

# **OPTIMIZATION, VALIDATION AND APPLICATION OF RADIOIMMUNOASSAYS FOR PLANT GROWTH SUBSTANCES IN AVOCADO (*PERSEA AMERICANA* MILL.) FRUITS**

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## **ABSTRACT**

The objective was to develop, optimize and validate radioimmunoassays (RIA) for several plant growth substances (PGS) and then apply the RIA's to determine PGS trends in 'Fuerte' avocado fruits from fruit set to fruit ripening.

Antibodies to the cytokinin isopentenyl adenosine (IPA) were obtained from rabbits inoculated with a periodate-derived IPA-BSA conjugate. The antiserum cross-reacted (25%) with only 2iP (isopentenyl adenine). The RIA measuring range was from 0, 1 to 100 ng. Anti-IPA serum was used to develop a RIA for 2iP, with a measuring range from 0,5 to 100 ng. Using Dowex 50W-X8 and cellulose TLC purified avocado fruit extract, 20 samples per day could be processed.

The RIA for abscisic acid (ABA) was developed from rabbit antibodies from an inoculated carbodiimide-derived ( $\pm$ ) ABA-BSA conjugates. The free active ABA component was isolated prior to quantitation by RIA by solvent partitioning and silica gel TLC.

The indole-acetic-acid (IAA) RIA was established from sheep-produced antibodies to a formaldehyde-derived IAA-BSA conjugate, after repeated inoculations. For both the ABA and IAA RIA's, contaminants in the avocado tissue were removed by solvent partitioning.

Developing avocado fruits, and especially young fruits were rich sources of IAA and 2iP in particular with seed concentrations exceeding those of the fruit flesh. The concentration of ABA rose throughout fruit development, reaching 100 ng g<sup>-1</sup> in the flesh at fruit maturity. Just prior to seed and fruit maturity, relatively high levels of IAA, 2iP and IPA were associated with the thick, fleshy testa, rapidly declining to zero as the testa dried. The avocado fruit is physiologically dependent on the seed right up to this stage, and testa maturity correlates well with minimum "legal" maturity of 80% moisture content of flesh.

In ripening avocado fruit, the concentration of free ABA rose as softening progressed. Total ABA concentrations fell initially, and then rose after a firmometer reading between 50 and 60 (100 coincides with eating ripeness). Later -harvested fruit had double the

ABA concentration of early harvested fruit. The ripening stimulus appeared to be related to moisture stress in the fruit.

This study has confirmed the prime advantages of sensitivity, specificity and rapidity of RIA, as well as its usefulness in multi-PGS studies and batch-type analysis.