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Regulation of isoprenoid metabolism and Hass avocado fruit: An overview

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ABSTRACT

Studies have shown that isoprenoid metabolism plays an essential role in normal fruit growth and development. Thus in an attempt to examine the metabolic events that contribute to the appearance of the Hass small-fruit phenotype, isoprenoid metabolism has been studied in detail in both normal and small-fruit phenotypes. 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR; EC 1.1.1.34) catalyses the formation of mevalonic acid (MVA) from 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which is the committed step in isoprenoid biosynthesis. The small-fruit phenotype is characterised by reduced HMGR and cell division cycle activity, and increased levels of abscisic acid (ABA) metabolic activity (which is associated with increased active oxygen species (AOS) production). The small-fruit phenotype also has reduced total C6 soluble sugar content and increased glucose content. Work carried out thus far shows a relationship between HMGR activity, isoprenoid biosynthesis, sugar metabolism of both C6 and C7 sugars, and AOS production. Results are concluded in the form of an hypothesis/scheme in an attempt to show the relationship between these various factors.

INTRODUCTION

Avocado (*Persea americana* Mill. cv Hass) produces normal and phenotypically small fruit (Cowan *et al.* 1997; Moore-Gordon *et al.* 1998; Zilkah and Klein, 1987) when grown under stressful conditions. The "Hass small-fruit problem" is a phrase that every avocado producer in South Africa is very familiar with. This small-fruit problem has serious consequences for growers as up to 50% of the Hass crop may be undersized in any one year, and since these small fruit cannot be exported, this results in decreased profits. The work that is being conducted is firstly trying to determine why small Hass fruit are small (Cowan *et al.* 1998; Cripps *et al.* 1999) and leading on from this, it is hoped that a solution to the Hass small-fruit problem will be found.

In grower terms, the small-fruit phenotype is characterised by fruit size — the preferred commercial fruit size is about 250 g to 300 g (Bergh, 1986) and fruit under 200 g are considered small. The small-fruit phenotype is also characterised by early seed coat senescence. Normal fruit will only have a senescenced or dead seed coat, once they

reach horticultural maturity. Work carried out by Cowan *et al.* (1997) showed that the mesocarp of small fruit was limited by cell number, not by cell size. Therefore the mesocarp of small fruit, has fewer cells, but these cells do expand to the same size as those of normal fruit mesocarp. Based on this work, it would appear that in the small-fruit phenotype cell cycle activity is being affected, possibly by products of isoprenoid metabolism, such as cytokinins (CKs) and abscisic acid (ABA).

Isoprenoid biosynthesis/metabolism is essential for normal fruit growth and development (Cowan *et al.* 1997; Gillaspy *et al.* 1993; Narita and Gruissem, 1989). This biochemical pathway supplies compounds such as phytosterols, carotenoids and many regulatory molecules, including brassinolides, ABA, gibberellins (GAs) and the side chain of CK (Campos and Boronat, 1995). The enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR; EC 1.1.1.34), catalyses the formation of mevalonic acid (MVA) from 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) (Chappell, 1995). HMGR activity is thought to be the main regulatory point of cytosolic isoprenoid biosynthesis and is sometimes referred to as the "rate-limiting enzyme of isoprenoid biosynthesis" (Moore and Oishi, 1994), although there is no unequivocal evidence to support this idea (Gray, 1987).

Based on this brief introduction to the isoprenoid pathway and isoprenoid metabolism, why has such a great emphasis been placed on this biochemical pathway, and how does this relate to Hass avocado fruit growth and more specifically to the Hass small-fruit problem?

Studies carried out by Cowan *et al.* (1997) and Moore-Gordon *et al.* (1998) demonstrated that the Hass small-fruit variant had reduced HMGR activity, increased ABA content and an altered CK:ABA ratio. The important role that isoprenoid metabolism and HMGR activity plays in fruit development is shown when young Hass fruit are injected with mevastatin (which is a competitive inhibitor of HMGR). These fruit show reduced growth, have a senescenced seed coat and increased ABA content, which are all characteristics of the Hass small-fruit phenotype (Cowan *et al.* 1997).

Thus based on the characterisation of the Hass small-fruit phenotype by Cowan *et al.* (1997) and Moore-Gordon *et al.* (1998), it was shown that isoprenoid metabolism is the common link between the small fruit characteristics. Since HMGR activity is thought to be the regulatory point of cytosolic isoprenoid metabolism, it would appear that a change in HMGR activity must play an important role in the Hass small-fruit phenomenon.

INVESTIGATING HMGR ACTIVITY IN HASS AVOCADO

Three seasons' data confirmed that HMGR activity was reduced in the seed tissue of the Hass small-fruit variant (Cowan *et al.* 1998; Cripps *et al.* 1999; Richings *et al.* 2000). Interestingly, these investigations showed that HMGR activity in mesocarp of untreated normal and small-fruit was similar. These results also confirmed those of Cowan *et al.* (1997) and Moore-Gordon *et al.* (1998) which showed that when fruit are treated with mevastatin (an inhibitor of HMGR) during the linear phase of growth, HMGR activity is reduced, and is at a similar level to that found in the small-fruit variant.

Further evidence supporting the important role of isoprenoid metabolism, and in particular HMGR activity for normal Hass fruit growth, was shown when co-treatment of mevastatin with mevalonic acid lactone, farnesyl pyrophosphate and geranylgeranyl pyrophosphate (all intermediates of the isoprenoid pathway) reversed mevastatin-induced inhibition of seed HMGR activity and restored fruit growth (Cowan *et al.* 1998; Richings *et al.* 2000). Thus, all of these results again emphasized the role of HMGR activity and isoprenoid metabolism for the development of normal Hass fruit.

INVESTIGATING THE LINK BETWEEN ISOPRENOID AND SUGAR METABOLISM

As previously mentioned, Moore-Gordon *et al.* (1998) suggested that an alteration in the CK:ABA ratio may be a crucial determinant in the expression of Hass avocado fruit size. These investigations also provided evidence to suggest that a change in the CK:ABA ratio occurred concomitant with changes in symplastic solute movement (noticeably with plasmodesmatal structure and function). The requirement for isoprenoid metabolism and HMGR activity during fruit growth has already been shown (Cowan *et al.* 1997; Gillaspy *et al.* 1993; Narita and Gruissem, 1989), while other studies suggest that control of sugar metabolism is important in the control of fruit size (Klann *et al.* 1996). There is also evidence to suggest that hormone and sugar metabolism impact on one another (Ackerson 1985; Perata *et al.* 1997; Schaffer *et al.* 1987; Wingler *et al.* 1998). Based on this work, as well as current literature, it was important to investigate changes in isoprenoid metabolism, HMGR activity and sugar content and composition, in an attempt to link isoprenoid and sugar metabolism in the control of Hass fruit growth. Thus carbohydrate, isoprenoid and ABA metabolism were compared and contrasted in developing normal and small Hass avocado fruit.

Carbohydrate metabolism was investigated by comparing and contrasting C6 sugar content and composition and activity of the sugar metabolizing enzyme, invertase (ß-D-fructofuranosidase, EC 3.2.1.26), in seed and mesocarp tissue of developing normal and small fruit. Analysis of soluble C6 sugar content and composition in seed and mesocarp tissue of the normal and small-fruit variant showed that differences were most pronounced in the seed tissue (Cowan *et al.* 1998; Cripps *et al.* 1999; Richings *et al.* 2000). The total sugar content was reduced by approximately 50% in the small-fruit variant. While seed of small Hass fruit contained 85% glucose compared to 12% in seed of normal fruit, indicative of glucose accumulation at the expense of both sucrose and fructose. The seed of small fruit also had substantially reduced fructose content, as opposed to that found in the seed of normal fruit.

A comparison of invertase activity in normal and small-fruit revealed a dramatic increase in insoluble acid invertase activity in the seed of the small-fruit variant (Cowan *et al.* 1998; Cripps *et al.* 1999; Richings *et al.* 2000). Insoluble acid invertase was reduced in mesocarp tissue from the small-fruit phenotype. When the supply of metabolizable sugar is limited, Koch *et al.* (1992) state that the genes for invertase are expressed optimally. Thus since the small-fruit variant has been shown to have reduced total soluble C6 sugar content, as well as loss of symplastic solute transport, the observed increase in insoluble acid invertase in seed of the small-fruit variant seemed a likely occurence.

It has previously been shown that small fruit have higher ABA levels than those found in the normal fruit (Cowan *et al.* 1997). Thus a detailed analysis of ABA metabolism (looking at levels of ABA as well as its major catabolites) in seed and mesocarp of normal and small fruit was undertaken (Cowan *et al.* 1998; Richings *et al.* 2000). In both the small-fruit seed and mesocarp tissue, the content of dihydrophaseic acid (DPA), ABA-glucose ester (ABA-GE) and ABA-glucoside (ABACS) was greater, indicating increased ABA metabolism. The ABA content of seed of the small-fruit was approximately double that of normal fruit, while there was no significant difference in mesocarp ABA content between normal fruit and small fruit. Various investigations have revealed a link between exogenous applications of ABA and an increase in invertase activity, and glucose and fructose concentration (Ackerson, 1985; Kojima *et al.* 1995). Thus, the high ABA content of seed of the small-fruit variant together with increased insoluble acid invertase activity and higher glucose concentration, suggested that in Hass avocado seed tissue, sugar metabolism may be linked to ABA turnover.

In a further attempt to link sugar and isoprenoid metabolism in the regulation of Hass avocado fruit growth, the C6 sugar content and composition of mevastatin-treated fruit was determined (Cripps *et al.* 1999; Richings *et al.* 2000). Mevastatin has been shown to decrease HMGR activity and fruit growth, and increase ABA content and thus it seemed likely that if sugars and hormones are interacting, that the sugar content and composition of mevastatin-treated fruit would resemble that of the small-fruit phenotype. In the seed tissue of mevastatin-treated fruit, total soluble sugar content was reduced, while glucose as a percentage of total soluble sugars, increased from 5 to 57%. These results resembled closely those for sugar content and composition obtained from seed of the small-fruit variant.

Thus to summarize the main results obtained in these investigations: the seed of the small-fruit variant was shown to have reduced HMGR activity, and enhanced insoluble acid invertase activity which was associated with glucose accumulation, sucrose depletion, and increased levels of ABA metabolism. These results suggested an interaction between glucose accumulation (relative to total soluble sugars) and/or sucrose depletion, increased ABA metabolism, and a reduction in HMGR activity in the control of Hass avocado fruit growth. It was proposed that sugar and isoprenoid metabolism/hormone signals interact to regulate expression and/or activity of HMGR in the growth and development of Hass avocado fruit (Figure 1).

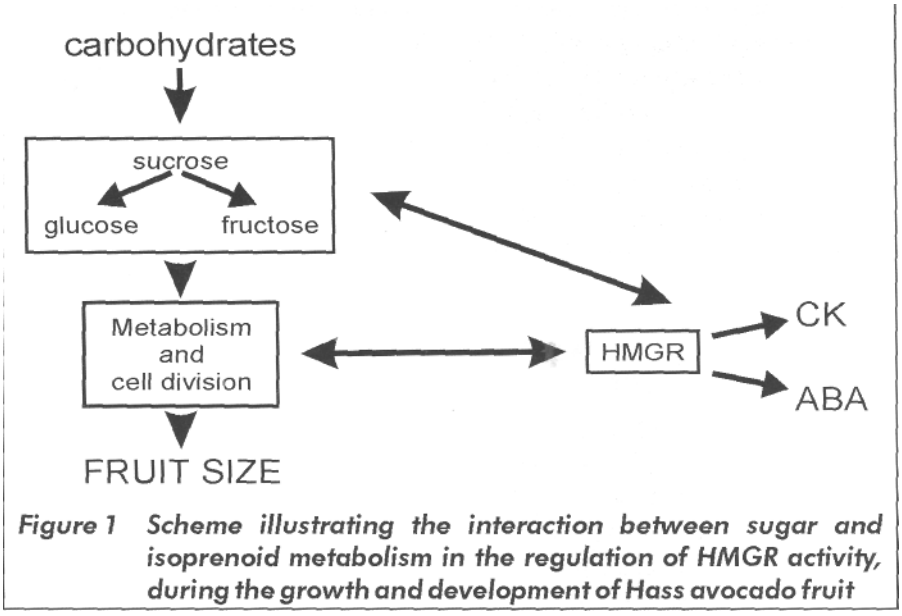

Figure 1 Scheme illustrating the interaction between sugar and isoprenoid metabolism in the regulation of HMGR activity, during the growth and development of Hass avocado fruit

Further evidence for the interaction between HMGR activity and sugar metabolism is provided by the recent information that suggests that HMGR kinase is a member of the sucrose non-fermenting-1 (SNF1)-related protein kinase 1 (SnRK1)-type subfamily (Barker *et al.* 1996; Halford and Hardie, 1998). These protein kinases catalyse reversible protein phosphorylation, which is one of the most important mechanisms by which cell activity is regulated. In a process termed carbon catabolite repression (Gancedo, 1992), several genes are repressed when sufficient glucose is available and the SNF1 proteins are required for derepression of these glucose repressed genes (Jang and Sheen, 1997). Since the expression of a wide spectrum of genes is either repressed or induced by glucose (Sheen, 1994; Koch, 1996; Jang and Sheen, 1997), it is likely that SnRK1 activity would be regulated by glucose or other C6 sugars. Therefore the effect of an exogenous glucose application on HMGR activity was investigated, as well as SnRK1 activity between normal and small Hass fruit.

To determine the effect of glucose on HMGR activity, freshly harvested intact fruit were pulsed with a solution of glucose via the pedicel and then incubated for periods up to 48 hours (Cripps *et al.* 1999; Richings *et al.* 2000). Within 6 hours of glucose application, HMGR activity of seed tissue had declined by 52%, and by 24 hours, seed HMGR activity was still ± 20% below that of the control. Thus from this study, it would appear that a change in HMGR activity and a change in the glucose content could be important factors in the development of the small-fruit phenotype. When SnRK1 activity was investigated in normal and small-fruit variants, it was found that there is approximately double the SnRK1 activity in the normal fruit as opposed to that found in the small-fruit variant (Richings and Cowan, unpublished data). Thus these results again link HMGR activity (and therefore isoprenoid metabolism) with sugar metabolism in the control of Hass avocado fruit growth.

INVESTIGATING C7 SUGARS AND ACTIVE OXYGEN SPECIES

Up until this point in the research, only the C6 sugars (namely sucrose, glucose and fructose) had been investigated in relation to the regulation of isoprenoid metabolism in Hass avocado. There are large quantities of the C7 sugar alcohol, D-*glycero*-D-*galacto*-heptitol (perseitol) and the related C7 sugar, D-*manno*-heptulose in avocado (La Forge, 1917; Richtmyer, 1970), and since D-*manno*-heptulose has been implicated in the sugar signal transduction pathway (Jang and Sheen, 1994; Salas, *et al.* l965) the potential role of these C7 sugars in the regulation of Hass fruit growth was also investigated.

Perseitol and D-*manno*-heptulose were quantified in seed and mesocarp tissue of both normal and small-fruit phenotypes (Table 1).

Table 1 ***C7 sugar content (mg g DW^{-1}) and composition (% of total) of seed and mesocarp tissue from developing normal and small Hass avocado fruit***

Type	Tissue	Sugar content (mg g DW^{-1})		
		Perseitol	D-*manno*-heptulose	Total
Normal	Seed	80.58 (69)	36.32 (31)	116.90 (100)
	Mesocarp	75.97 (30)	181.45 (70)	257.42 (100)
Small	Seed	88.21 (62)	54.98 (38)	143.19 (100)
	Mesocarp	43.87 (24)	138.75 (76)	182.62 (100)

In the mesocarp of normal fruit, both the perseitol and D-*manno*-heptulose content was greater than that found in the mesocarp tissue of the small-fruit variant. While in the seed of normal fruit, both the perseitol and D-*manno*-heptulose was lower than that found in the seed tissue of the small-fruit variant. Thus these differences in C7 sugar content between normal and small-fruit must be indicative of a role of the C7 sugars, in the control of Hass avocado fruit size.

The penultimate step in abscisic acid biosynthesis is catalysed by an aldehyde oxidase (Cowan and Richardson, 1997; Milborrow, *et al.* 1997) and activity of plant aldehyde oxidase, may generate active oxygen species (AOS), such as hydrogen peroxide (H_2O_2), hydroxyl radicals and superoxide anions. Active oxygen species are routinely generated at low levels in plant cells that are involved in reduction-oxidation processes and are also responsible for causing cellular damage, ageing and mutagenesis, as well as lipid breakdown and nucleic acid damage (Mehdy, 1994).

Work carried out by Omkumar and Ramasarma (1993) showed that H_2O_2, when in excess, caused a concentration-dependent inactivation of HMGR in rat liver microsomal preparations and reduced cell division cycle activity. Furthermore, Burdon (1996) found that processes leading to either abnormally high or abnormally low levels of H_2O_2 depressed rates of cell proliferation and increased programmed cell death. Therefore it was of interest to determine the effect of *in vivo* and *in vitro* applications of AOS on HMGR activity of Hass avocado fruit.

Firstly the effect of AOS on endogenous HMGR activity was investigated. As shown in Figure 2, treatment of fruit with solutions of either (a) *tert*-butylhydroperoxide or (b) H_2O_2, reduced endogenous HMGR activity in seed tissue of avocado.

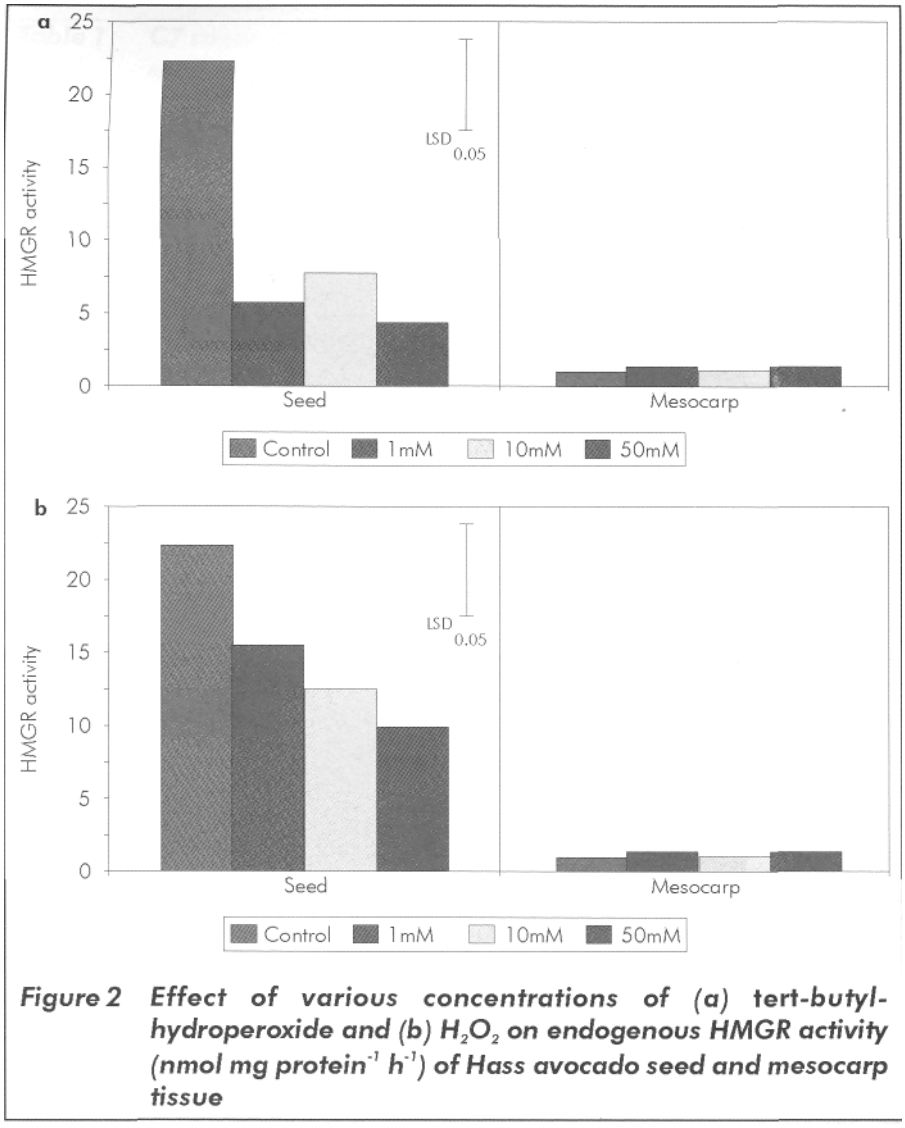

Figure 2 ***Effect of various concentrations of (a) tert-butyl-hydroperoxide and (b) H_2O_2 on endogenous HMGR activity (nmol mg protein^{-1} h^{-1}) of Hass avocado seed and mesocarp tissue***

In response to increasing concentrations of AOS, the degree of HMGR inactivation was more severe and appeared to reach completion at about 10 mM. Secondly, in order to investigate the effect of AOS on HMGR activity in more detail, the effect of AOS on HMGR activity *in vitro* was examined. Again it was shown that (a) *tert*-butyl-hydroperoxide and (b) H_2O_2 + EDTA reduced in vitro HMGR activity (Figure 3).

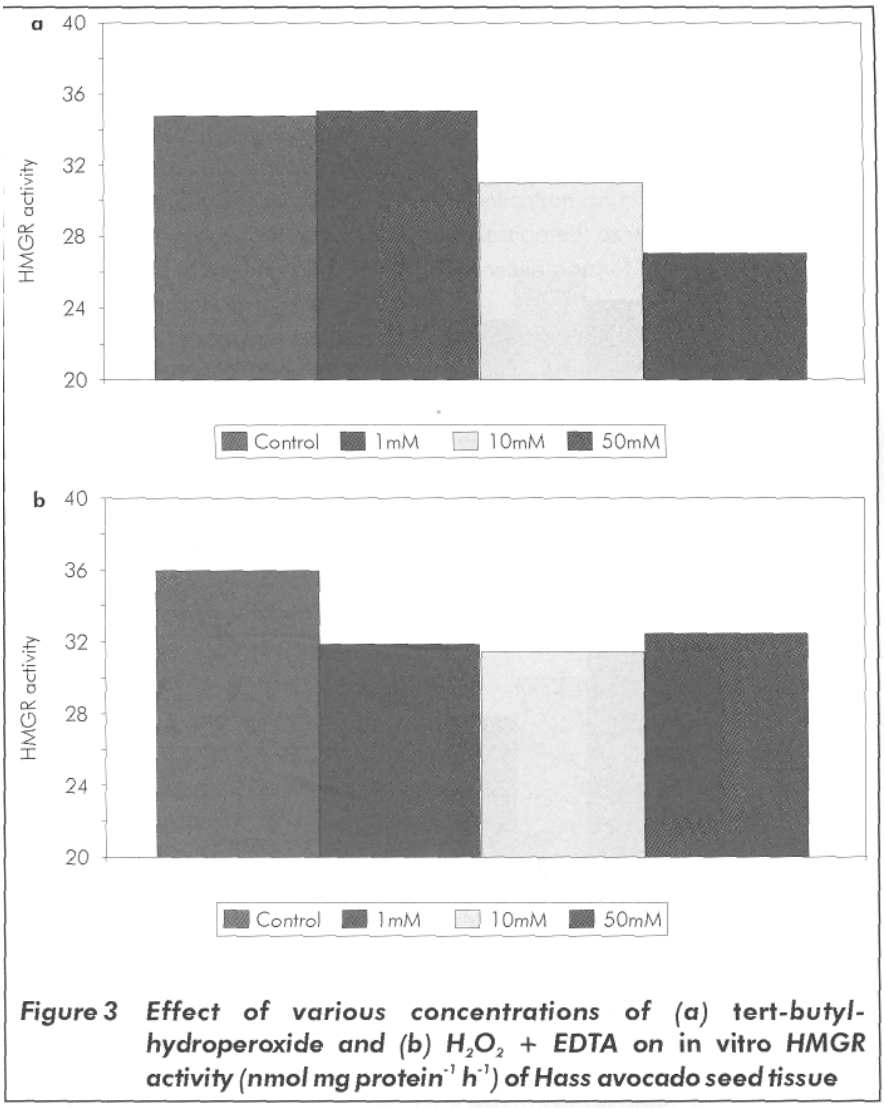

Figure 3 ***Effect of various concentrations of (a) tert-butyl-hydroperoxide and (b) H_2O_2 + EDTA on in vitro HMGR activity (nmol mg protein^{-1} h^{-1}) of Hass avocado seed tissue***

Sugar alcohols have been shown to be scavengers of AOS that are usually formed in response to pathogen invasion and severe stress (Pharr, *et al.* 1995; Smirnoff and Cumbes, 1989). Thus it was hypothesised that the C7 sugar alcohol, perseitol, may have a role of scavenging AOS, that might otherwise inactivate HMGR during Hass fruit development, leading to the appearance of the small-fruit variant. To test this hypothesis, the effect of exogenous perseitol and D-*manno*-heptulose on HMGR activity, and perseitol and D-*manno*-heptulose content of seed tissue was investigated (Richings and Cowan, unpublished data). These results showed that treatment of fruit with 1 mg mL^{-1} perseitol had no effect on either HMGR activity or the D-*manno*-heptulose content. As expected the level of perseitol in the seed tissue increased following the application of perseitol. When fruit were treated with 1.25 and 2.5 mg mL^{-1} D-*manno*-heptulose, HMGR activity of seed tissue increased by 10 and 47% respectively. It is interesting to note that the D-*manno*-heptulose content of seed tissue from D-*manno*-heptulose-treated fruit declined, whereas the concentration of perseitol increased. This suggested that perseitol may function to protect important enzymes,

such as HMGR, that are required for normal growth and development, from oxidative damage or AOS-induced inactivation.

CONCLUSIONS

The investigations outlined above have shown that HMGR activity in avocado seed tissue is sensitive to AOS, and it would appear that there is an interaction between AOS and C7 sugars in avocado and that this interaction impacts on HMGR activity. Based on the results presented, a scheme is proposed to illustrate the possible interaction between AOS, C7 sugars and HMGR activity during Hass avocado fruit growth (Figure 4).

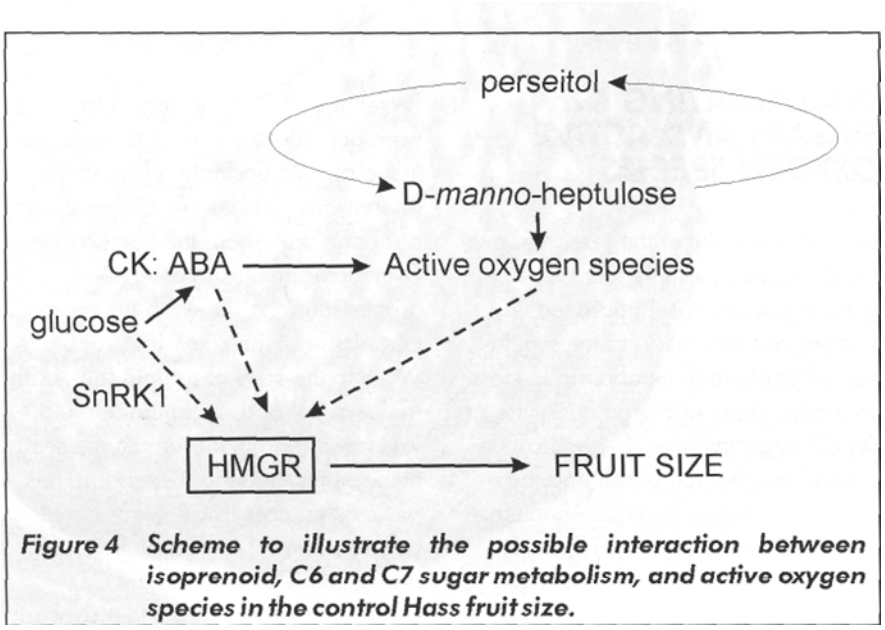

Figure 4 ***Scheme to illustrate the possible interaction between isoprenoid, C6 and C7 sugar metabolism, and active oxygen species in the control Hass fruit size.***

It is hypothesized that interconversion of perseitol and D-*manno*-heptulose may scavenge AOS that would otherwise inactivate HMGR during the normal course of avocado fruit growth, leading to reduced cell division and the appearance of phenotypically small fruit.

In conclusion, the scheme shown in Figure 4 links together the various aspects and results discussed thus far: the C6 sugars (most importantly glucose) and its effect on SnRK1 activity; the C7 sugars (most importantly the inter-conversion between perseitol and D-*manno*-heptulose), the CK:ABA ratio and its link to AOS and HMGR activity, and finally how all of these factors will affect Hass avocado fruit size. Thus this research has firstly demonstrated that the regulation of isoprenoid metabolism is a complex process which is affected by many factors. Secondly it has shown that isoprenoid metabolism is essential for normal fruit growth and development, and that an alteration in isoprenoid metabolism plays an important role in the development of the Hass small-fruit phenotype.

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LITERATURE CITED

ACKERSON, R.C. 1985. Invertase activity and abscisic acid in relation to carbohydrate status in developing soybean reproductive structures. *Crop Science* 25: 615-618.

BARKER, J.H.A., SLOCOMBE, S.P., BALL, K.L., HARDIE, D.G., SHEWRY, P.R. & HALFORD, N.G. 1996. Evidence that barley 3-hydroxy-3-methylglutaryl-coenzyme A reductase kinase is a member of the sucrose nonfermenting-1-related protein kinase family. *Plant Physiology* 112: 1141-1149.

BERGH, B.O. 1986. *Persea americana*. In Halevy, A.H. (Ed) Handbook of Flowering. CRC Press (Boca Raton) pp 253-268.

BURDON, R.H. 1996. Control of cell proliferation by reactive oxygen species. *Biochemical Society Transactions* 24: 1028-1032.

CAMPOS, N. & BORONAT, A. 1 995. Targeting and topology in the membrane of plant 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *The Plant Cell* 7: 2163-2174.

CHAPPELL, J. 1995. Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 46: 521-547.

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 catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Plant Physiology* 114: 511-518.

COWAN, A.K. & RICHARDSON, G.R. 1997. Carotenogenic and abscisic acid biosynthesizing activity in a cell-free system. *Physiologica Plantarum* 99: 371-378.

COWAN, A.K., RICHINGS, E.W. & CRIPPS, R.F. 1998. Metabolic control of Hass avocado fruit growth. *South African Avocado Growers' Association Yearbook* 21: 48-51.

CRIPPS, R.F., RICHINGS, E.W., TAYLOR, N.J. & COWAN, A.K. 1999. The Hass small fruit syndrome: solving a 50 million rand per season problem. South *African Avocado Growers' Association Yearbook* 22: 1-6.

GANCEDO, J.M. 1992. Carbon catabolite repression in yeast. *European Journal of Biochemistry* 206: 297-313.

GILLASPY, G., BEN-DAVID, H. & GRUISSEM, W 1993. Fruits: a developmental perspective. *The Plant Cell* 5: 1439-1451.

GRAY, J.C. 1987. Control of isoprenoid biosynthesis in higher plants. *Advances in Botanical Research* 14: 27-91.

HALFORD, N.G. & HARDIE, D.G. 1998. SNF1-related protein kinases: Global regulators of carbon metabolism in plants? *Plant Molecular Biology* 37: 735-748.

JANG, J-C. & SHEEN, J. 1994. Sugar sensing in higher plants. *The Plant Cell* 6: 1665-1679.

JANG, J-C. & SHEEN, J. 1997. Sugar sensing in higher plants. *Trends in Plant Science* 2: 208-214.

KLANN, E.M., HALL, B. & BENNETT, A. 1996. Antisense acid invertase *(TIV1)* gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiology* 112: 1321- 330.

KOCH, K.E. 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 47: 509-540.

KOCH, K.E., NOLTE, K.D., DUKE, E.R., McCARTY, D.R. & AVIGNE, W.T. 1992. Sugar levels modulate differential expression of maize sucrose synthase genes. *The Plant Cell* 4: 59-69.

KOJIMA, K., YAMADA, Y. & YAMAMOTO, M. 1995. Effects of abscisic acid injection on sugar and organic acid contents of citrus fruits. *Journal of the Japanese Society of Horticultural Science* 64: 17-21.

LA FORGE, F.B. 1917. D-mannoketo-heptose, a new sugar from the avocado. *Journal of Biological Chemistry* 28: 511-522.

MEHDY, M.C. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiology* 105: 467-472.

MILBORROW, B.V, BURDON, R.S. & TAYLOR, H.F. 1997. The conversion of 2-cis-[^{14}C] xanthoxic acid into [^{14}C] ABA. *Phytochemistry* 45: 257-260.

MOORE, K.B. & OISHI, K.K. 1994. 3-Hydroxy-methylglutaryl-coenzyme A reductase activity in the endosperm of maize vivipary mutants. *Plant Physiology* 105: 119-125.

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 and Cell Physiology* 39: 1027-1038.

NARITA, J.O. & GRUISSEM, W. 1989. Tomato hydroxy methylglutaryl-CoA reductase is required early in fruit development but not during ripening. *The Plant Cell* 1: 181-190.

OMKUMAR, R.V. & RAMASARMA, T. 1993. Irreversible inactivation of 3-hydroxy-3-methylglutaryl-CoA reductase by H_2O_2. *Biochimica et Biophysica Acta* 1156: 267-274.

PERATA, P., MATSUKURA, C., VERNIERI, P. & YAMAGUCHI, J. 1997. Sugar repression of a gibberellin-dependent signalling pathway in barley embryos. *The Plant Cell* 9: 2197-2208.

PHARR, D.M., STOOP, J.M.H., WILLIAMSON, J.D., STUDER FEUSI, M.E., MASSEL, M.O. & CONKL1NG, M.A. 1995. The dual role of mannitol as osmoprotectant and photo assimilate in celery. *HortScience* 30: 1182-1188.

RICHINGS, E.W., CRIPPS, R.F. & COWAN, A.K. 2000. Factors affecting 'Hass' avocado fruit size: carbohydrate, abscisic acid and isoprenoid metabolism in normal and phenotypically small fruit. *Physiologica Plantarum* 109: 81-89.

RICHTMYER, N.K. 1970. The isolation of volemitol and other polyhydric alcohols from avocado seeds. *Carbohydrate Research* 12: 135-138.

SALAS, J., SALAS, M., VINUELA, E. & SOLS, A. 1965. Glucokinase of rabbit liver. *Journal of Biological Chemistry* 240: 1014-1018.

SCHAFFER, A., SAGEE, O., GOLDSCHMIDT, E.E. & GOREN, R. 1987. Invertase and sucrose synthase activity, carbohydrate status and endogenous IAA levels during *Cucumis* leaf development. *Phytochemistry* 26: 1883-1887.

SHEEN, J. 1994. Feedback control of gene expression. *Photosynthesis Research* 39:

427-438.
SMIRNOFF, N. & CUMBES, Q.J. 1989. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28: 1057-1060.
WINGLER, A., VON SCHAEWEN, A., LEEGOOD, R.C., LEA, P.J. & QUICK, P.W. 1998. Regulation of leaf senescence by cytokinins, sugars, and light. *Plant Physiology* 116: 329-335.
ZILKAH, S. & KLEIN, I. 1987. Growth kinetics and determination of shape and size of small and large avocado fruits of cultivar 'Hass' on the tree. *Scientia Horticulturae* 32: 195-202.