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CHANGES IN THE LEVELS OF COMMON ENDOGENOUS POLYAMINES IN THE PERICARP AND SEEDS OF MANGO FRUITS DURING DEVELOPMENT

G.S.R. MURTI* AND K.K. UPRETI

Plant Hormones Laboratory, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore – 560 089

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SUMMARY

Levels of common free polyamines in pericarp and seeds of developing fruits of mango cv. Alphonso were analyzed to examine their relationship with fruit growth and development. Putrescine was the major polyamine in fruit pericarp and spermine in the seed. The levels of total polyamines and individual polyamines, putrescine and spermine increased in the pericarp and were highest at 45 and 30 days during the years 2000 and 2001, respectively. In seeds, total polyamines, spermine and spermidine levels were high at 90 and 75 days after fruit set during the same years. The high polyamine concentration in fruit preceded its active growth phase and in case of seed it coincided with the active growth phase.

***Key words*: Fruit development, mango, pericarp, polyamines, seed.**

INTRODUCTION

Polyamines are among the most widespread of the low molecular weight, nitrogenous non-protein metabolites with growth regulatory functions reported to be involved in various physiological processes. The titer and metabolism of polyamines are known to change during fruit development and ripening (Evan and Malmberg 1989, Martin-Tanguy 1997). However, the pattern of change in polyamines differs in fruits of different species. While in case of apple (Biasi *et al.* 1988), avocado (Kushad *et al.* 1988), pear (Toumadji and Richardson 1988), grapes (Shiozaki *et al.* 2000), pepper (Serrano *et al.* 1995) and strawberry (Ponappa and Miller 1996), the polyamine levels were high in the early phase of fruit development and were found to decline gradually towards the completion of fruit development, their levels in fruits like mandarin (Nathan *et al.* 1984), orange (Hasdai *et al.* 1988) and cherimoya (Escribano and Merodio 1994) reportedly increased during maturity and ripening. Mango is an important fruit crop of India exhibiting wide variation in the pattern of flowering and fruiting. Investigations have been carried out in this laboratory to examine the changes in the levels of plant growth substances such as ABA, ethylene, IAA and cytokinins in relation to fruit growth and development in mango (Murti and Upreti 1995, 1997). However, information in respect of polyamines in relation to fruit development in mango is lacking. In the present study, an attempt has been made to (i) analyze the level of free polyamines in the pericarp and seeds of mango fruits cv. Alphonso at different growth stages, and (ii) to examine their relationship with fruit growth and development.

MATERIALS AND METHODS

Experiments were conducted in the mango (*Mangifera indica* L.) orchard of the Indian Institute of Horticultural Research, Hessaraghatta. A large number of fruits were labeled during January, 2000 and 2001. The samples were drawn from different trees and pooled appropriately from fruit set (fruitlets at mustard stage) to maturity at 15 days interval. The average fresh weight of

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*Corresponding author: email: gsrm@iihr.kar.nic.in fruits and seeds was recorded. The fruits were analyzed for free polyamine levels in the pericarp and seeds. The polyamines were analyzed by HPLC using the procedure of Flores and Galston (1982). Five gram sample of fruits/ seeds was homogenized with 20 ml of chilled 5% (v/v) perchloric acid. The acidic extract was kept overnight at 4°C and then centrifuged (3200g, 30 min, 4°C). The supernatant containing the free polyamines was stored in glass vials at 0°C. Following the transfer of 1.0 ml of the supernatant in a test tube, 2.0 ml of 2N NaOH and 50µl of benzoyl chloride (99% w/v) were added, vortexed thoroughly for 1 minute, and then allowed to stand for 30 minute at room temperature. Following this, 4.0 ml of saturated NaCl was added and the benzoylated polyamines were extracted in 6.0 ml of chilled diethyl ether. The ether phase was aspirated out, dried under N_2 stream and redissolved in 50 µl of methanol for HPLC. A reverse phase bondapak C_{18} column was used for HPLC and the analysis was done in the isocratic mode (62% methanol containing 1% acetic acid) at a flow rate of 1.0 ml/min with UV detector (Waters M 486) set at 282 nm. Under these conditions, benzoylated polyamines, putrescine, spermidine and spermine were completely resolved and eluted at the retention times of 5.37, 7.55 and 11.53 minutes, respectively. The quantification of free polyamines was carried out on peak area basis using polyamine standards (Sigma make) benzoylated similarly.

RESULTS AND DISCUSSION

Fruit growth and seed development: The fruit growth in terms of its fresh weight exhibited a single sigmoidal curve (Fig 1). The growth was slow upto 45-60 days following fruit set and increased rapidly thereafter till 120 days. The observed growth response confirmed earlier findings in developing fruits of mango cv. Alphonso (Murti and Upreti 1995, 1997). The initial slow growth may be attributed to cell division activity at the early stages, while the relatively rapid increase in fruit weight subsequently may result from the phase of active cell elongation. This assumption needs an investigation. The seed was visible following 45 days of fruit set (Fig. 1). The pattern of seed growth was similar to that observed for the fruits. The seed fresh weight increased sharply during 60 to 105 days, and the period of active growth of seed broadly coincided with that of the fruit.

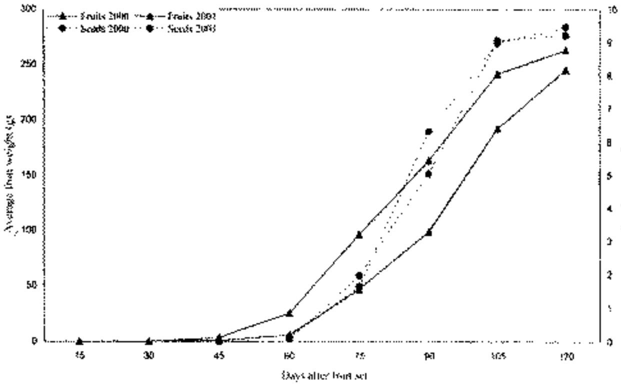

Fig. 1. Increase in fresh weight of fruits and seeds in mango cv. Alphonso

Changes in polyamines: Putrescine, spermidine and spermine were the major polyamines detected in the extracts of pericarp and seeds of developing mango fruits. Putrescine was found to be the dominant polymine in the extract of fruit pericarp during most of the growth stages, while in case of seed it was spermine. The levels of total as well as individual polyamines exhibited marked variation, both in fruits and seeds of different growth stages (Table 1). In the pericarp, total and individual polyamines were found to increase after fruit set in the samples from both the years, with total polyamines, putrescine and spermine showing a peak at 45 and 30 days during the year 2000 and 2001, respectively. Spermidine levels were highest in fruit samples at 60 days in both the years (Table 1), later on the polyamine levels declined up to 105 days. In case of seeds, the total polyamines as well as spermine and spermidine levels increased from 60 days with highest values being recorded at 90 days in the year 2000 (Table 1). In contrast, the putrescine concentration in seeds was high initially (60 days) and declined steadily with fruit maturity. During the year 2001, the total and the individual polyamines levels were found to increase from 60 days and peaked at 75 days, followed by a decline (Table 1). There was slight increase in the seed spermine concentration after 105 days.

The results show that the polyamine concentration during the development of fruit and seed is high at initial stages. The high polyamine concentration in seed broadly coincided with the active phase of fruit growth; however during this period, the polyamine concentration in fruit

Table 1. Polyamines (nmol/g fresh wt) in relation to fruit and seed development in mango cv. Alphonso.

Days after fruit set & fruit part	Putrescine			Spermidine			Spermine			Total polyamines		
	2000	2001	Average	2000	2001	Average	2000	2001	Average	2000	2001	Average
Fruit 15	187.0	94.5	140.8	57.0	36.3	46.7	124.5	226.6	175.6	368.5	357.4	363.0
Seed												
Fruit 30	350.3	552.0	451.2	58.3	30.2	44.3	183.5	353.2	268.4	592.1	935.4	763.8
Seed												
Fruit 45	420.7	222.1	321.4	157.9	63.0	110.5	299.3	181.3	240.3	877.9	466.4	672.2
Seed												
Fruit 60	260.9	129.7	195.3	218.5	154.6	186.6	200.8	91.8	146.3	680.2	376.1	528.2
Seed	255.6	209.2	232.4	100.4	54.2	77.3	183.4	293.5	238.5	539.4	556.9	548.2
Fruit 75	156.1	91.4	123.8	120.4	70.0	95.2	129.3	67.0	98.2	405.8	228.4	317.1
Seed	213.5	323.3	268.4	207.6	196.9	202.3	306.6	453.8	380.2	727.7	974.0	850.9
Fruit 90	97.0	98.2	97.6	63.0	54.6	58.8	92.3	56.1	74.2	252.3	208.9	230.6
Seed	124.0	187.6	155.8	293.0	119.2	206.1	397.4	210.3	303.9	814.4	517.1	665.8
Fruit 105	162.7	143.3	153.0	44.9	33.7	39.3	117.2	64.0	90.6	324.8	241.0	282.9
Seed	92.6	116.7	104.7	196.6	74.5	135.6	337.3	171.9	254.6	626.5	363.1	494.8
Fruit 120	203.8	87.5	145.7	36.0	50.0	43.0	114.2	94.0	104.1	354.0	231.5	292.8
Seed	106.5	113.1	109.8	114.9	69.7	92.3	264.9	237.4	251.2	486.3	420.2	453.3

pericarp recorded a decline, possibly due to the dilution effect as a consequence of increase in the rate of fruit growth. Similar trends were witnessed in the fruits of apple, avocado, pear, etc. The highest concentration of total polyamines, spermine and putrescine in the pericarp preceded the period of rapid growth, while that in case of spermidine coincided with the actual period of growth. In seeds, however, the peak of total as well as all individual polyamines coincided with the active phase of seed growth. The high concentration of polyamines at initial stages of fruit and seed growth may be associated with the period of cell division during this phase, as polyamines are known modulators of this event (Kumar *et al.* 1997). In earlier studies, higher cytokinin levels have been reported during initial stages of fruit development (Murti and Upreti 1997), and the cytokinins have been reported to exert positive influence on polyamines (Suresh *et al.* 1978, Cho 1983). The observed higher levels of polyamines at initial stage of fruit development could result from such an influence. Also, high ethylene production has been reported in fruits during 60 to 105 days of growth (Murti and Upreti 1995). The polyamine concentration in the pericarp during this period was found to be low in this study. Ethylene and polyamines share a common intermediate metabolite SAM (S-adenosylmethionine) in their biosynthetic pathway (Evans and Malmberg 1989); thus the lower polyamine concentration in the pericarp may be a consequence of greater channeling of SAM for ethylene production, in preparation for the processes of fruit maturity and ripening.

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