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## **Role of oxidative metabolism in the onset of senescence in Plant Storage**

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### **Introduction**

The research program undertook to examine and develop the concept that the aging process (senescence) in plant storage organs is an oxidative phenomenon.

Plant storage organs (fruit, tubers) were selected as test systems since:

- a) they function as independent organs and, therefore, free of metabolic interactions with other plant parts;
- b) they display uniformity in anatomy and metabolism, and therefore, the aging process as reflected in tissue masses can be studied as cellular aging, and
- c) the onset of senescence in these tissues can be strictly regulated.

To support the hypothesis that senescence is an oxidative process it is necessary to show the formation and the activity of active oxygen forms, because molecular oxygen has low kinetic activity and does not readily react with metabolites. In contrast active oxygen species, including  $\text{H}_2\text{O}_2$ ,  $\text{HO}_2$ , or  $\text{HO}\cdot$ , are singlet compounds and can attack cellular constituents (16).  $\text{H}_2\text{O}_2$  was selected as a likely oxidant leading to senescence because the compound is relatively stable (15) and its utilization depends mostly on the activity of enzymes (peroxidases) (15, 31). These conditions are compatible with the concept that senescence in storage organs is a regulated phenomenon depending on the *de novo* synthesis of enzymes (10) and hormonal action (28).

The following sections present the evidence in support of the above hypothesis, and, forecast some of the possible future work.

### **Background work**

#### **A. Relation of $\text{H}_2\text{O}_2$ to the onset of senescence**

a) The onset of fruit senescence is normally accompanied by a pronounced increase in  $\text{H}_2\text{O}_2$  (1, 11). Conditions favoring the formation of the peroxides, e.g. application of  $\text{H}_2\text{O}_2$  substrates, stimulate senescence, and conversely, inhibition in the synthesis of  $\text{H}_2\text{O}_2$  leads to the deferral of fruit senescence (1). Similarly, inhibition of peroxidase and, thereby, the utilization of  $\text{H}_2\text{O}_2$  also resulted in a corresponding inhibition of fruit senescence (32). Identical results were obtained also in senescing leaves (24).

b) Compounds which accelerate senescence processes in storage organs (e.g. ethylene) have been shown to stimulate the formation of  $\text{H}_2\text{O}_2$

(1, 3, 4), suggesting that their action consist, in part, of the activation of oxygen, as shown in the formation of  $H_2O_2$ .

c) High concentrations of  $O_2$  augment the action of ethylene, or other senescence factors (3, 4, 20, 26, 27). These data is consistant with stimulatory effect of  $O_2$  on  $H_2O_2$  forming enzymes (30). It thus appears that senescence promoting factors act, in part, by stimulating the metabolic activation of oxygen.

d) This concept is applied in practice to the regulation of senescence. Storage at low oxygen concentrations is used to defer the onset of senescence in plant storage organs. In contrast, high  $O_2$  concentrations are employed to accelerate fruit senescence (5, 7) and can overcome the natural resistance to senescence in non-senescent fruit mutants (12).

### *B. The metabolic effects of $H_2O_2$*

a) The increase in the level of  $H_2O_2$  leads to a significant decline in sulfhydryl group (19) and apparently to the oxidation of other reducing functional groups. Decline in sulfhydryl group in turn, markedly accelerates the onset of fruit senescence (6).

b)  $H_2O_2$  may also be involved in hormonal turnover. Indole acetic acid (IAA) is a senescence inhibitor in fruit (8) whereas the IAA oxidation products are senescence promoters (5, 9).  $H_2O_2$  is utilized by peroxidase, to oxidize IAA (2, 31) and thereby, depreciate the action of a senescence retardant (IAA) and simultaneously result in the formation of a senescence promoter, and thus promotion of senescence.

c)  $H_2O_2$  is implicated in the biosynthesis of ethylene (11, 21) a potent accelerator of senescence in plants.

d) The increase in  $H_2O_2$  in senescing fruit is (1) accompanied by a similar increase in lipid peroxides (11). The latter may represent the oxidation of membraneous system by  $H_2O_2$  and the loss of cellular organization.

The outlined results show that senescence processes in plant storage organs are accompanied, and influenced, by the formation of  $H_2O_2$ . The peroxide is the product of the metabolic activation of oxygen, as regulated by the action of ethylene and other factors. Preliminary results also show some of the metabolic effects initiated by the oxidative action of  $H_2O_2$ .

The free radical theory of aging has been previously proposed in mammalian systems (13, 14, 17, 18, 22, 23, 25, 29). The work at Rutgers supports the concept as it applies to plant systems, and indicates clear advantages of utilizing plant test systems to further test and develop the concept.

### **Future work**

To establish the concept that senescence in plants is an oxidative phenomenon it is required to show that the onset of the process is accompanied by the increase in the relative abundance of oxygen in the cellular matrix and that  $H_2O_2$  is the oxygen species functioning as the active species in oxidation.

It is also required to identify defined metabolic systems which can undergo a change in function by changes in the redox state, and can lead to the onset of senescence.

Finally, it is necessary to identify the pathway (s) leading to the activation of molecular oxygen (formation of  $H_2O_2$ , or other active oxygen species) and establish how they are regulated.

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