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Progress Report on Study of Sclereid Formation in Avocado Fruit Pericarp

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The development of sclereids, or stone cells, in fruit tissue is a common characteristic of many horticultural fruit species. The nuts as a group, such as walnuts, pecans, and hazelnuts, have their hard "shell" composed entirely of closely packed, highly lignified stone cells. Many "soft" fruits, however, actually develop stone cells to various degrees; but these cells generally are not a major constituent of the edible portion of these soft fruits. Pears of such varieties as Bartlett have a few stone cells. A noticeable quantity of clusters of stone cells is found in the so-called "sand pears" and Kieffer pears (2). Stone cells are also found in some tissues of such soft fruits as the avocado and sapodilla. The development of excessive stone cell clusters in otherwise soft fruit tissue can adversely influence the eating quality of the fruit. Selection of superior horticultural varieties generally involves the avoidance of such "gritty," hard cell clusters which could detract from the eating quality of the particular fruit.

The very unusual observance of avocado fruit with excessive stone cells in the pericarp or edible fruit wall (1) warrants continued investigation into the description and causative factors of the aberrant fruit tissue. There is need to investigate all possible factors which might be associated with this physiological and morphological modification of the fruit tissue. The previous report on this observation of so-called "stony fruit" (1) resulted in the development of the term "sclerocarpelosis" which adequately describes the basic problem. Some of the normally soft, edible cellular structure of the fruit pericarp in this particular case is converted into hard, closely packed, gritty cellular masses which would generally be considered inedible. This stony tissue is composed almost entirely of masses of sclereids, or simple stone cells.

The normal development and structural features of the avocado fruit have been described in the literature (1, 3, 4). The avocado fruit is a botanical berry, having a soft edible wall of generally uniform structure throughout and a single large seed. The thick fruit wall in some varieties contains modified tissue near the outer skin surface in the form of sclereids which range from none to small clusters located just beneath the epidermis. Fruits of the Mexican varieties have none to few sclereids while some of the "thick-shelled" or "thick-skinned" types characteristic of Guatemalan varieties are characterized by a thick and substantial layer of stone cells in the outer subepidermal tissues. Such thick-skinned varieties attain a thickness of more than 3/8" in extreme cases. Frequently, a single layer of thick-walled lignified sclereids can be identified in the cellular layer, the endocarp, immediately surrounding the seed. This layer is generally only one cell in thickness and it becomes indistinguishable as the fruit reaches maturity and softens.

Aside from the vascular strands which sometimes darken in mature fruits of some avocado varieties, the major tissue which comprises the edible, soft pericarp wall is the parenchyma. This tissue consists of simple, thin-walled cells. These cells are isodiametric in shape. This tissue contains the oil droplets and other constituents which are of major commercial value in the fruit. A single parenchymatous cell is characterized by a thin wall and by its potentiality to undergo division at nearly all stages. The fact that many of these parenchymatous cells are in stages of active division as long as the fruit is attached to the tree causes the avocado fruit to be unique among our common fleshy fruits. Thus cell division in the pericarp parenchyma results in continuous enlargement of the fruit through the developmental life of the fruit from fruit set to maturity.

A series of experiments was designed to investigate the effect of specific herbicide molecules on cell and tissue differentiation of tissue explants taken from avocado pericarp. This technique of growing avocado fruit callus in *vitro* has been well demonstrated. The usual response of such cultures is the development of cellular proliferation and formation of a simple callus with little or no cellular differentiation. In the present investigations, a series of concentrations of herbicides alone and in mixtures were incorporated in the tissue culture media. These herbicides are currently used extensively in the field for weed control. Among the herbicides used in the media were Rhodamine, Repel, Roundup, Cygon, Princep, Krovar, and Vendex. A simple water extract of the soil obtained under herbicide sprayed trees was also used in some of the media. Following a period of six weeks to three months incubation, sections of the callus were made for microscopic examination.

The results of many observations can be summarized in that extensive callus resulted in most instances, except where very high concentration of herbicide resulted in death of the mother tissue disc. It was noted in many specimens which had been growing for two or more months that several instances of tracheid formation to various degrees were evident. The tracheids in these instances are characterized by thickened cell walls which become highly lignified. The pitting of the tracheid walls is very prominent, consisting of large simple pits. Such tracheids appear in linear sequence, in rows, or in scattered clusters suggestive of the sequence in development of a primitive xylem tissue.

Occasionally, there was observed in such callus tissue isolated thick-walled parenchyma with simple small pits which suggest the potential development of a sclereid. The cell wall in these few cases was not very thick, but was highly lignified. Very few of such cells were noted. The more prominent tracheid type of cellular differentiation was more commonly noted.

The appearance of sclereids in nature is rather widespread in various tissues of stem, leaf, fruit, and roots. The factors which initiate and govern the development of these specialized cells are not well defined or actually known. Wounding of tissues sometimes can induce sclereid formation. High degrees of metabolic activity and high osmotic pressures or high sugar concentration have been associated with some sclereid formation. High enzyme activity involving cytochrome oxidase also has been related to sclereid formation. Calcium metabolism may possibly be related to cell wall thickness under conditions where the addition of calcium increases the cell wall rigidity, the result of cross-linkage between chains of calcium pectate, while auxin reduces the activity of

calcium causing the cell wall to become soft.

The tentative conclusion drawn from the present series of investigations in relation to the stony avocado problem is that the several herbicides tested proved toxic in some instances of high concentration, but no evidence of excessive cell wall thickening could be detected. The toxicity level was not determined in the present experimental series. There was no evidence of a specific response to a given herbicide by the formation of a sclereid cell. Some isolated cases of cellular differentiation which possibly might be interpreted as sclereid formation were noted, but these were not reproducible within the present experimental conditions. Cellular differentiation in the form of tracheid formation, sometimes rather extensive in nature, was noted in many sections exposed to herbicide molecules. Tracheids are commonly observed in tissue cultures of avocado pericarp.

Literature Cited

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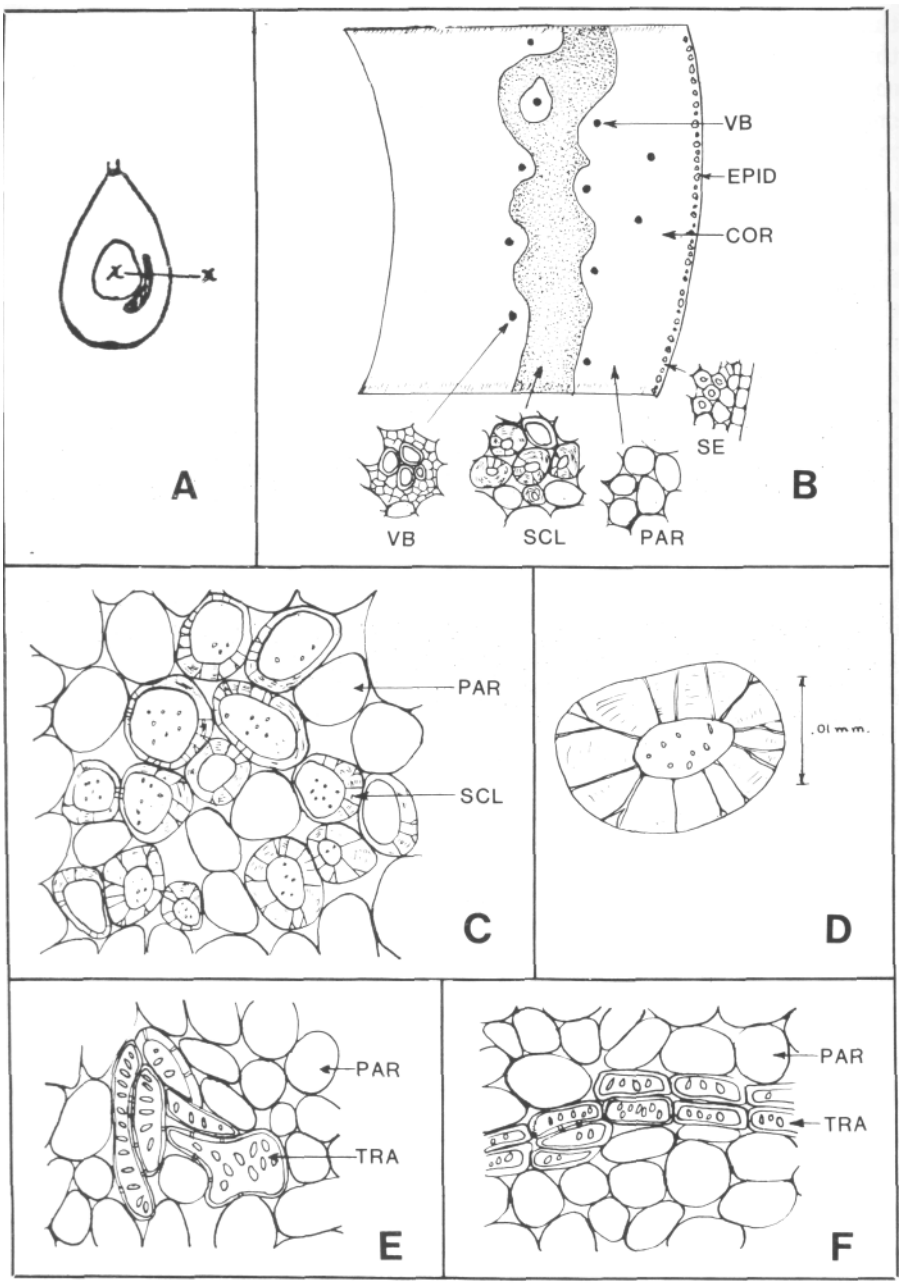

Figure 1: *A—Area of avocado fruit pericarp. B—Type of tissue represented in cross-section of pericarp at point x-x. C—Early stage of stone cell differentiation in pericarp of stony avocado. D—Single, fully developed sclereid. E-F—Tracheid development in callus from tissue culture of pericarp parenchyma. VB—Vascular bundle, EP—Epidermis, COR—Cortex, SCL—Sclereid, PAR—Parenchyma, TRA—Tracheids, SE—Subepidermal layer.*