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Histochemical Studies of Wound Periderm Formation

IV. Changes in Activity of Phenol Oxidases

By

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It has previously been shown (BABA, 1953 and 1955) that the activity of nadi oxidase has some close connection with the occurrence of cell division in the wounded tuber tissues of *Solanum tuberosum* and *Helianthus tuberosus*.

In the tubers of *Solanum tuberosum*, the polyphenol oxidase has been considered the principal terminal oxidase of their respiration (BOSWELL, 1945 and 1950; BOSWELL et al., 1938). As to *Helianthus tuberosus*, BELVAL and LEGRAND (1946 a and b) have found the presence of phenol oxidase in the tuber. While, the polyphenol oxidase system has found to be activated after wounding (*Cf.* VAN FLEET, 1952). From these observations mentioned above, the changes in activity of phenol oxidase in wounded regions may also be expected to occur during a wound periderm formation. Therefore an attempt is made in this study to see the relation between the occurrence of cell divisions and the activity of some phenol oxidases during the wound periderm formation.

Material and Methods

Tubers of *Solanum tuberosum* and *Helianthus tuberosus*, roots of *Raphanus sativus* were used as material. Material, cut with a sharp knife, was kept in a moist container at a temperature of about 30°C. for 24, 48, 72 and 96 hours. Tissues about 0.5 mm. in thickness, were excised parallel to the cut surface. One gm. of the freshly excised tissues was homogenized in 9 ml. of M/100 phosphate buffer solution at pH 5.5. The phenol oxidase activity was measured with a WARBURG manometric apparatus. The components of the test vessel for the phenol oxidase preparation were as follows:

vessel: 1.0 ml. of the homogenate and 1.4 ml. of M/100 phosphate buffer solution of pH 5.5.
side arm: 0.6 ml. of aqueous solution of the substrate (phenol compound), which will be described in appropriate places in this paper.
midwell: 1 ml. of 20% aqueous potassium hydroxide solution.

The components added into the control vessel were the same as those given in the test vessel with one exception: the side arm had 0.6 ml. of distilled water instead of the substrate.

The vessels, prepared as above, were equilibrate for 15 minutes in a constant temperature bath at 30°C., and the phenol compound solution in the side arm was added into the main vessel in which the homogenate had been suspended in the phosphate buffer solution. Just after the addition, the volume of oxygen consumption was followed at 10 minute intervals for an hour, and the total volume of the oxygen consumption for an hour was measured.

Results

The results of the morphological observation of the tissue during the wound periderm formation were the same as those given in the previous paper (BABA, 1955), and only the results obtained in the oxidase measurements will be given below.

As the volume of oxygen consumption in the control vessel (the homogenate alone) in this experiment was about 10 microliters per hr. per 1.0 ml. of the homogenate at its maximum, the phenol oxidase activity was obtained by deducting the volume of oxygen consumption of the control vessel from that of the respective test vessel, as shown in the Diagram 1, 2, 3 and 4.

1) *Pyrocatechol oxidase*

The activity of pyrocatechol oxidase in the tissue homogenate was determined by using M/30 pyrocatechol aqueous solution as a substrate. Diagram 1 shows the changes in pyrocatechol oxidase activity within 96 hours after the cutting. In *Solanum tuberosum* and *Helianthus tuberosus* immediately after the cutting, the addition of pyrocatechol into the homogenate results in an increase of oxygen consumption, however, in *Raphanus sativus* such an increase did not occur. The activity of pyrocatechol oxidase in *Solanum tuberosum* and *Helianthus tuberosus* shows a marked increase in the lapse of time after the cutting (Diagram 1). On the contrary, little activity was observed in *Raphanus sativus* within 96 hours after the cutting. Cell divisions leading to the wound peri-

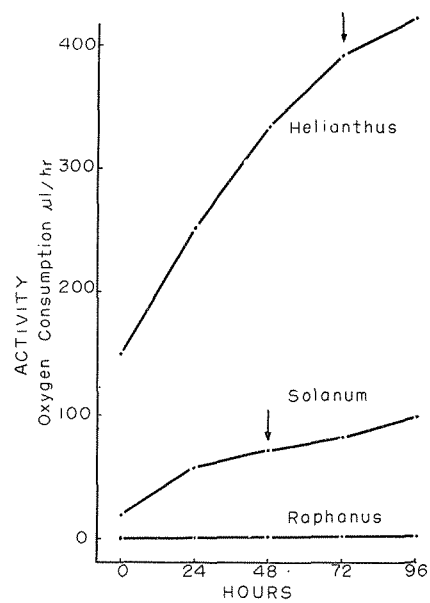

Diagram 1. Diagram showing the relation between the pyrocatechol oxidase activity and the lapse of time after the cutting.

The ordinates represent the oxidase activities (see text) of the preparation; 1 ml. of the homogenate, 1.4 ml. of the phosphate buffer solution and 0.6 ml. of M/30 pyrocatechol aqueous solution. The abscissas represent the times after the cutting. The arrows mark the time at which the first cell divisions are observed, whether locally or entirely along the cut surface.

derm are observed entirely along the cut surface at 48, 72 and 96 hours after the cutting in *Solanum tuberosum* (BABA, 1955) and partly along the cut surface at 72 and 96 hours in *Helianthus tuberosus* (BABA, 1955). In *Raphanus sativus*, such cell divisions were not observed within 96 hours after the cutting (BABA, 1955).

2) *Cresol oxidase*

The activity of cresol oxidase in the tissue homogenate was determined by using an aqueous solution of 0.025% o-cresol, m-cresol or p-cresol as a substrate. In the homogenate immediately after the cutting, the activity of p-cresol is rather strong in *Solanum tuberosum* and *Helianthus tuberosus*, while in *Raphanus sativus* the activity was not observed. In *Solanum tuberosum* within 24 hours after the cutting, the activity of p-cresol oxidase shows a little increase as compared with that immediately after the cutting, and the increased activity is maintained in the lapse of time after the first 24 hours. The activity of p-cresol oxidase in *Helianthus tuberosus* in the lapse of time after the cutting is nearly the same as that immediately after the cutting. In *Raphanus sativus* in the lapse of time after the cutting, the addition of p-cresol into the homogenate did not result in an increase of oxygen consumption. These results are shown in Diagram 2. In the case of o- or m-cresol, the homogenate prepared from all kinds of the materials at any time after the cutting did not show any oxidase activity by adding one of these substrates.

3) *Tyrosinase*

The activity of tyrosinase in the tissue homogenate was determined by using 0.02% aqueous solution of l-tyrosine as a substrate. The results of this experiment are shown in Diagram 3. In *Solanum tuberosum*

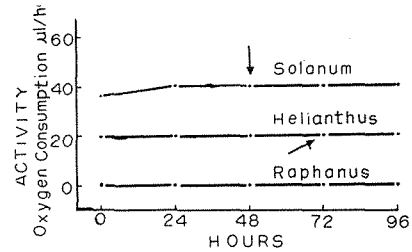

Diagram 2. Diagram showing the relation between the p-cresol oxidase activity and the lapse of time after the cutting.

The ordinates represent the oxidase activities (see text) of the preparation; 1 ml. of the homogenate, 1.4 ml. of the phosphate buffer solution and 0.6 ml. of 0.025% p-cresol aqueous solution. The abscissas represent the times after the cutting. The arrows mark the time at which the first cell divisions are observed, whether locally or entirely along the cut surface.

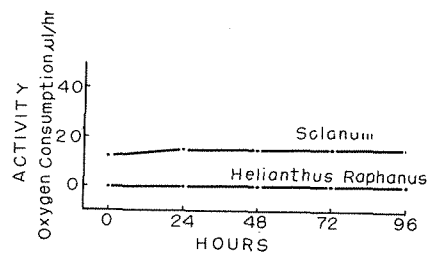

Diagram 3. Diagram showing the relation between the tyrosinase activity and the lapse of time after the cutting.

The ordinates represent the oxidase activities (see text) of the preparation; 1 ml. of the homogenate, 1.4 ml. of the phophate buffer solution and 0.6 ml. of 0.02% tyrosine aqueous solution. The abscissas represent the times after the cutting.

at any time after the cutting the tyrosinase activity is strong, while in *Helianthus tuberosus* and *Raphanus sativus* it was not observed.

4) *Dopa oxidase*

This oxidase activity was determined using 0.05% 3, 4-dihydroxyphenyl alanine (Dopa) aqueous solution as a substrate. The changes in the activity of dopa oxidase within 96 hours after the cutting are shown in Diagram 4. As shown in this diagram, in both *Solanum tuberosum* and *Helianthus tuberosus*, the dopa oxidase activity is pretty strong immediately after the cutting, and the activity shows an increase within 24 hours after the cutting. Furthermore the increased activity remains unchanged after the first 24 hours. On the contrary, in *Raphanus sativus* the activity is little or null at any time after the cutting.

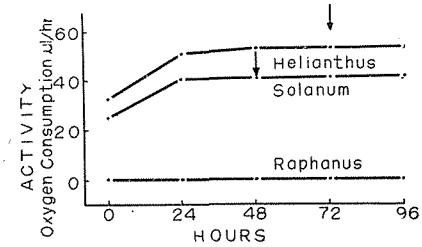

Diagram 4. Diagram showing the relation between the dopa oxidase activity and the lapse of time after the cutting.

The ordinates represent the oxidase activities (see text) of the preparation; 1 ml. of the homogenate, 1.4 ml. of the phosphate buffer solution and 0.6 ml. of 0.05% dopa aqueous solution. The abscissas represent the times after the cutting. The arrows mark the time at which the first cell divisions are observed, whether locally or entirely along the cut surface.

Conclusion

The activities of pyrocatechol oxidase, p-cresol oxidase, tyrosinase and dopa oxidase in *Solanum tuberosum* and those of pyrocatechol oxidase, p-cresol oxidase and dopa oxidase in *Helianthus tuberosus* are demonstrated to exist in the homogenate immediately after the cutting. However, in *Raphanus sativus* the activities of these phenol oxidases were hardly confirmed immediately after the cutting. O- and m-cresol oxidase did not show any activity in all the materials studied. These facts are harmonious with the results that the polyphenol oxidase has been considered as the principal terminal oxidase of respiration in the tuber of *Solanum tuberosum* (BOSWELL, 1945 and 1950; BOSWELL et al., 1938) and that the phenol oxidase has been found in the tuber of *Helianthus tuberosus* (BELVAL and LEGRAND, 1946 a and b).

Among these phenol oxidases mentioned above, the activity of pyrocatechol oxidase increases with the lapse of time after the cutting in *Solanum tuberosum* and *Helianthus tuberosus* (Diagram 1), in both of which cell divisions take place resulting in the wound periderm formation (BABA, 1955). Viz., the activity becomes fairly strong by the time the first cell divisions are observed, and the activity increases further as many divided cells are observed (Diagram 1). Furthermore, in *Raphanus sativus*, in which cell divisions did not take place within 96 hours after the cutting (BABA, 1955), the pyrocatechol oxidase did not show any activity at

any time after the cutting. From these results it may be concluded that the pyrocatechol oxidase activity has some close connection with the occurrence of cell division during the wound periderm formation (*Cf.* The case of nadi oxidase. BABA, 1955). It is an interesting fact, however, that catechol is one of the effective compounds for inducing many layers of the regenerated cells in the cut tuber of *Solanum tuberosum* (SIMONDS et al., 1953).

Though dopa oxidase shows an increase within 24 hours after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*, the activity remains unchanged in the lapse of time after the first 24 hours. The activity of p-cresol oxidase and tyrosinase in *Solanum tuberosum* and that of p-cresol oxidase in *Helianthus tuberosus*, did not show any remarkable change in the lapse of time after the cutting.

Summary

1) The oxygen consumption by the homogenate in the presence of pyrocatechol, p-cresol, o-cresol, m-cresol, tyrosine and dopa was measured by employing the manometric technique in the tubers of *Solanum tuberosum* and *Helianthus tuberosus* and the root of *Raphanus sativus* within 96 hours after the cutting.

2) The activities of pyrocatechol oxidase, p-cresol oxidase, tyrosinase and dopa oxidase in *Solanum tuberosum* and those of pyrocathecol oxidase, p-cresol oxidase and dopa oxidase in *Helianthus tuberosus* were detected in the homogenate immediately after the cutting. In *Raphanus sativus*, however, the oxidase activity of all the phenols used was hardly detected.

3) The activity of pyrocatechol oxidase increases with the lapse of time after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*. In these materials, the cell divisions take place resulting in the wound periderm formation (BABA, 1955). Therefore, it may be concluded that the pyrocatechol oxidase activity has some close connection with the occurrence of cell divisions.

4) The activity of dopa oxidase shows an increase within 24 hours after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*, but the activity remains unchanged after the first 24 hours.

5) The activities of p-cresol oxidase and tyrosinase in *Solanum tuberosum* and those of p-cresol oxidase in *Helianthus tuberosus* did not change remarkably in the lapse of time after the cutting.

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