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# Avoidance of phenol interference with the cytochemical reaction for esterase activity in plant tissues

G. O. C. ONYIA

## RÉSUMÉ

ONYIA, G. O. C. (1984). Abolition de l'interférence phénolique avec la réaction enzymatique de l'activité estérasique dans les tissus végétaux. *Saussurea* 15: 55-59. En anglais, résumé français.

L'analyse cytochimique quantitative de l'activité estérasique dans les tissus du bois indique que l'interférence phénolique avec la réaction enzymatique peut être abolie par un pré-traitement des coupes avec l'aniline.

## ABSTRACT

ONYIA, G. O. C. (1984). Avoidance of phenol interference with the cytochemical reaction for esterase activity in plant tissues. *Saussurea* 15: 55-59. In English, French abstract.

A quantitative cytochemical analysis of esterase activity in woody tissues shows that phenolic interference with the enzyme reaction may be overcome by pretreatment of sections with diazotized aniline.

## Introduction

1. *Materials.* – Stems, root apices and petioles from *Acer pseudoplatanus*, *Aesculus hippocastum*, *Vicia faba* and *Pisum sativum* were used for the preparation of unfixed, transverse or longitudinal, frozen sections (GAHAN & al., 1967), of non-woody material, and razor-cut sections from woody tissues.

2. *Esterase reaction.* – Sections were incubated at 37°C for 1 h in the full reaction medium (McLEAN & GAHAN, 1970) using naphthol AS-D acetate as substrate and fast blue BB as the diazonium salt.

3. *Diazotized aniline.* – One volume (2.33 ml or 0.256 moles) of aniline were added to 20 ml of 2.5N HCl in an ice-bath. Sodium nitrite (1.73 g) was

dissolved in 5 ml of distilled water, precooled in an ice-bath and added dropwise to the acidified aniline which was stirred continuously for 10-15 min. The completion of the reaction was controlled with potassium iodine-starch paper (FINAR, 1973). The solution was diluted initially to a volume of 50 ml with ice-cold distilled water to give a 0.5 M solution at pH 1-2. This was further diluted (1:10) with ice-cold distilled water, and a further 1:10 with 0.2M acetate buffer at pH 5.0 giving a final concentration of  $5 \times 10^{-3}$ M. The pH was adjusted to pH 4.7 with the addition of drops of 2.5N HCl. A range of solutions of concentrations between  $5 \times 10^{-3}$  and  $12.5 \times 10^{-3}$ M were prepared in similar manner.

4. *Coupling reaction.* – Ten drops of diazotized aniline were placed on air-dried sections on a slide and allowed to react with any endogenous phenols for 8 min at room temperature. The diazotized aniline was drained from the sections, which were rinsed briefly in 0.1M tris-HCl buffer at pH 6.5 prior to use for the cytochemical reaction (2 above).

5. *Controls.* – Sections of tissue similar to that used in 4 above were reacted a) in the full incubation medium without treatment with diazotized aniline; or b) without diazotization in the reaction medium which lacked substrate. All reactions were performed for 1 h at 37°C.

Serial sections of root apices from *V. faba* and *P. sativum* were pretreated with diazotized aniline and compared with those not so treated to determine the effect of the pretreatment on the esterase activity present in the sections.

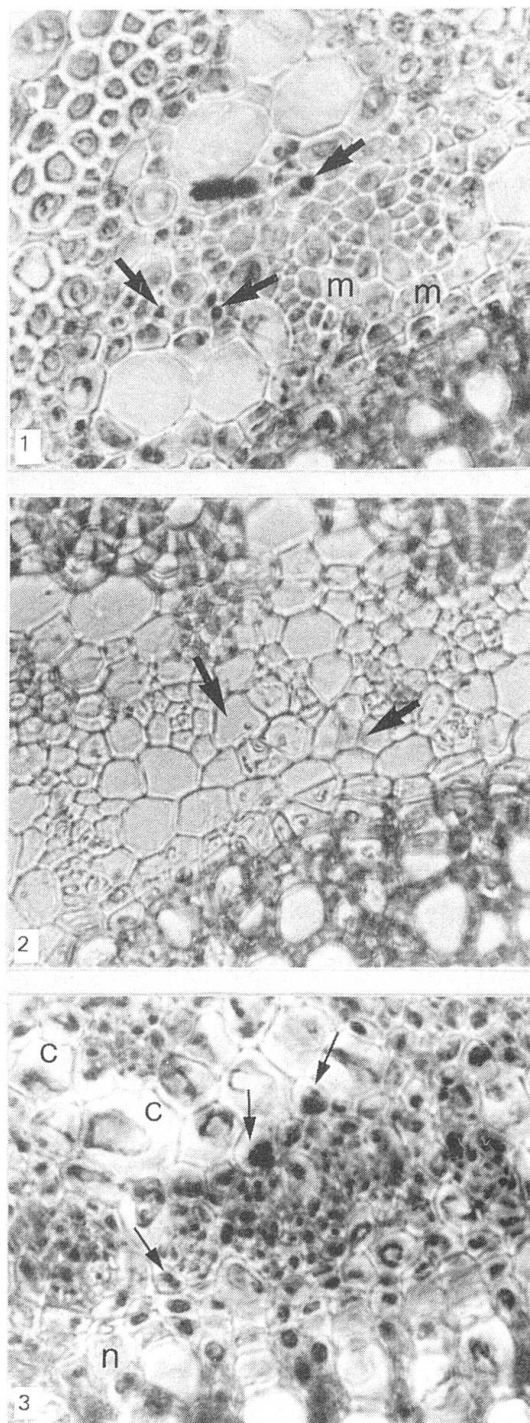
6. *Quantification of the cytochemical reaction.* – Esterase reaction product was measured with a Vicker's M86 integrating microdensitometer (Vicker's Instruments, York, U.K.) at 640 nm, objective  $\times 20$ , spot number 2 and diaphragm B3. Readings were expressed as absolute integrated optical density units per  $\mu\text{m}^2$  of reacting cytoplasm (AIOD/ $\mu\text{m}^2$ ; GAHAN & al., 1983; GAHAN, 1984).

## Results

Untreated sections reacted for esterase activity showed reaction product together with a heavy deposition of phenolic diazonium salt complex which masked much of the esterase reaction product (Fig. 1). However, sections incubated in the absence of substrate gave only the phenolic response (Fig. 2) indicating that the blue reaction product was due to the esterase activity.

Treatment of the sections with diazotized aniline at all concentrations prior to reacting for esterase activity showed a small amount of reddish deposit which permitted the visualization of the esterase reaction product (Fig. 3). Measurements made on phloem cells in *A. hypocaustum* sections, showed that the higher concentration of diazotized aniline yielded the greater amount of esterase reaction product, i.e.  $5 \times 10^{-3}$ M diazotized aniline gave  $0.39 \pm 0.05$  AIOD/ $\mu\text{m}^2$  and  $12.5 \times 10^{-3}$ M gave  $0.54 \pm 0.01$  AIOD/ $\mu\text{m}^2$ , a significant difference ( $P < 0.01$ ).

Attempts to quantify the esterase activity in different cell types without pretreatment with diazotized aniline were unsuccessful due to the masking of the enzyme reaction product by the phenolic complexes (Table 1). Thus 50-60% of the esterase activity was not available for measurement without the diazotized aniline pretreatment.



Figs. 1-3. - Unfixed razor-cut transverse sections of *Acer pseudoplatanus* (petiole).

Fig. 1. - Reacted for esterase activity at 37°C for 1 hour showing low deposits of visible esterase reaction product (arrow). Endogenous phenol-diazonium salt complex masked esterase reaction product (m). Magnification  $\times 350$ .

Fig. 2. - Incubated at 37°C for 1 hour in the absence of substrate showing patches of (reddish) discoloration due to endogenous phenolic response (arrow). Magnification  $\times 350$ .

Fig. 3. - Pretreated with  $5 \times 10^{-3}$ M diazotized aniline at 20°C for 8 minutes prior to esterase reaction at 37°C for 1 hour showing large deposits of esterase reaction product (arrow) against a clear background (c), and little (reddish) deposit of endogenous phenol-diazonium salt complex (n). Magnification  $\times 350$ .

<i>Species</i>	<i>Esterase reaction</i>	<i>Diazotized aniline esterase reaction</i>	<i>%Increase of visible and quantifiable esterase reaction product due to diazotized aniline pretreatment</i>
<i>Acer pseudoplatanus</i>	0.25 ± 0.09	0.51 ± 0.07	104%
<i>Aesculus hyppocastum</i>	0.16 ± 0.04	0.41 ± 0.14	156%

Table 1. – Esterase reaction in phloem cells of *Acer pseudoplatanus* and *Aesculum hyppocastum* in section with or without pretreatment with  $5 \times 10^{-3}$ M diazotized aniline. Measurements are means of 20 readings for each section and are expressed in absolute optical density units (AIOD) per  $\mu\text{m}^2$  of reactive cytoplasm ± standard deviation.

<i>Species</i>	<i>Esterase reaction</i>	<i>Diazotized aniline + esterase reaction</i>
<i>Vicia faba</i> . . . . .	0.95 ± 0.13	1.02 ± 0.11
<i>Pisum sativum</i> . . . . .	1.32 ± 0.29	1.29 ± 0.29

Table 2. – The effects of diazotized aniline on esterase activity in the stele region of root apices of *Vicia faba* and *Pisum sativum*. Measurements are expressed in absolute optical density units (AIOD) per  $\mu\text{m}^2$  of reacting cytoplasm. Figures given are means of 20 readings per section ± standard deviation.

Comparison of the esterase reaction in root apex sections, with and without the pretreatment (Table 2), showed no difference in the measurable esterase reaction product and hence the diazotized aniline would appear to cause no measurable inhibition of the enzyme activity.

### Discussion

Sections reacted for esterase activity in tissues containing high levels of endogenous phenolics yield little observable reaction product. This is due in part, at least, to the heavy precipitate of phenolic-diazonium salt complex in the cells so masking most of such esterase reaction product (Table 1: Fig. 1). It is known that phenolics will interfere with enzyme activities during biochemical isolation and purification (LOOMIS & al., 1963; ANDERSON & al., 1967; ANDERSEN & al., 1968), though this effect may be blocked by the addition of reducing reagents (STOKES & al., 1968; JAMES & SMITH, 1972) or polyvinylpyrrolidone (ANDERSEN & al., 1968). Therefore, it is possible that the phenolics in untreated sections may result in a reduced esterase activity due to phenolic interference. However, this effect may be reduced in the cytochemical reaction due to the different compartmentation of phenolics and esterases in a cell. It is clear from Tables 1 and 2 that on blocking the phenolic reaction with diazotized aniline, the level of esterase activity is not significantly impaired, and is greatly enhanced in cells containing phenolics. Thus, any phenolic inhibition of enzyme activity is readily reversed by the addition of diazotized aniline, the reaction of which depends upon the concentration of the benzene-diazonium cation which is the active coupling component (FINAR, 1973).

It is suggested that the pretreatment of sections containing high concentrations of endogenous phenolics with diazotized aniline may be used for the assessment of esterase activities, and may also be helpful in studies of other hydrolases employing the diazonium salt reaction.

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