Evidence of acidic Invertase as a control step of sucrose level during ripening of two diploid banana fruit

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INTRODUCTION AND OBJECTIVES

• Sucrose metabolism is vital to multicellular plants. Its affects the behavior of organoleptic trait in fruit, the allocation of crucial carbon resources and the initiation of hexose-based sugar signals in importing structures.

• Sucrose Phosphate Synthase (SPS), Invertase (IV) and reversible Sucrose Synthase (SuSy) catalyze the sucrose metabolism pathway in vivo. Based on four (cooking and dessert) diploid banana varieties, we previously shown that i) dessert varieties accumulated more sucrose than cooking ones and that ii) acidic Invertase (AIV) could be one of the main determinants of the sugar composition of diploid banana fruit by correlating the ratio sucrose / hexoses (see Fils-Lycaon et al., poster).

• In the prospect of improvement of banana fruit quality throughout conventional breeding and marker assisted selection, identification of major or candidate gene(s) of which expression regulate the sucrose metabolism during banana fruit is an essential step for identification of related marker (s).

• Using two contrasted varieties as model, we investigate here the sucrose metabolism pathway at molecular level, in order to identify these major genes.


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MATERIALS AND METHODS

▪ Two diploid contrasted banana varieties were used, IDN110 (dessert, AA) (left pictures) and Sowmuk (cooking, AA) (right pictures).

▪ For both varieties, fruits were taken at two green physiological stages namely immature (IMG) and mature stages (MG). For late ripening stages, MG fruits ripen in air after acetylene-treated (10000ppm/24h/20°C) were taken at breaker (Br), more breaker (Br+), yellow (Yw) and ripe (Ri).

▪ RACE-PCR method and the online BLAST program were used for genes isolation and for sequence homology analysis, respectively.

▪ Genes expression was performed throughout real-time quantitative PCR using ABprism 7000 apparatus (Applied Biosystems, Courtaboeuf, France).

▪ Enzymes activities and, sucrose glucose and fructose were measured as described Fils-Lycaon et al. (see Fils-Lycaon et al., poster).


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RESULTS

▪ Isolation of sucrose metabolism genes

Sequence ID	Size (pb)	E-value	ORF (aa)	Blast Best Hit Description
MaCWI	2049	1148 E 0.0	586	cell wall invertase [Musa acuminata]
MaSuSy	1810	937 E 0.0	523	sucrose synthase [Oncidium cv. 'Goldiana']
MaSPS	1504	462 E-129	403	sucrose phosphate synthase [Oncidium cv. Goldiana']

Table 1: Sequence analysis of sucrose metabolism genes isolated from banana

▪ SPS and acidic invertase activities, and sucrose/hexoses ratio in pulp tissue of banana

	Physiological Stages of fruit	IMG	MG	Br	Br+	Yw	Ri
IDN110	SPS	nd	nd	106 7±9 6	168 6±14 8	119±3 9	106 1±11 6
	AINV	nd	nd	9 6±1 7	16 5±2 3	22 2±4 1	25 8±4 5
	Sucrose/hexoses	nd	nd	2 1	3 6	3 7	0 85
Sowmuk	SPS	nd	nd	120 7±17 8	177 6±11	202 1±11 9	222 2±24 9
	AINV	nd	nd	189±17 7	207±28 8	200±24 1	174 2±37
	Sucrose/hexoses	nd	nd	0 07	0 17	0 087	0 04

Table 2: Activities of sucrose metabolism enzymes and sucrose/hexoses (glucose +fructose) rat o during ripening of IDN and Sowmuk varieties. Enzymes activities are express inμmole.g⁻¹ of fresh weight.h⁻¹.

▪ Expression of sucrose metabolism genes in pulp tissue of banana

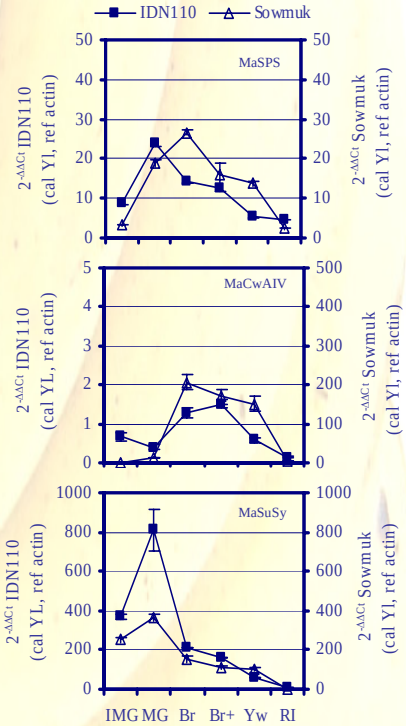

Fig 1: mRNA accumulation of sucrose metabolism genes during development and post-harvested ripening of two varieties banana (IDN110 AA dessert and Sowmuk AA cooking).

The y axis represents the relative fold d fference of mRNA level and was calculated using the 2-ΔΔCt formula with actin and young banana leaves as reference and calibrator respectively.

Each data point is the mean of va ues obtained from qPCR reaction performed in triplicate on one sample. Each sample was prepared from four fruits originated from three replicate bunches. Vertical bars indicate standard deviation (S.D.). When no bar is shown S.D. was smaller than the symbol.

DISCUSSION AND CONCLUSIONS

• The pattern of MaSPS, MaCWI, MaSuSy mRNA accumulation, during fruit development and ripening, was similar in both varieties.

• At equivalent stages, MaCw-AIV mRNA level was increased approximately 100-fold more in Sowmuk than in IDN110, concomitantly with AIV activity and conversely with sucrose level. No significant changes were observed for neutral invertase and Susy activities during late ripening stage (data not shown).

• Expression of MaCWI cDNA contributes to the high level of acidic Invertase measured during ripening. It appears as a key step that controls the sucrose level during ripening of diploid banana fruit.

• MaCWI cDNA can be considered as a major and candidate gene for identification of functional marker in the prospect of improvement of banana fruit quality traits throughout breeding program.

PERSPECTIVES

• Examine throughout genes isolation and characterization the impact of other cell wall and/or vacuolar invertase genes on acidic Invertase.

• Investigate on a large samples of cooking and dessert banana varieties, the relationship between structural diversity of the major AIV genes – their expression – AIV activity – sucrose level, in order to identify the candidate genes and functional marker for breeding program.