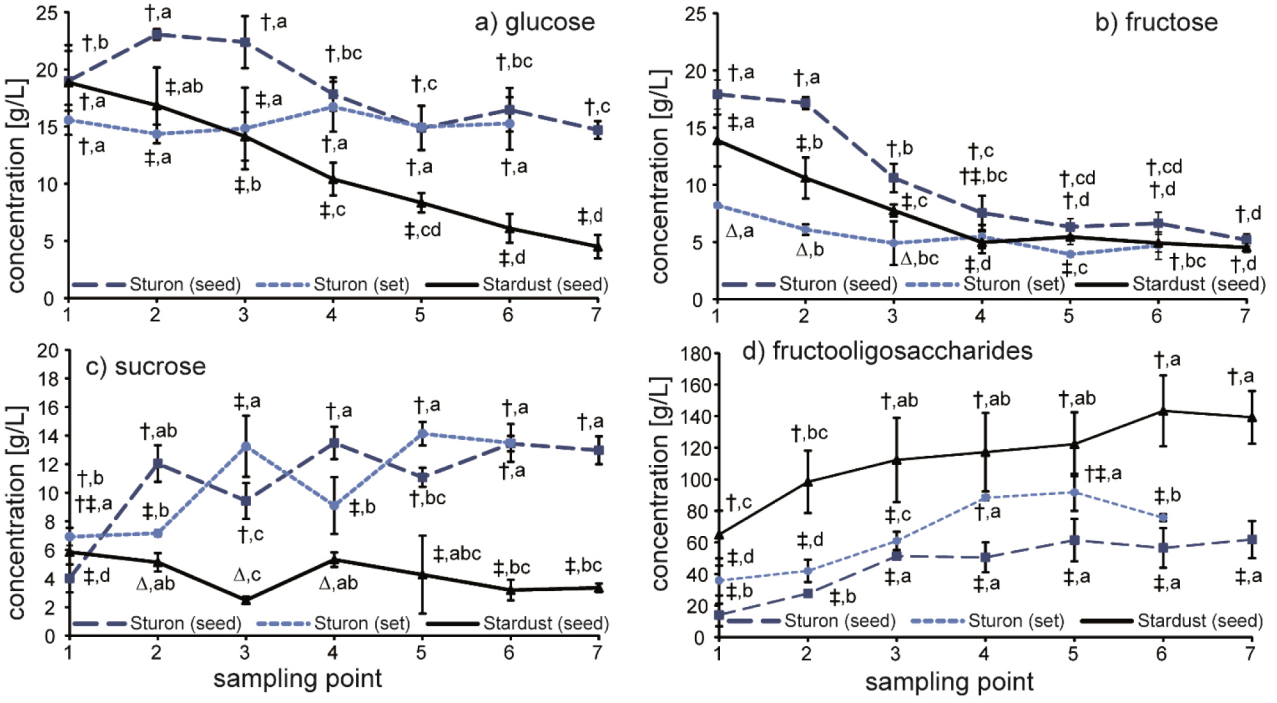

Fig. 2: Glucose a), fructose b), sucrose c) and d) total fructooligosccharides (FOS) concentration in different stages of maturity in onions of the cultivars 'Sturon' (grown from sets), 'Sturon' (grown from seeds) and 'Stardust' (grown from seeds). Sampling point 1 represents the time point when first signs of bulb development were observed. Subsequent time points represent weekly sampling intervals, continued until leaves had fallen, i.e. until regular harvest of onions. On each sampling date, samples of five maturing onion bulbs were collected from 3 parcels per variant, i.e. 15 bulbs per sampling date and variant. Symbols †, ‡ and Δ indicate significant differences of means ($p < 0.05$) between individual variants Sturon (grown from seeds), Sturon (grown from sets) and Stardust (grown from seeds) at different sampling points. Different letters (a, b, c, d) indicate significant differences of means ($p < 0.05$) within individual variants, sampled at different stages of maturation.

by sucrose:sucrose-fructosyltranferase activity. Hence, sucrose is constantly synthetized and consumed, possibly explaining the fluctuating concentrations observed during our study.

Fructooligosaccharides (FOS)

Fructooligosaccharides (FOS) were found in all stages of bulb development in all three variants. Initial total concentrations of FOS in set grown onions (35.9 ± 9.5 g/L) were higher than those in their seed grown counterparts (14.1 ± 7.2 g/L). However, highest initial concentrations (65.0 ± 15.1 g/L) were found in the seed grown dehydrator onions (cv. 'Stardust'). All variants accumulated FOS during the bulbing process to reach a final FOS concentration of 75.7 ± 2.2 g/L (cv. 'Sturon' set grown), 61.8 ± 11.7 (cv. 'Sturon' seed grown), and 139.32 ± 16.6 (cv. 'Stardust' seed grown). However, FOS accumulation was highest in dehydrator onions (+74.3 g/L, cv. 'Stardust'), while seed grown (+47.8 g/L) and set grown (+39.8 g/L) common onions accumulated lower FOS concentrations. In contrast to seed grown onions, FOS contents of set grown onions dropped from 91.8 ± 11.8 to 75.7 ± 2.2 g/L in the week before harvest (Fig. 2d).

The course of individual FOS levels is illustrated in Fig. 3a-h and revealed that the concentration of individual FOS approached an individual plateau, as similarly found for fructose and glucose. In common onions, this potential steady state is first reached for low degrees of polymerisation and, after progressing maturation, later at higher degrees of polymerization. Concentrations of FOS with a DP of 3 increased during the first 3 weeks of growth in common onions from 10.8 ± 2.2 to 17.7 ± 1.1 g/L (set grown) and from 5.6 ± 2.2 to 14.7 ± 0.8 g/L (seed grown), regardless of the cultivation method.

Contrarily, levels of FOS with DP3 dropped in dehydrator onions from their highest concentration in week 2 (13.5 ± 2.7 g/L) to their lowest concentration at harvest (7.9 ± 1.3 g/L).

By analogy, FOS having a DP of 4 increased during bulb development in bulbs of the common onion cv. 'Sturon' (Fig. 3b). In set grown onions (cv. 'Sturon'), FOS levels increased from 9.4 ± 2.5 to 19.3 ± 0.7 g/L in the first 4 weeks, while those of their seed grown counterpart accumulated from 3.7 ± 1.6 to reach their maximum levels of 13.7 ± 0.6 g/L within 3 weeks. FOS (DP4) concentrations in dehydrator onions increased from 11.6 ± 2.4 at the first sampling date to 18.3 ± 3.7 g/L one week later, subsequently dropping to 12.4 ± 1.9 g/L at harvest. Likewise, in common onions, concentrations of FOS with DP5 and DP6 exhibited a characteristic increase during the first weeks of accumulation, all reaching a steady plateau after 4 weeks (Figs. 3c and 3d). Thereafter, FOS levels of DP 5 and 6 of dehydrator onions showed a slight decline at harvest (Figs. 3a to 3c). In contrast to plateauing FOS with DP < 6, accumulation of FOS with DP greater than 7 continued until their late stages of growth. Overall, levels of FOS with a DP of *n+1* were consistently lower than those of their corresponding precursors with a DP of *n*. DP of 10 was found to be the maximum DP for common onions, irrespective of the cultivation method. In contrast, the accumulation of FOS with still higher DPs of 10-15 was observed in dehydrator onions. FOS with highest degrees of polymerisation (DP11-15) were only reached in the last stages of growth as illustrated by Fig. 3h. FOS in dehydrator onions were also reaching plateau levels of individual FOS. However, the highest steady state levels were not reached for DP3 as shown for common onions, but for DP 7 (27.0 ± 3.2 g/L, Fig. 3e). This divergent pattern differentiates dehydrator onions clearly from common cultivars.

However, set grown onions contained slightly higher steady state concentrations of FOS as compared to seed grown onions, indicating the influence of the cultivation method. The outstanding accumulation pattern of FOS in dehydrator onions might be related to different enzymatic activities. These observations support our hypothesis that sun exposure and growth duration had influenced the observed differences between seed and set grown onions, although substantial genotype-dependant differences were also found, particularly regarding the outstanding dehydrator cultivars. Shiomi et al. (1997) discovered higher activities of 1^F-fructosyltransferase and 6^G-fructosyltransferase in cultivars high in total soluble solids. Oku et al. (2019) showed both, FOS accumulation in aerial leaf tissues and anabolic enzyme activity, to be coequal in Japanese 'Kita-momiji 2000' and 'Pole Star' onions. However, their diverging final FOS concentrations were due to augmented hydrolytic activities of 1-fructan exohydrolase (EC 3.2.1.153), 6-fructan exohydrolase (6-FEH) (EC 3.2.1.154), and invertase (EC 3.2.1.26) prior to bulbing in 'Pole Star' onions. Our findings are also in accordance with Bufler et al. (2003), who also found higher concentrations of FOS in transplanted onions (cv. 'Sturon') than in seed grown onions (cv. 'Sturon'), sown simultaneously. Interestingly, the difference in FOS between transplanted and seed grown onions disappeared when transplanting was delayed by four weeks. In this case concentrations in seed and set grown onions were the same (Bufler et al., 2003).

Pyruvic acid accumulation

Initial concentrations of pyruvic acid (PA), being indicative of pungent alk(en)yl cysteine sulfoxides (ACSOs), were 3.0 ± 0.7 µmol/mL, 4.7 ± 0.4 µmol/mL, and 5.2 ± 0.9 µmol/mL in common set grown, common seed grown onions, and in seed grown dehydrator onions, respectively (Fig. 1d). Sulphur containing fertilizer (superphosphate) was used at two different dates, initially, before sowing and transplanting (April 21), and subsequently at bulbing initiation (July 07, sampling point 3) for set grown onions and July 14 for seed grown onions to stimulate the concentration of sulphur containing ACSOs according to commercial practise. Accordingly, in set grown onions, PA levels resulting from ACSO-degradation increased after the second fertilization with superphosphate, resulting in a final PA concentration of 6.2 ± 0.1 µmol/mL (Fig. 1d; sampling point 6). In seed grown onions of cvs. 'Sturon' and 'Stardust', a marked increase in PA concentrations was observed after the second fertilization, resulting in higher final PA concentrations in common onions (8.9 ± 0.5) and dehydrator onions (9.7 ± 0.6) (Fig. 1d; sampling point 7). Hence, the accumulation of ACSOs was most pronounced during the period between sampling point 1 and 2 for seed grown onions and during the period from sampling point 4 to 5 for set grown onions, being most likely due to the second fertilization applied prior to this period. However, in set grown onions (sampling points 5 and 6), PA levels even dropped after the second fertilization, from 6.5 ± 0.4 to 6.2 ± 0.1 µmol/mL. Likewise, levels dropped from 9.5 ± 1.0 to 8.9 ± 0.5 µmol/mL and from 11.8 ± 0.8 to 9.7 ± 0.6 µmol/mL in seed grown common onions (sampling points 3 and 7) and in seed grown dehydrator onions (sampling points 2 and 7), respectively. Decreases after the initial or fertilization-induced increase might have been caused by the ongoing growth and a lack of available sulphur in the soil. The second fertilization of set grown onions was carried out within the last development stages, while the second fertilisation of seed grown onions was conducted at the beginning of bulb development. Thus, final concentrations were 42 and 57% higher in seed grown common onions (cv. 'Sturon') and in dehydrator onions (cv. 'Stardust') as in set grown common onions (cv. 'Sturon'). For reference, the pyruvic acid concentration released from harvest-mature set grown cv. 'Sturon' (6.2 µmol/mL) was similar to that described in an earlier study (ca. 6 µmol/g FW, Romo Pérez et al., 2018).

Our data suggest that onions during bulb weight accumulation had an enhanced uptake of sulphur and, thus, a higher accumulation of ACSOs. Comparable pyruvic acid concentrations in seed and set grown onions have been observed by our previous study, also following the same fertilization protocol (Pöhnl et al., 2018). Gene-

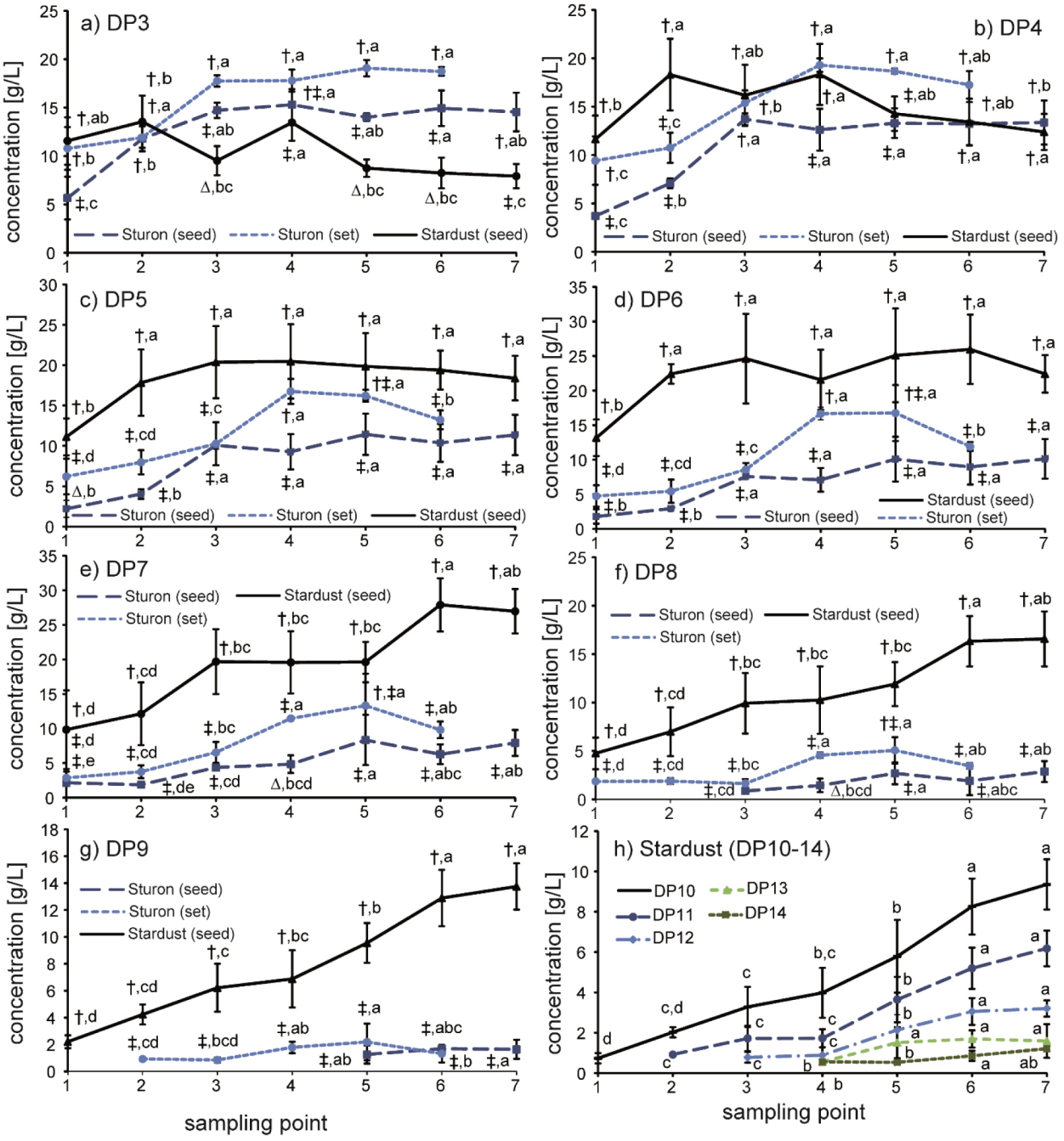

Fig. 3: Concentration of fructooligosaccharides (FOS) with a degree of polymerization (DP) between 3 and 9 (a - g) in different stages of maturity in onions of the cultivars 'Sturon' (grown from sets), 'Sturon' (grown from seeds) and 'Stardust' (grown from seeds) and higher polymerized FOS (DP 10 to 14; h) in the cv. 'Stardust'. Sampling point 1 represents the time point when first signs of bulb development were observed. Subsequent time points represent weekly sampling intervals, continued until leaves had fallen, i.e. until regular harvest of onions. On each sampling date, samples of five maturing onion bulbs were collected from 3 parcels per variant, i.e. 15 bulbs per sampling date and variant. Symbols †, ‡ and Δ indicate significant differences of means ($p < 0.05$) between individual variants Sturon (grown from seeds), Sturon (grown from sets) and Stardust (grown from seeds) at different sampling points. Different letters (a, b, c, d) indicate significant differences of means ($p < 0.05$) within individual variants, sampled at different stages of maturation.

rally, sulphur fertilization is known to increase the content of pungent ACSOs (RANDLE and BUSSARD, 1993; RESEMANN et al., 2004). Therefore, we suggest that the timing of the application of sulphurous fertilizers is important for modulating ACSO levels in onions, while the effect of the growing method (seed *vs*. set) appears to be negligible.

Conclusions

Carbohydrate accumulation in set grown onions was expectedly ca. 3 weeks ahead of that of seed grown onions, yielding temporarily divergent but finally similar FOS profiles when considering the same cultivar. Cultivar-related differences became most obvious when considering dehydrator cultivars, which were characterized by higher-polymerized FOS (mainly DP > 7), lower glucose, and higher total

FOS concentrations as compared to those of common onions. Thus, the selection of the cultivar was clearly a most important influence factor on the yielded FOS levels, although cultivation method (set *vs*. seed) and the date of sowing or transplanting was also shown to modulate the composition of carbohydrates in onions. Particularly, longer growing periods and sun light exposure resulted in increased FOS levels at harvest maturity. Regarding the pungent principles of onion, i.e. the ACSOs, no clear differentiation between seed and set onions was observed in agreement with our previous study (Pöhnl et al., 2018). Highlighting the importance of agricultural practices, a clear impact of the timing of sulphur fertilization on the ACSO concentrations at harvest was suggested by our study. Further study on the expression of the involved genes encoding both FOS and ACSO biosynthetic enzymes is needed.

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