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The role of cytokinin, auxin and abscisic acid metabolism in the control of Hass avocado fruit size

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ABSTRACT

Avocado fruit growth is the result of sustained cell division and cell expansion. Studies have revealed that cell division is the limiting factor in the control of fruit growth and that the endogenous cytokinin to auxin to abscisic acid ratio is critical in supporting continued fruit growth. Detailed biochemical studies have led to the proposal that cytokinin regulates auxin and abscisic acid metabolism by impacting on the molybdenum cofactor-requiring aldehyde oxidase, responsible for catalysing the conversion of indole-3-acetaldehyde to indole-3-acetic acid and abscisic aldehyde to abscisic acid. In order to evaluate in more detail the biochemical interrelationship between cytokinin, auxin and abscisic acid metabolism a number of enzymes involved in all three processes were evaluated in both normal Hass fruit and its small-fruit phenotype, together with a number of compounds that form the vital link between these three metabolic pathways. Results indicate that the cytokinin and auxin level relative to the abscisic acid level is lowered in small-fruit compared to normal fruit.

INTRODUCTION

The limiting factor in Hass avocado fruit growth has been found to be cell number (Cowan *et al.* 1997), which implies that any factor reducing cell division cycle activity will contribute to the development of the small-fruit phenotype. A crucial factor in the control of cell division and fruit growth appears to be the CK:ABA ratio, as evidence has been found that an imbalance in this ratio is pivotal in seed coat senescence and retardation of fruit growth (Moore-Gordon *et al.* 1998). Cytokinin and auxin are known to interact to control cell division (Coenen & Lomax, 1997) and thus the ratio of plant hormones controlling cell division can be extended to include auxin. As such, it is the ratio of CK and IAA to ABA that is postulated to play a key role in the control of fruit size. By studying hormone metabolism in the normal and small-fruit phenotype, the relationship between changes in the CK:IAA:ABA ratio can be evaluated in relation to fruit size.

The importance of the CK:IAA:ABA ratio is further emphasised by the possible interaction of the cytokinin, IAA and ABA biosynthetic pathways (Figure 1).

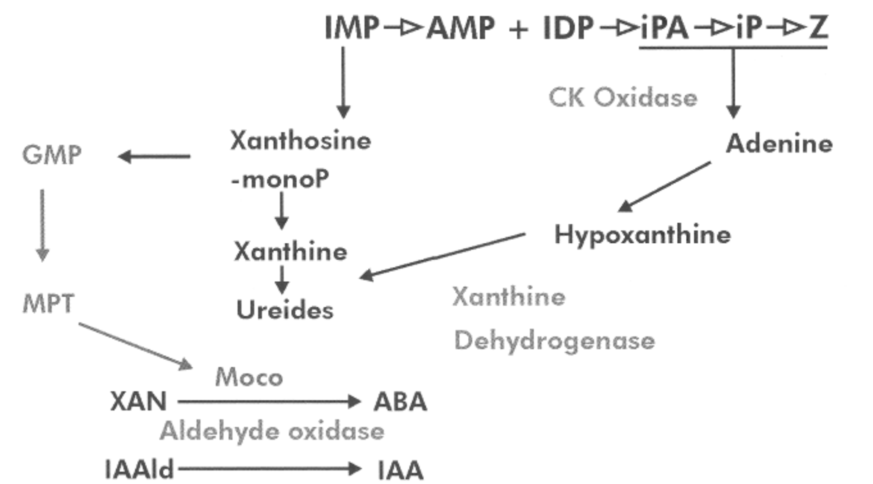

Figure 1 *Proposed scheme illustrating the relationship between cytokinin and ABA biosynthetic pathways (adapted from Cowan et al., 1999)*

This interaction is thought to occur through the production of a molybdenum cofactor (MoCo), required by the enzyme catalysing the final step of both IAA and ABA biosynthesis. This cofactor is synthesised from GTP (Mendel, 1997), a precursor to purines and thus cytokinin biosynthesis. Changes in cytokinin metabolism will therefore impact on the levels of GMP and thus the rate at which the MoCo can be synthesised and utilized. This will in turn impact on the activity of the enzyme catalysing the final step of IAA and ABA biosynthesis and thus changes in cytokinin metabolism can be linked to changes in IAA and ABA biosynthesis.

HORMONE HOMEOSTASIS IN HASS AVOCADO FRUIT

In an attempt to verify that changes in CK metabolism do affect ABA levels, fruit were treated with two cytokinin analogues. Results indicate that overall ABA metabolism is enhanced by cytokinin analogues (Table 1).

Table 1 *The effect of cytokinin analogues on ABA metabolism in ripening mesocarp tissue of normal Hass avocado fruit*

Treatment	ABA	PA	DPA	Total
			$ng\ g^{-1}$ FW (% control)	
Control	650.10 (100)	189.40 (100)	441.38 (100)	1280.88 (100)
6-chloropurine	1269.38* (195)	781.73* (413)	569.08* (129)	2620.19* (205)
2,6-dichloropurine	1110.15* (171)	312.25 (165)	529.00 (120)	1951.40* (117)

Values followed by * are significantly different ($P \leq 0.05$) from the control

This is illustrated by the fact that ABA and its breakdown products, phaseic and dihydrophaseic acid, all increased in fruit treated with cytokinin analogues. This helps to partly confirm the theory that there is a link between the cytokinin and ABA biosynthetic pathways.

Through the study of the enzymes involved in the biosynthetic pathways of CK, ABA and IAA in normal and small-fruit, an indication of how the CK:IAA:ABA ratio changes in these two fruit can be obtained. The enzymes chosen were 1) cytokinin oxidase (CK oxidase) and 2) aldehyde oxidase (AO). CK oxidase is responsible for the irreversible breakdown of cytokinins and as a result of this action it is thought to control the total cytokinin activity in tissues (Kaminek, *et al*. 1997). AO, on the other hand, is the enzyme responsible for catalysing the final steps of the biosynthesis of ABA (Walker-Simmons *et al.* 1989; Sindhu *et al.* 1990; Leydecker *et al*. 1995) and IAA (Koshiba *et al.* 1996; Lips *et al.* 1999). It is a molybdenum cofactor-requiring-enzyme and changes in CK metabolism are thought to impact on this enzyme (Cowan *et al.* 1999).

There does not appear to be any significant difference in CK oxidase activity between normal and small-fruit (Table 2).

Table 2 The activity of cytokinin oxidase, ABAld oxidase and IAAld oxidase in normal and small fruit. (ND = activity not detected)

Enzyme	NORMAL		SMALL	
	seed	mesocarp	seed	mesocarp
	enzyme activity mg^{-1} protein			
cytokinin oxidase	250.86	7.00	256.79	9.27
ABAld oxidase	6.64	7.48	19.58	8.71
IAAld oxidase	29.78	ND	2.51	ND

In both cases CK oxidase activity was higher in the seed than in the mesocarp. The activity of the enzyme, catalysing the final step in ABA production (ABAId oxidase), was generally higher in small-fruit than normal fruit, whilst the enzyme, catalysing the final step in IAA production (IAAId oxidase), was lower in small-fruit than normal fruit. This suggests increased ABA levels, but decreased IAA levels in small-fruit compared to normal fruit.

From the activity of these enzymes it is proposed that in normal fruit the CK and IAA level is elevated relative to the ABA level, whilst in small-fruit the CK and IAA level is reduced relative to the ABA level. The result of this is a favourable environment for cell division in normal fruit, but an unfavourable environment for cell division in small-fruit.

ENDOGENOUS PURINE LEVELS

If the proposed scheme of hormone interaction in Figure 1 is correct, then the change in hormone homeostasis, between small and normal fruit, should be reflected by a changed rate of purine metabolism and thus purine levels.

Small-fruit had much lower adenine and xanthine levels than normal fruit, but much higher hypoxanthine, GMP and IMP levels (Table 3).

Table 3 ***Purine levels in small and normal fruit***

tissue	adenine	xanthine	hypoxanthine	GMP	IMP
			μmol/g DW		
small fruit	691	13	9554	2618	553
normal fruit	8779	1464	4171	82	495

The lower adenine and xanthine levels in small-fruit is indicative of increased purine metabolism, as xanthine is a substrate for the key enzyme in purine metabolism. On the other hand, the higher GMP and IMP levels in small-fruit is indicative of a greater potential for MoCo production, which could lead to elevated activity of the MoCo-requiring enzymes, AO, xanthine dehydrogenase (XDH) and nitrate reductase (NR) in small-fruit.

As XDH is the key enzyme in purine metabolism, changes in purine levels should be reflected in changed XDH activity. XDH activity was higher in small-fruit than normal fruit, which confirms the measured purine levels in small and normal fruit and the proposed increase in purine metabolism in this fruit (Figure 2).

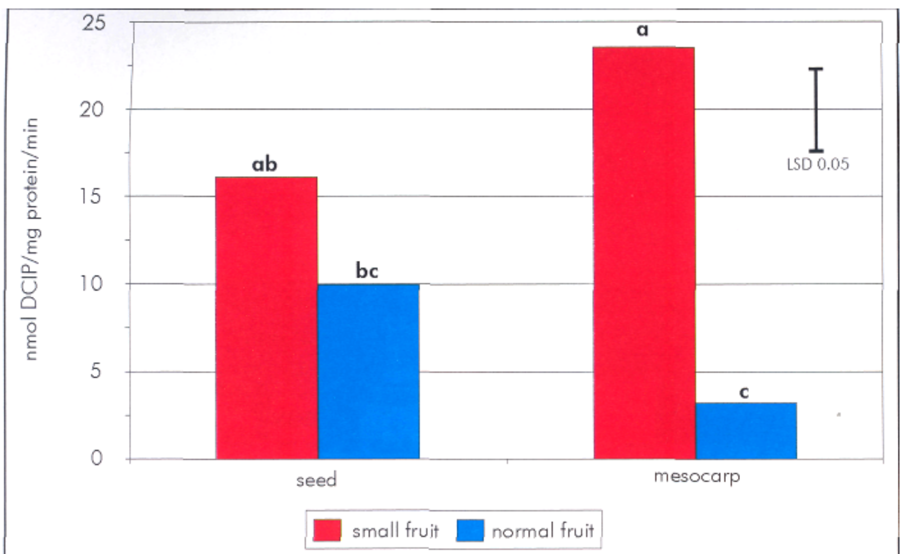

Figure 2 ***XDH activity in small and normal fruit***

CONCLUSION

To date, the control of hormone homeostasis, that will result in normal or reduced levels of cell division activity, is thought to occur as in Figure 3.

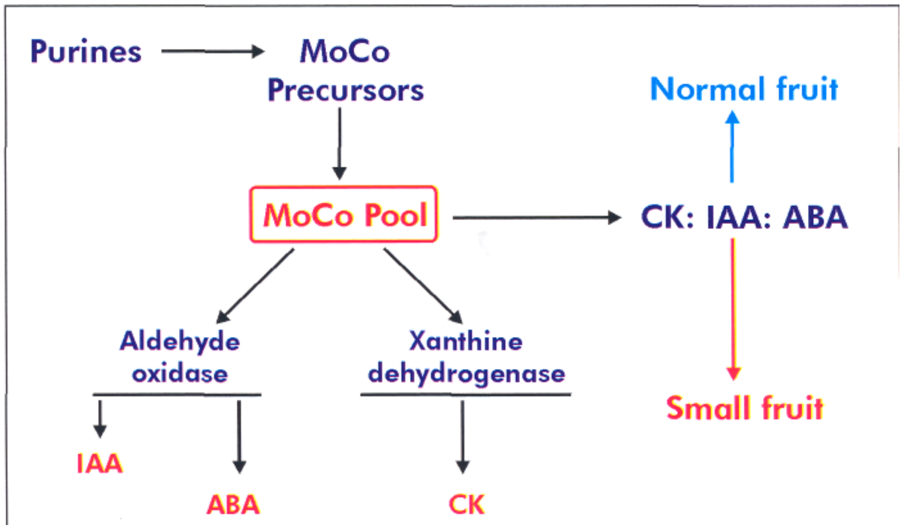

Figure 3 Proposed scheme for the control of hormone homeostasis in small and normal fruit

A change in purine levels will impact on the level of MoCo precursors needed for the synthesis of the MoCo. If these MoCo precursors are increased then the size of the MoCo pool will increase, which can then be allocated to the MoCo-requiring enzymes, AO and XDH. Depending on the relative allocation of the MoCo to these enzymes, hormone homeostasis will be altered. If the CK and IAA levels increase relative to ABA, it is proposed that cell division will be sustained and normal fruit will result, but if the CK and IAA levels decrease relative to ABA, then it is proposed that cell division will be reduced and small-fruit will result.

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