Available online at www.sciencedirect.com

SciVerse ScienceDirect

South African Journal of Botany 79 (2012) 159 – 165

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

D-mannoheptulose and perseitol in 'Hass' avocado: Metabolism in seed and mesocarp tissue

S.Z. Tesfay *, I. Bertling, J.P. Bower

Horticultural Science, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Received 24 June 2011; received in revised form 7 October 2011; accepted 15 October 2011

Abstract

Mature avocado plants produce a higher amount of the heptose D-mannoheptulose and its polyol form, perseitol, than hexoses. These heptoses have various functions as anti-oxidants and energy sources. Although C7 sugars are abundantly produced in avocado, knowledge of their metabolism and synthesis is limited. Therefore the synthesis of these sugars in seed and mesocarp tissue was investigated, in particular the interconversions of perseitol to D- mannoheptulose and of D-mannoheptulose to perseitol were followed in different tissues. The ability of cotyledons of etiolated seedlings to convert perseitol to D-mannoheptulose was significantly higher than that of the cotyledons of germinating seedlings grown in the light as well as that of dormant seeds. The cotyledons of light-grown, germinating plants had a greater ability to convert D-mannoheptulose to perseitol than the cotyledons of etiolated plants and the dormant seed. The cotyledons of etiolated plants showed higher activity in converting perseitol to D-mannoheptulose than the cotyledons of light-grown plants; whereas the cotyledons of light-grown plants had a greater ability to convert D-mannoheptulose to perseitol than cotyledons of etiolated seedlings. In mesocarp tissue, a higher percentage of perseitol was converted to D-mannoheptulose than vice versa. Furthermore, aldolases could be identified in mesocarp as well as in dry seed tissue. Therefore, the production of these heptoses and their enzymatic inter-conversion is dependent on the plant's ontogenic stage. This paper describes the following basic findings a) conversion of D-mannoheptulose to perseitol as well as conversion of perseitol to D-mannoheptulose in dry seed, cotyledons and mesocarp tissue b) detection of aldolase enzymes and c) interconversion of perseitol and D-mannoheptulose in bark exudates.

Keywords: Perseitol; *D*-mannoheptulose; *Persea americana* Mill

1. Introduction

The major non-structural carbohydrates in avocado (*Persea americana* Mill.) are the heptoses *D*-mannoheptulose and perseitol (Liu et al., 1999, 2002). Although there have been reports on photosynthetic assimilation, translocation and storage of carbon, reducing power, and protection against different types of stresses (Bertling et al., 2007; Bieleski, 1982; Cowan, 2004;Lewis, 1984; Loescher and Everard, 1996; Stoop et al., 1996;), little is known about their physiology and metabolism in avocado. However, the biosynthetic pathways of most pentols and hexitols in higher plants have been elucidated (Flora and Madore, 1993; Moing et al., 1992). Häfliger et al. (1999) investigated the metabolism of the C7 sugar alcohol volemitol and characterized a novel ketose reductase enzyme in the horticultural hybrid polyanthus (*Primula* x *polyantha*).

Isomers of the heptoses volemitol, perseitol, as well as its hydrogenated form *D*-mannoheptulose, have been reported to be produced in substantial amounts in avocado (Liu et al., 1999). However, avocado seeds also contain the common carbohydrate storage forms sucrose and starch (Liu et al., 2002), which is commonly the case in plants producing rare carbohydrates (Häfliger et al., 1999). In avocado the sugar profile changes when seeds and seedlings are grown in light versus dark (Kazama et al., 1978). It has been postulated that avocado plants use *D*-mannoheptulose for a variety of purposes, ranging from

* Tel.: +27 033 260 5442; fax: +27 033 260 5073.
E-mail address: tesfay@ukzn.ac.za (S.Z. Tesfay).

0254-6299/$ - see front matter © 2011 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2011.10.006

energy sources to anti-oxidants and transport sugars (Liu et al., 2002; Tesfay et al., 2010), and that perseitol, the reduced form of *D*-mannoheptulose, may also function as storage and/or as transport carbohydrate.

It has been proposed by Häfliger et al. (1999) that the heptose formation in avocado is catalysed by three known enzymatic reactions that will form C7 intermediates; a) an aldolase reaction—erythrose-4-P + dihydroxyacetone-P sedoheptulose-1,7-bis-P; b) a transketolase reaction—xylulose-5-P + ribose-5-P sedoheptulose-7-P + glyceraldehyde-3-P; or c) a transaldolase reaction—fructose-6-P + erythrose-4-P sedoheptulose- 7-P + glyceraldehyde-3-P (Liu et al., 2002). This formation of heptoses via these pathways requires NADPH/$NADP^+$ as redox power (Häfliger et al., 1999).

Negm et al. (2001) speculated that heptoses are synthesized by aldolase condensation of a dihydroxyacetone with erythrose-4-phosphate to form sedoheptulose-1-7-bis-phosphate and this reaction, in avocado, is followed by isomerization to a phosphorylated *D*-mannoheptulose derivative, probably perseitol. Avocado seeds, as storage organs, contain starch as a storage carbohydrate (Liu et al., 2002) but also significant amounts of the C7 sugar perseitol, a likely storage form of *D*-mannoheptulose (Tesfay et al., 2010).

The current experiment was designed to investigate formations of heptoses, especially *D*-mannoheptulose-perseitol interconversions, in light- and dark-grown seedlings.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained either from Sigma-Aldrich®, Saarchem®, Fluka®, or Glycoteam GmbH.

2.2. Plant materials

2.2.1. Dry seed

Mature 'Hass' avocado fruit of uniform appearance and size were collected from trees in the KwaZulu-Natal Midlands (30°16′E, 29°28′S), the exo- and mesocarp removed, the seed snap-frozen in liquid nitrogen and freeze-dried and stored at −75 °C for further analysis. Avocado fruit size expansion is dependent on sustained cell division in the mesocarp (Cowan et al., 1997), a process under hormonal regulation, on particular cytokinins, which have only been detected in seed (Gazit and Blumenfeld, 1970). Taylor and Cowan (2001) also suggested that the seed is the primary source of the hormone.

2.2.2. Seed germination

Seeds of the same size from the same batch of fruit were imbibed by submerging them in tap water, which was replaced every five days for 6–8 weeks until seeds cracked, a sign of germination. Seeds were then transferred to heated propagation beds (26 °C) for seedling development, for about eight weeks, until the seedling reached a height of 30 cm. Seedlings which had not yet developed leaves, but only roots and stems, were then transferred to a dark growth room for eight weeks to be etiolated. When such plants reached a height of 100 cm, they had pale leaves of reduced size, an indication of restricted photosynthesis. Thereby it was ensured that the energy for seedling development was only obtained from CHOs derived from the cotyledons. Cotyledon portions were sampled from (a) plants grown under light and (60 days exposure to light) (b) etiolated plants (60 days exposure to darkness) while they were germinating. Cotyledon samples were flush-frozen, freeze-dried, ground and kept at −75 °C for further analysis.

2.2.3. Fruit

Physiologically mature fruit were sampled from the same orchard; the mesocarp immediately separated from other fruit tissues and snap-frozen. Samples were stored at −75 °C until further analysis.

2.3. Enzyme extraction and activity determination of dry seed and cotyledon tissue

The extraction of proteins was carried out according to Helmerhorst and Stokes (1980). Milled, frozen fruit tissue (1.0 g) was thawed and extracted on ice in a glass homogenizer containing 5 volumes of extraction buffer (20 mM Hepes/KOH, pH 7.5, 5 mM DTT, 5 mM MgCl2, 2% (w/v) PEG-20,000, and 2% (w/v) PVP K30). The mixture was allowed to stand on ice for 15 min and then centrifuged at 20,000 × *g* for 20 min. The supernatant was passed through Miracloth® and immediately centrifuge-desalted through Sephadex G-25 pre-equilibrated with the appropriate assay buffer. The Bradford microassay (Bradford, 1976) was used to determine the protein concentration of samples.

2.4. Electrophoretic detection of mesocarp and seed aldolase proteins

Crude mesocarp and seed protein extracts were loaded onto a 12% acrylamide running gel and a 5% acrylamide stacking gel, and were separated by SDS-PAGE using a Mini-PROTEAN® Tetra Cell (Bio-Rad, USA) at 200 V. An amount of 100 μg of seed protein and 50 μg of mesocarp protein were loaded. Spinach aldolase was used as a standard for identification of aldolases in the samples.

2.5. Carbohydrate composition of exudates from girdled branches

Several branches (10–15 cm diameter) of different trees were girdled using Optima Girdling Pliers (Optima, RSA). Exudates from the cut branches were collected on Whatman® no. 1 filter paper, which was placed onto the girdled area immediately after the girdling process. The filter paper was removed after 30 min and rinsed with 5 ml 80% (v/v) ethanol. The liquid collected in a 20 ml glass vial. Sample was collected during the early hours of the morning and sugars quantified according to Liu et al. (1999). Furthermore, the dry residue which had formed on the girdled branches, was collected seven days after the

girdling process. The residue was suspended in ultra-pure water and filtered through 0.45 μm filters before analysis by HPLC.

2.6. Statistical analysis

The experiment was conducted twice, consisting of twenty (20) samples, assigned into four (4) lots. Each lot represented a replication, containing five (5) samples. Analyses of variance were performed using GenStat version 9.1 (VSN International, Hemel Hempstead, UK). Standard error values were calculated and differences among treatments were separated by the least significant difference at $P<0.05$ level.

3. Results

Cotyledons of seedlings germinating under light had a three times greater ability than mature, dormant seeds to convert the proposed C7 sugar transport form, *D*-mannoheptulose to perseitol, the likely C7 storage form (Fig. 2B). These, however, could only convert perseitol to *D*-mannoheptulose at 20% of the rate of the cotyledons of light-grown seedlings. The cotyledons of etiolated seedlings, however, displayed a four to five times greater ability to convert perseitol to *D*-mannoheptulose than the cotyledons of germinating seedlings (Fig. 2A). An attempt was also made to elucidate whether there was a correlation between enzyme activity and actual tissue carbohydrate

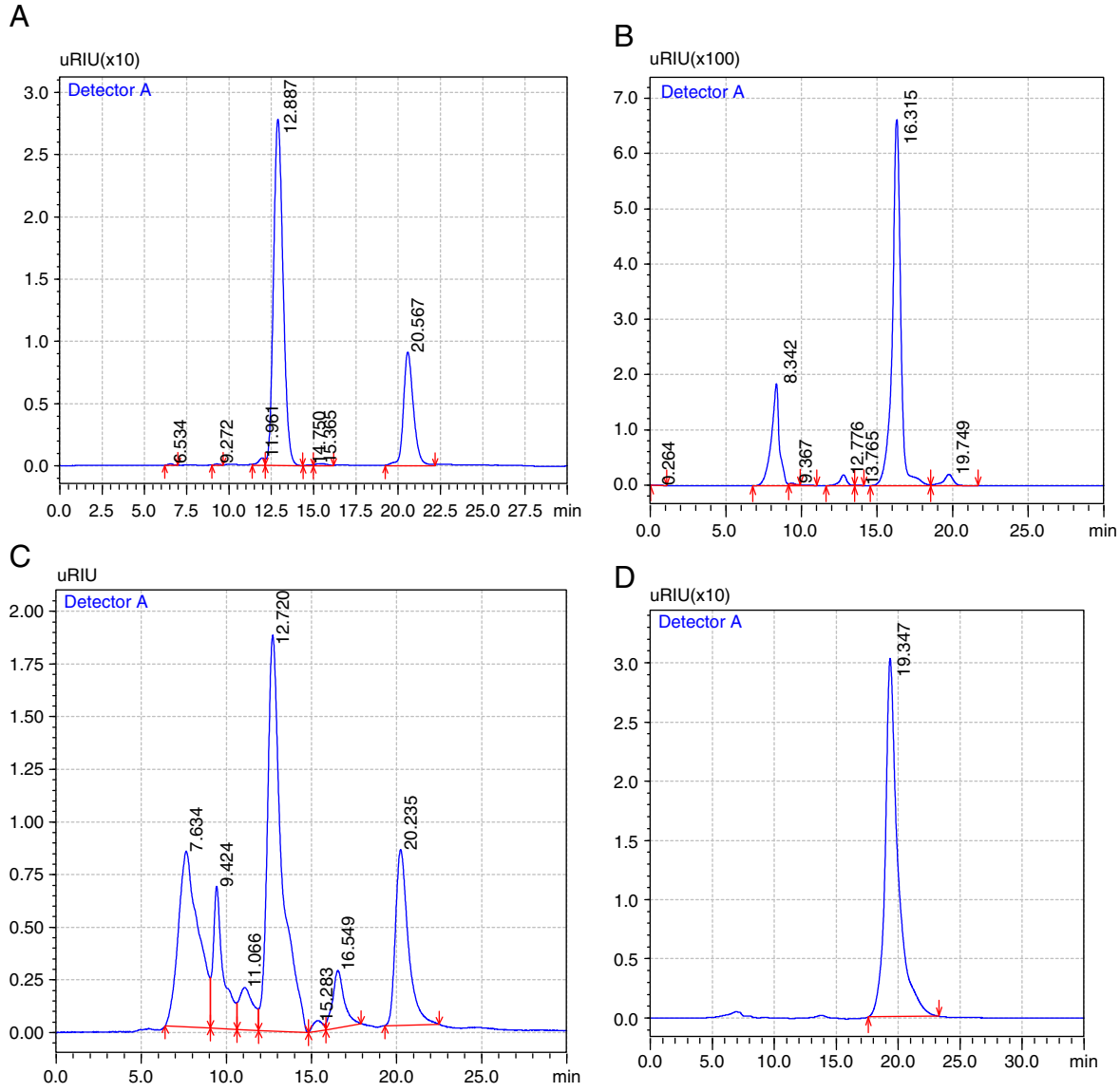

Fig. 1. HPLC chromatograms of water-soluble carbohydrate standards detected isocratically using HPLC/RID-10A (A); substrate-enzymatic reaction products (B); branch exudate collected immediately after girdling (C); dry exudates collected 7 days after girdling (D). Standards: 1. *D*-mannoheptulose (12.7 min), 2. Perseitol (19.3–20.3 min).

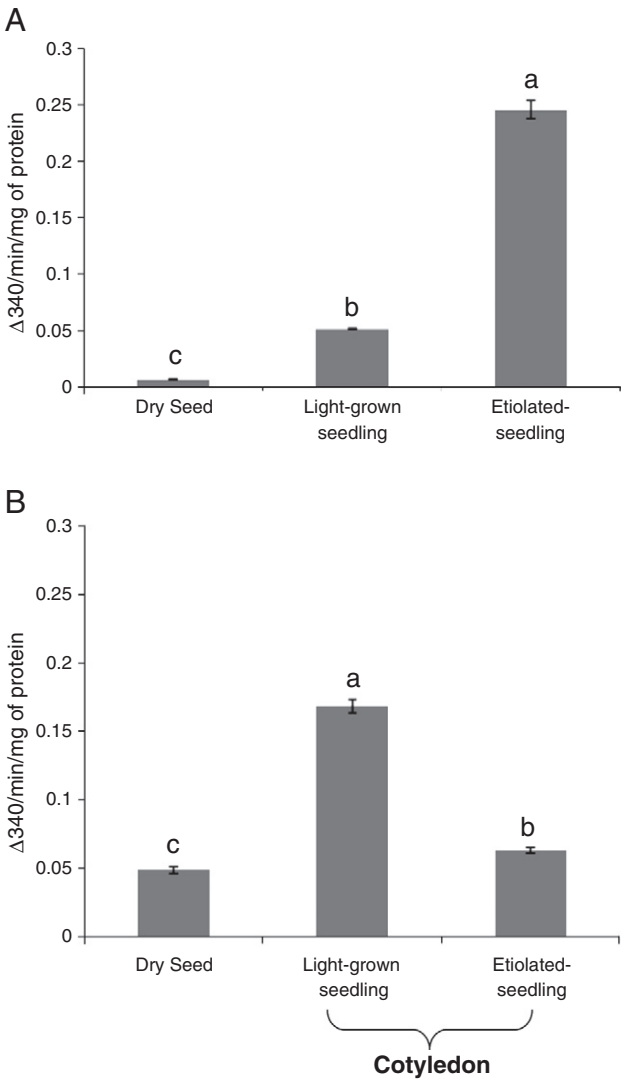

Fig. 2. A. Enzyme activities of crude desalted protein extracts used to catalyze the conversion of perseitol into *D*-mannoheptulose, $NADP^+$ was a co-factor added into the reaction mixture. The spectrophotometric quantification of NADPH as a byproduct was at wavelength 340 nm. The more spectrophotometric absorbance implies higher enzyme activity, more NADPH and finally higher conversion percentage (%). $LSD_{(0.05)}=0.01$ (n=5). B. Enzyme activities of crude desalted protein extracts used to catalyze the conversion of *D*-mannoheptulose into perseitol, NADPH was a co-factor added into the reaction mixture. The spectrophotometric quantification of $NADP^+$ as a byproduct was at wavelength 340 nm. The more spectrophotometric absorbance implies higher enzyme activity, more NADP and finally higher conversion percentage (%). $LSD_{(0.05)}=0.038$ (n=5).

concentrations. Dormant seeds had higher perseitol than *D*-mannoheptulose concentrations, whereas the cotyledons of seedlings germinating in the light had a different pattern, similar to those germinating in the dark: *D*-mannoheptulose concentrations were higher than perseitol concentrations (Fig. 3).

In mesocarp tissue, a higher percentage of *D*-mannoheptulose was converted to perseitol than perseitol to *D*-mannoheptulose (data not presented). Therefore, the enzyme catalyzing the reduction of *D*-mannoheptulose to perseitol in this tissue, had a relatively higher activity than the enzyme catalysing the oxidation of perseitol to *D*-mannoheptulose. Fresh branch exudates

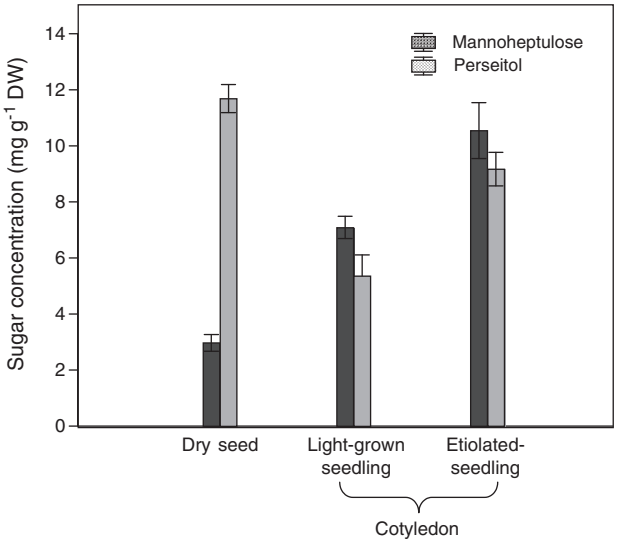

Fig. 3. Comparison of *D*-mannoheptulose and perseitol concentrations of avocado seeds; dry seeds versus cotyledons germinated under light and exclusion of light 'etiolated'. Bars are standard error (SE) of different means (n=5).

collected immediately after girdling contained *D*-mannoheptulose and perseitol, while only perseitol was detected in the dry exudates collected seven days after girdling (Table 1; Fig. 1C, D).

Electrophoresis of mesocarp and seed protein extracts (Fig. 4) showed that various aldolases were present. Spiking the protein extracts with spinach aldolase resulted as very intense protein bands, with aldolases being detected in sample extracts. In mature, dormant seed aldolase was significantly more expressed than in the cotyledons of etiolated seedlings as well as light-grown seedlings (Fig. 4).

4. Discussion

The C7 sugars are a special group of carbohydrates, which are abundantly produced in avocado plants and have been proposed to fulfil a variety of functions, such as carbon and energy storage, as well as anti-oxidants (Tesfay et al., 2010). At the onset of germination the embryo of the avocado seed contains perseitol, and even more so, *D*-mannoheptulose (Fig. 3). As most seeds accumulate carbohydrates as storage compounds (Duffus and Duffus, 1984), perseitol has been postulated to be the likely reserve carbohydrate of avocado (Cowan, 2004), which would, at the onset of germination, be converted into *D*-mannoheptulose by an aldolase. Both sugars were found in and exuded from the stem of seedlings (Fig. 1C), indicating that both sugars are transportable, as described by Liu et al. (2002). However, while perseitol is the major C7 sugar in the seed, and could have been transported from the seed into the developing seedling, *D*-mannoheptulose was not present in substantial amounts in the seed. It is likely, that this C7 sugar was released from its reduced form, perseitol, which, therefore, acted as the storage form of *D*-mannoheptulose.

As demonstrated by Liu et al. (2002)*D*-mannoheptulose is a primary photosynthetic product in avocado; hence, these authors postulated that this C7 sugar acts as an energy source,

Table 1
Absolute and relative concentrations of soluble, non-structural carbohydrates from avocado branch exudates. The branch exudates are fresh if collected immediately after girdling (while it is still wet). Note: nd=not detected.

Exudates	Branch	Sugars (mg mL^{-1})			%		
		Sucrose	Mannoheptulose	Perseitol	Sucrose	Mannoheptulose	Perseitol
Fresh	1	0.106	0.403	0.184	15.31	58.12	26.57
	2	0.168	0.450	0.212	20.20	54.19	25.61
	3	0.158	0.451	0.209	19.31	55.17	25.52
Dry	1	nd	nd	7.713	0	0	100
	2	nd	nd	7.689	0	0	100
	3	nd	nd	7.349	0	0	100

stored in the form of sucrose and/or starch. As the plant utilizes the stored carbohydrates, these are broken down into simpler units, normally glucose, which is readily available for energy generation. Since *D*-mannoheptulose was found to be a primary photosynthetic product, this heptose might be catalysed by transaldolases existing within the Calvin Cycle, to form the C7 storage product, perseitol. As the protein extract from seed as well as mesocarp tissues was able to convert *D*-mannoheptulose into perseitol and *vice versa*, these reactions might have occurred via an existing aldolase enzyme of the Calvin Cycle, possibly transaldolase. However, this aldolase presence in seed protein extracts indicates that this enzyme is not (only) translocated from the mesocarp, but also manufactured 'de novo' in the seed (Fig. 4).

Both heptoses are transported in the phloem (Liu et al., 2002) and can, therefore, be moved from source to sink. Perseitol is the more likely source of energy, at least for the young, fast growing seedling, than *D*-mannoheptulose, as under light exclusion, and, therefore, without any additional energy input via photosynthesis, it is easier converted into *D*-mannoheptulose than *D*-mannoheptulose is into perseitol (Fig. 2A). Perseitol, on the other hand, is predominantly occurring in the cotyledons, a typical feature of a storage carbohydrate. Additionally, etiolated seedlings can derive their energy

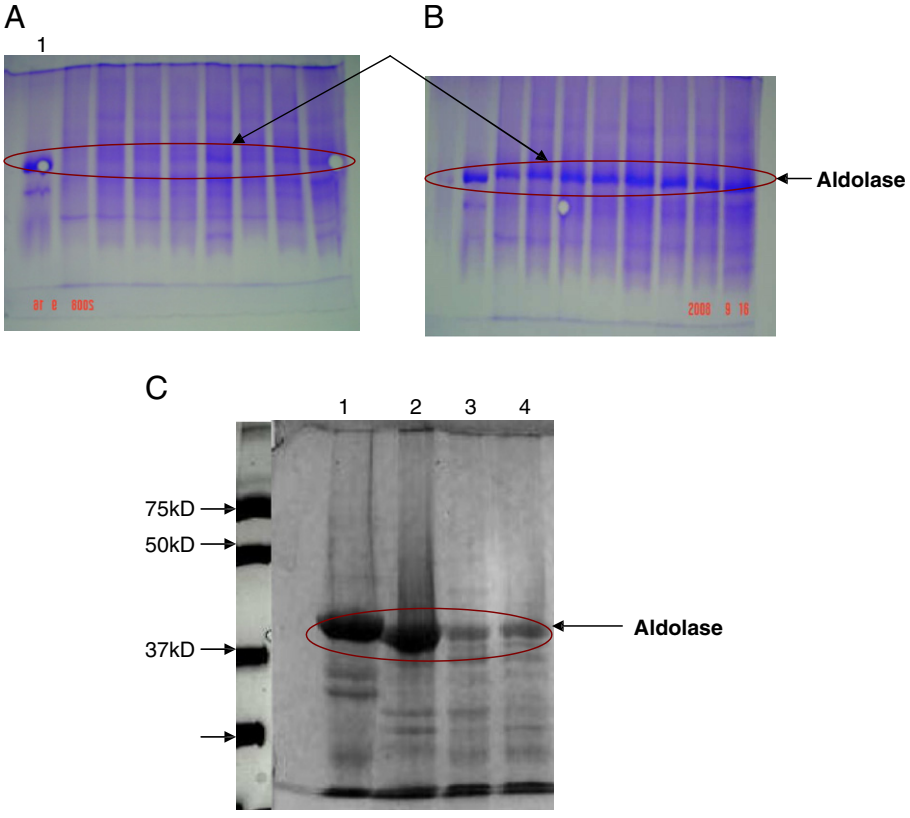

Fig. 4. 12% SDS-PAGE, of crude protein extract of mesocarp tissues. Gel A, all lanes are circled to indicate proposed mescarp aldolases aligned to crude spinach aldolase standard (Sigma) (Lane 1). Gel B, proposed mesocarp aldolases spiked with spinach aldolase (Sigma), thick bands are aldolases (sample and spinach aldolase mix). The protein amount loaded in each lane was 25–50 µg. Gel C. 12% SDS-PAGE was performed to identify aldolase in various avocado tissues. Lane 1: aldolase standard; Lane 2: mature dry seed; Lane 3: cotyledons of light-grown seedlings seed; Lane 4: cotyledons of dark-grown seedlings. The aldolase has a molecular weight of 40 kD.

only from the seed (Kazama et al., 1978), further substantiating our hypothesis that perseitol is the likely storage product in avocado; contradicting the postulation by Liu et al. (1999) that *D*-mannoheptulose is the energy source sustaining plant growth.

Furthermore, the seed supplies energy from its reserve carbohydrates to maintain its development and to form energy source compounds for the germination process. Avocado seed predominantly stores perseitol and, to a lesser degree, sucrose as soluble non-structural carbohydrates, as well as starch as an insoluble, non-structural carbohydrate. Perseitol may be a readily available energy source that can be converted to *D*-mannoheptulose as an energy-providing compound as well as a transport carbohydrate. Perseitol accumulation might, therefore, be related to an increased 'de novo' synthesis of C7 sugars, particularly in the dry seed, further strengthening the hypothesis that perseitol is a storage carbohydrate.

The two major heptoses of avocado are synthesized in different parts of the plant, probably through different pathways. It has been reported that C7 compounds are formed through the condensation of carbon 3 (C3) and carbon 4 (C4) compounds in the Calvin Cycle, mainly in photosynthetically active tissues in a reaction catalysed by aldolases (Liu et al., 2002). In the Pentose Phosphate Pathway, heptoses can also be further transformed into different isomers, such as mannoheptulose and perseitol, through the process of oxidation and reduction. Mesocarp and seed cells of avocado, therefore, possibly use the co-factors $NADP^+$/NADPH as a redox power, as demonstrated in polyanthus (*Primula* x *polyantha*) by Häfliger et al. (1999). In avocado, the formation of these sugars is seemingly affected by both, the amount of $NADP^+$/NADPH available and the presence of responsible enzymes. Our results suggest that it is crucial to investigate means to manipulate the production of $NADP^+$/NADPH, thereby creating a condition to regulate expression of these enzymes, resulting in an increased concentration of the C7 sugars.

Besides morphological unique features, the avocado fruit is relatively large and contains a large-sized seed (Barlow, 2002). The provision of solutes from the seed to other fruit tissues depends on the ability to transport these through the seed coat (Steyn et al., 1993). This seed coat can, therefore, channel solutes, including mineral nutrients, carbohydrates and hormones from the seed to other fruit via the plasma membrane and plasmodesmata. Via these structures the avocado seed is able to supply the required energy compounds as well as possible building blocks of structural carbohydrates to the mesocarp. Therefore, early senescence of the seed coat results in small-sized 'Hass' avocado fruit, particularly due to the lack of transporting channels for growth stimulating compounds, such as cytokinins (Moore-Gordon et al., 1998).

Like in other fruit, size increase in avocado is possible due to cell expansion; however, the sustained cell division of mesocarp cells also allows for ongoing fruit growth (Moore-Gordon et al., 1998). This increase in mesocarp cell number is made possible by cytokinin activity (Gazit and Blumenfeld, 1970), an activity balanced by the presence of ABA (Cowan et al., 1997). A certain cytokinin to ABA ratio in the seed is, therefore, the likely trigger for the sustained viability of the seedcoat, which, in turn, facilitates the supply of the necessary resources, such as building blocks, hormones and energy sources, to the mesocarp, allowing for its further expansion. The presence of an aldolase enzyme (Fig. 4) and its ability of convert perseitol, stored in the cotyledons, to *D*-mannoheptulose would allow for the sustained supply of the C7 transport sugar *D*-mannoheptulose to the mesocarp.

Our study provides clear evidence that an aldolase enzyme plays a role in the interconversion/formation of the main sugars in avocado, the heptoses *D*-mannoheptulose and perseitol. Such an enzyme, probably transaldolase, is produced in a variety of tissues of avocado (Fig. 4), indicating its importance in the species, probably to convert the C7 sugar storage form (perseitol) into a C7 sugar transport from (*D*-mannoheptulose). However, further research is needed to identify, and, if novel, characterize this enzyme in order to further elucidate the heptose carbohydrate metabolism in avocado.

Acknowledgement

Financial support by the South African Avocado Growers' Association (SAAGA) for this research is gratefully acknowledged.

References

Barlow, C., 2002. Fruit development. In: Whiley, A., Schaffer, B., Wolstenholme, B. (Eds.), The Avocado: Botany, Production and Uses. CABI Publishing, Oxon, UK, Cambridge, MA, USA, p. 32.

Bertling, I., Tesfay, S.Z., Bower, J.P., 2007. Antioxidants in 'Hass' avocado. South African Avocado Growers' Association Yearbook, 30, pp. 17–19.

Bieleski, R.L., 1982. Sugar alcohols. In: Loewus, F.A., Tanner, W. (Eds.), Plant Carbohydrates I: Intracellular Carbohydrates. : New Series, Vol 13A. Springer-Verlag, Berlin, pp. 158–192.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Cowan, A.K., 2004. Metabolic control of avocado fruit growth: 3-hydroxy-3-methylglutaryl coenzyme a reductase, active oxygen species and the role of C7 sugars. South African Journal of Botany 70, 75–82.

Cowan, A.K., Moore-Gordon, C.S., Bertling, I., Wolstenholme, B.N., 1997. Metabolic control of avocado fruit growth: isoprenoid growth regulators and the reaction catalysed by 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Physiology 114, 511–518.

Duffus, C.M., Duffus, J.H., 1984. Carbohydrate Metabolism in Plants. Longman Inc, NY, USA. pp. 183.

Flora, L.L., Madore, M.A., 1993. Stachyose and mannitol transport in olive (*Olea europaea* L). Planta 189, 484–490.

Gazit, S., Blumenfeld, A., 1970. Cytokinin and inhibitor activities in the avocado fruit mesocarp. Plant Physiology 46, 334–336.

Häfliger, B., Kindhauser, E., Keller, F., 1999. Metabolism of *D*-glycero-*D*-heptitol, volemitol, in polyanthus. Discovery of a novel ketose reductase. Plant Physiology 119, 191–197.

Helmerhorst, E., Stokes, G.B., 1980. Microcentrifuge desalting: a rapid, quantitative method for desalting small amounts of protein. Analytical Biochemistry 104, 130–135.

Kazama, H., Katsumi, M., Ueda, K., 1978. Light-controlled sugar-starch interconversion in epidermal chloroplasts of light-grown cucumber hypocotyl sections and its relationship to cell elongation. Journal of Plant Research 91, 121–130.

Lewis, D.H., 1984. Physiology and metabolism of alditol. In: Lewis, D.H. (Ed.), Storage Carbohydrates in Vascular Plants. Cambridge University Press, Cambridge, UK, pp. 157–180.

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L., 1999. 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. Journal of the American Society for Horticultural Science 124, 676–681.

Liu, X., Sievert, J., Arpaia, M.L., Madore, M.A., 2002. Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. Journal of the American Society for Horticultural Science 127, 108–114.

Loescher, W.H., Everard, J.D., 1996. Sugar alcohol metabolism in sinks and sources. In: Zamski, E., Schaffer, A.A. (Eds.), Photoassimilate Distribution in Plants and Crops, Source–Sink Relationships. Marcel Dekker, New York, pp. 185–207.

Moing, A., Carbonne, F., Rashad, M.H., Gaudillere, J.P., 1992. Carbon fluxes in mature peach leaves. Plant Physiology 100, 1878–1884.

Moore-Gordon, C.S., Cowan, A.K., Bertling, I., Botha, C.E.J., Cross, R.H.M., 1998. Symplastic solute transport and avocado fruit development: a decline in cytokinin/ABA ratio is related to appearance of the Hass small fruit variant. Plant & Cell Physiology 39, 1027–1038.

Negm, F.A., Arpaia, M.L., Madore, M.A., 2001. Synthesis of seven-carbon sugars in avocado (*Persea Americana* Mill.)website http://www.plantphysiology.com2001Poster: enzymology/metabolism Accessed on 26-11-2009.

Steyn, E.M.A., Robertse, P.J., Smith, D., 1993. An anatomical study of ovary-to-cuke development in consistently low-producing trees of 'Fuerte' avocado (*Persea americana* Mill.) with special reference to seed abortion. Sexual Plant Reproduction 6, 87–97.

Stoop, J.M.H., Williamson, J.D., Pharr, D.M., 1996. Mannitol metabolism in plants: a method for coping with stress. Trends in Plant Science 1, 139–144.

Taylor, N., Cowan, K., 2001. Plant hormone homeostasis and the control of avocado fruit size. Plant Growth Regulation 35, 247–255.

Tesfay, S.Z., Bertling, I., Bower, J.P., 2010. Levels of anti-oxidants in various tissues during maturation of 'Hass' avocado (*Persea americana* Mill.). The Journal of Horticultural Science and Biotechnology 85, 106–112.

Edited by AK Cowan