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# Pyrophosphatase, Peroxidase and Polyphenoloxidase Activities during Leaf Development and Senescence[1]

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## ABSTRACT

**Inorganic pyrophosphatase, peroxidase, and polyphenoloxidase activities were studied as the function of leaf insertion level in eight monocotyledonous and eight dicotyledonous species. Alkaline inorganic pyrophosphatase shows a declining activity toward the end of senescence whereas no regular drift in either peroxidase or polyphenoloxidase activities was noticed during senescence of attached leaves. In the primary leaves of rice, peroxidase and polyphenoloxidase activities were high in the senescent leaves and there exists a correlation between chlorophyll content and peroxidase activity though not with polyphenoloxidase activity. Upon detachment leaves exhibit increasing peroxidase and polyphenoloxidase activities with time. The distribution of the enzyme activities during senescence of attached leaves is suggested to be species-specific, and an increase in peroxidase and polyphenoloxidase activities cannot be taken as an indicator of leaf senescence.**

A number of reports are available regarding the increase (1, 2, 4, 7–10, 14, 16, 19) and/or decrease (4, 10, 12, 19, 20) in activities of several hydrolyzing and oxidative enzymes during senescence of leaves. Differences in the senescence pattern of attached leaves compared to detached leaves or leaf discs have been reported (13, 14, 24, 25).

Both acid and alkaline inorganic pyrophosphatase (EC 3.6.1.1) occur in higher plants (17). It has been suggested that the alkaline inorganic pyrophosphatase, having extremely high affinity for pyrophosphate, is associated with the anabolic process in the leaf, while acid inorganic pyrophosphatase participates in the catabolic process (7, 21, 23). However, there are several conflicting reports on increase and decrease in inorganic pyrophosphatase activity during senescence of attached and detached leaves. For instance, Kisban *et al.* (10) reported an increase in alkaline inorganic pyrophosphatase activity in detached senescing leaves of barley and wheat and Rauser (21) detected that both acid and alkaline inorganic pyrophosphatase activities declined with plant age in attached leaves of bean, tobacco, and sunflower. However, our previous investigations (7, 9) suggested an opposite trend in the activities of acid and alkaline inorganic pyrophosphatase during senescence of rice leaves.

Peroxidase (EC 1.11.1.6), on the other hand, exhibits irregular drift in its activity during development and subsequent senescence. There is a considerable loss of protein in senescing leaves, whereas peroxidase is not subjected to turnover (1, 8, 14) and its absolute activity increases during senescence. Parish (19) suggested that the increase in peroxidase activity in senescing tobacco leaf discs was associated with protein synthesis, since protein synthesis inhibitors inhibit the increase in its activity. He further suggested that the increase in peroxidase activity can be taken as a reliable indicator of leaf senescence. With cucumber, a significant increase in peroxidase activity was achieved by delaying protein and Chl loss, either by detopping the shoots above the cotyledons (22) or by kinetin and benzimidazole treatment (8). Previous investigation (8) in this laboratory demonstrated an increase in peroxidase and polyphenoloxidase (EC 1.10.3.1) activities of both attached and detached rice leaves during senescence. Results obtained with attached rice leaves of various ages contradict those reported for senescing cucumber cotyledons (5) and suggest that the pattern of change in oxidative activities during senescence may be species-specific.

This investigation was undertaken to study the distribution pattern of inorganic pyrophosphatase, peroxidase, and polyphenoloxidase activities in attached leaves of different physiological ages and to determine if the changes in peroxidase and polyphenoloxidase activities during leaf senescence can be considered to be species-specific.

## MATERIALS AND METHODS

The following species were investigated: monocotyledons: *Amomum aromaticum* Roxb., *Eleusine corocana* Gaertn., *Hordeum vulgare* L., *Oryza sativa* L., *Pennisetum typhoideum* Rich., *Sorghum vulgare* Pers., *Triticum vulgare* Villars., and *Zea mays* L.; dicotyledons: *Arachis hypogaea* L., *Boerhaavia diffusa* L., *Chenopodium album* L., *Crotolaria striata* DC., *Hibiscus micranthus* L., *Nicotiana plumbaginifolia* Viv., *Raphanus sativus* L., and *Tabernaemontana coronaria* Br.

Healthy plant samples were collected on sunny days at 10 AM and the approximate age of the organs determined by the method of Kornilov *et al.* (11). The samples were transported to the laboratory in polyethylene bags covered with crushed ice. The leaves were numbered from the apex downward so as to obtain subsamples representative of various developmental stages. The leaves were excised within 15 min of collection, washed thoroughly with tap water, and then in distilled $H_2O$. The leaves were blotted, weighed, and stored in a refrigerator at 8 C for 30 min.

**Enzyme Extraction and Assay.** Enzyme extraction for the assay of inorganic pyrophosphatase activity was made as described previously (7) and the extraction procedure for peroxidase and polyphenoloxidase was followed. The chilled leaves were homogenized with cold 0.1 M phosphate buffer (pH 6.8) (100 mg fresh tissue-2 ml buffer) in a prechilled mortar and pestle. The crude homogenate was taken for the assay of peroxidase and polyphenoloxidase activities after proper dilution.

**Inorganic Pyrophosphatase Assay.** The assay mixture (5 ml) for acid inorganic pyrophosphatase contained: 300 μmol citrate buffer (pH 5.0), 5 μmol neutralized tetrasodium pyrophosphate, and 1 ml enzyme extract; the assay mixture (5 ml) for alkaline inorganic pyrophosphatase contained: 150 μmol Tris-HCl (pH 8.6), 5 μmol

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neutralized tetrasodium pyrophosphate, 100 μmol magnesium chloride, and 1 ml enzyme extract (diluted 5-fold). The reaction was allowed to proceed for 10 min at 37 C and the enzyme activity was stopped with the addition of 1 ml 20% perchloric acid. A zero time control was run at the same time with the enzyme extract added following perchloric acid addition. After centrifugation for 15 min at 3,000*g*, an aliquot of the supernatant was taken for inorganic phosphorus estimation (6). All enzyme activities were calculated on the basis of net inorganic phosphorus released during the incubation period.

One unit of the enzyme activity is defined as the amount of the enzyme which liberates 1 μmol inorganic phosphorus/min under the assay conditions described above.

**Peroxidase Assay.** The assay mixture (6 ml) contained: 300 μmol phosphate buffer (pH 6.8), 5 μmol pyrogallol, 50 μmol $H_2O_2$, and 1 ml enzyme extract diluted 100-fold. The reaction was allowed to proceed for 5 min at 25 C after which the reaction was stopped by adding 0.5 ml 5% (v/v) $H_2SO_4$. A zero time control was run at the same time with the enzyme extract added following $H_2SO_4$ addition. After centrifugation for 15 min at 3,000*g*, the amount of purpurogallin formed was determined from the *A* at 420 nm.

**Polyphenoloxidase Assay.** The assay mixture (6 ml) contained: the same components determined above for the peroxidase assay without $H_2O_2$. The amount of purpurogallin formed was determined from the *A* at 420 nm.

One unit of peroxidase or polyphenoloxidase activity was defined as that amount of purpurogallin formed which increases the *A* by 0.1/min under the assay conditions described above.

**Estimation of Chl and Protein.** The homogenate for the enzyme determination was mixed with 4 volumes of 100% ethanol, boiled in a water bath for 10 min, cooled, and centrifuged for 15 min at 3,000*g*. The pellet was extracted three times with 80% ethanol and centrifuged for 15 min at 3,000*g*. The supernatants were combined, made to volume (20 ml), and Chl determined from *A* at 665 nm (18).

The pellet was washed successively with 10% (w/v) cold trichloroacetic acid (twice), ethanol (once), ethanol-chloroform (3:1, v/v, twice), ethanol-diethylether (3:1, v/v, once), and finally with ether (once). The pellet was allowed to dry and protein was solubilized by boiling with 1 N NaOH for 15 min in a water bath. The sample was centrifuged for 15 min at 3,000*g* and an aliquot was taken for protein determination (15).

**Presentation of Data.** Leaves of similar developmental stages from three identical twigs were sampled separately for analyses and the experiments were repeated three times. The data presented in the tables are the mean of three independent experiments each of three replications.

## RESULTS

### DISTRIBUTION OF PYROPHOSPHATASE, PEROXIDASE, AND POLYPHENOLOXIDASE ACTIVITIES IN ATTACHED LEAVES

**Monocotyledons.** Distribution of alkaline inorganic pyrophosphatase, peroxidase, and polyphenoloxidase activities as the function of leaf insertion level was determined in eight monocot species (Table I). Two species, *A. aromaticum* and *Z. mays,* were in the vegetative stage and in those species leaves at nodes 3 to 7 represented fully mature leaves. Other species were in the reproductive stage with all leaves fully mature. Whether expressed on a fresh weight basis or on organ basis acid inorganic pyrophosphatase varies with the plant species (data not presented). Alkaline inorganic pyrophosphatase, however, exhibited a declining trend in its activities toward the basal end and the highest activity was detected in flag or middle leaves.

The increase in peroxidase activity was marked from the topmost to the basal senescing leaves in three species, *i.e. O. sativa, S. vulgare,* and *Z. mays,* whereas a decrease in peroxidase activity from the topmost to the basal senescing leaves was observed in *E. corocana, P. typhoideum,* and *T. vulgare. A. aromaticum* exhibited the highest peroxidase activity in the topmost and basal senescing leaves, but *H. vulgare* possessed the highest activity in middle leaves.

A similar trend of increasing polyphenoloxidase activity toward basal senescing leaves was noticed in *E. corocana, O. sativa, S. vulgare,* and *Z. mays. H. vulgare* and *P. typhoideum* showed decreased activity from the topmost leaves to the basal leaves. *A. aromaticum* possessed high polyphenoloxidase activity in middle mature leaves, but *T. vulgare* had no detectable polyphenoloxidase activity.

**Dicotyledons.** Distribution of alkaline inorganic pyrophosphatase, peroxidase, and polyphenoloxidase activities as the function of leaf insertion level was also determined in eight dicotyledonous species (Table II).

All species studied were vegetative and the middle leaf position represented mature leaves. Unlike the monocotyledonous species, all of the dicotyledonous species exhibited highest acid inorganic pyrophosphatase activities in the apical growing leaves; but there was never any uniform trend, either decreasing or increasing, in any of the species studied (data not presented). Alkaline inorganic pyrophosphatase, however, showed a regular drift from apical or subapical leaves toward basal senescing leaves. The low ratio of alkaline to acid enzyme was a feature in all basal senescing leaves except *B. diffusa* and also in growing leaves except *C. striata* and *H. micranthus* (data not presented). The low ratio of alkaline to acid inorganic pyrophosphatase activity in most of the species corroborated the findings of Rauser (21) for bean, tobacco, and sunflower plants.

Like monocotyledonous species, the dicotyledonous species did not show consistent patterns in peroxidase activities. Four species, *i.e. A. hypogaea, B. diffusa, H. micranthus,* and *R. sativus* showed increase in peroxidase activity toward the basal leaves, but the remaining six species exhibited high activity in middle leaves.

Increased polyphenoloxidase activity toward basal senescing leaves was also not a regular feature except in *A. hypogaea, B. diffusa, H. micranthus,* and *T. coronaria.* In two species, *C. album* and *R. sativus,* high activity was noted in the apical and the lowermost leaves but other species, like *C. striata* and *N. plumbaginifolia,* exhibited high activity in the middle leaves.

**Drift in Peroxidase and Polyphenoloxidase Activities during Senescence of Primary Leaves of Rice.** Peroxidase and polyphenoloxidase activities as a function of leaf age were studied in primary leaves of *O. sativa* L. cv. Ratna (Fig. 1). Each sample consisted of 20 leaves taken at one time. Peroxidase and polyphenoloxidase activities decreased uniformly from 5-day-old leaves to 11- and 13-day-old leaves, respectively. The same trend was also noticed when the activity was expressed on a per organ basis (data not presented). The Chl content increased at first and then remained almost constant for some time, after which it started decreasing from the 9-day-old leaves. This study clearly indicated that high activities of peroxidase and polyphenoloxidase in senescent leaves and low activity in mature leaves are specific features of the rice plant.

**Pyrophosphatase, Peroxidase, and Polyphenoloxidase Activities during Detached Flag Leaf Senescence.** The upper 5-cm segments of the flag leaves from several monocotyledonous species were floated on water in Petri dishes kept in the dark at 25 C ± 3 for 72 and 144 hr. Changes in the enzyme activity were determined at 72 and 144 hr and the data are presented in Table III. The level of acid inorganic pyrophosphatase activity was maximum at 72 hr of detachment except in rice leaves where the highest activity was noted at 144 hr of detachment (data not presented). However, there was a gradual decline in alkaline inorganic pyrophosphatase activity and Chl content upon detachment with time.

An increase in peroxidase and polyphenoloxidase activities upon detachment was observed in all of the species studied. The increase in peroxidase and polyphenoloxidase activities in de-

Table I. Levels of Inorganic Pyrophosphatase and Peroxidase and Polyphenoloxidase Activities and Protein and Chlorophyll Contents of Attached Senescing Leaves as Related to Leaf Insertion Level for Monocotyledonous Plants.

The leaves are numbered from the apex to the base.

| Leaf Position | Leaf Age (Weeks) | Enzyme Units: Alk PPase[1] (g fr.wt.$^{-1}$) | Peroxidase (g fr.wt.$^{-1}$) | Polyphenol-oxidase (g fr.wt.$^{-1}$) | Protein mg/g fr.wt. | Chloro-phyll $A_{665}$ |
|---|---|---|---|---|---|---|
| | | *Amomum aromaticum* | | | | |
| 1 | 4-5 | 0.83 | 540 | 40 | 38.2 | 0.63 |
| 3 | 7-8 | 1.11 | 530 | 44 | 42.0 | 0.72 |
| 5 | 10-12 | 0.83 | 530 | 60 | 38.4 | 0.72 |
| 7 | 12-13 | 0.55 | 490 | 56 | 23.6 | 0.41 |
| 8 | 13-14 | 0.55 | 560 | 44 | 14.8 | 0.12 |
| | | *Eleusine corocana* | | | | |
| 1(Flag) | 2-3 | 59.45 | 640 | 40 | 62.9 | 0.33 |
| 3 | 4-5 | 47.00 | 590 | 40 | 76.7 | 0.26 |
| 5 | 6-7 | 37.33 | 560 | 40 | 69.0 | 0.20 |
| 6 | 7-8 | 25.58 | 560 | 44 | 62.9 | 0.12 |
| 7 | 8-9 | 18.66 | 560 | 48 | 53.7 | 0.07 |
| | | *Hordeum vulgare* | | | | |
| 1(Flag) | 2-3 | 31.10 | 210 | 32 | 35.3 | 0.33 |
| 2 | 4-5 | 31.10 | 270 | 32 | 59.8 | 0.34 |
| 3 | 6-7 | 29.72 | 340 | 28 | 44.5 | 0.33 |
| 5 | 8-9 | 20.05 | 260 | 24 | 30.7 | 0.22 |
| 6 | 8-9 | 13.82 | 260 | 24 | 27.6 | 0.13 |
| | | *Oryza sativa* | | | | |
| 1(Flag) | 2-3 | 34.24 | 240 | 20 | 68.0 | 0.65 |
| 2 | 5-6 | 33.39 | 340 | 25 | 53.0 | 0.62 |
| 3 | 7-8 | 26.12 | 450 | 40 | 42.0 | 0.51 |
| 4 | 9-10 | 15.68 | 560 | 50 | 32.0 | 0.29 |
| | | *Pennisetum typhoideum* | | | | |
| 1(Flag) | 3-4 | 101.61 | 320 | 64 | 61.4 | 0.58 |
| 2 | 4-5 | 101.61 | 300 | 64 | 70.6 | 0.63 |
| 4 | 6-7 | 85.71 | 300 | 52 | 59.8 | 0.49 |
| 6 | 7-8 | 38.71 | 260 | 40 | 42.9 | 0.27 |
| 7 | 8-9 | 5.53 | 220 | 36 | 33.7 | 0.12 |
| | | *Sorghum vulgare* | | | | |
| 1(Flag) | 3-4 | 73.55 | 360 | 32 | 61.3 | 0.50 |
| 2 | 4-5 | 59.03 | 360 | 36 | 62.9 | 0.42 |
| 4 | 6-7 | 43.50 | 420 | 54 | 56.8 | 0.33 |
| 6 | 10-11 | 22.81 | 580 | 108 | 55.2 | 0.22 |
| | | *Triticum vulgare* | | | | |
| 1(Flag) | 2-3 | 49.77 | 310 | ND[2] | 53.7 | 0.36 |
| 2 | 4-5 | 35.94 | 270 | ND | 50.6 | 0.33 |
| 3 | 5-6 | 22.12 | 240 | ND | 39.9 | 0.26 |
| 4 | 6-7 | 15.21 | 140 | ND | 29.1 | 0.19 |
| | | *Zea mays* | | | | |
| 1 | 2-3 | 50.46 | 170 | 28 | 38.4 | 0.36 |
| 3 | 4-5 | 81.57 | 190 | 28 | 33.7 | 0.40 |
| 5 | 6-7 | 96.77 | 190 | 32 | 27.6 | 0.54 |
| 6 | 7-8 | 55.30 | 290 | 44 | 24.5 | 0.43 |
| 8 | 8-9 | 17.97 | 290 | 64 | 21.5 | 0.18 |

[1] Alk PPase - Alkaline Inorganic Pyrophosphatase

[2] ND - No activity detected

tached senescing leaves was not considered representative of the process as observed in attached senescing leaves (Tables I and II).

## DISCUSSION

The present work was undertaken to study the distribution pattern of pyrophosphatase, peroxidase, and polyphenoloxidase in developing and senescing leaf tissues and to determine whether the increase in peroxidase and polyphenoloxidase activities during senescence is species-specific.

Alkaline inorganic pyrophosphatase activity consistently decreased with increasing leaf age and there was a correlation between the decrease in alkaline inorganic pyrophosphatase activity and decrease in the Chl content. A considerable variation in the distribution pattern of acid inorganic pyrophosphatase in leaves was detected. Decreases in both acid and alkaline inorganic pyrophosphatases during senescence of attached leaves of bean, tobacco, and sunflower were reported by Rauser (21). However, during senescence of attached and detached rice leaves we found

Table II. Levels of Inorganic Pyrophosphatase and Peroxidase and Polyphenoloxidase Activities and Protein and Chlorophyll Contents of Attached Senecing Leaves as Related to Leaf Insertion Level for Dicotyledonous Plants.
The leaves are numbered from the apex to the base.

| Leaf Position | Leaf Age(Weeks) | Enzyme Units: Alk PPase (g fr.wt.$^{-1}$) | Peroxidase (g fr.wt.$^{-1}$) | Polyphenol-oxidase (g fr.wt.$^{-1}$) | Protein mg/g fr.wt. | Chlorophyll $A_{665}$ |
|---|---|---|---|---|---|---|
| | | | *Arachis hypogea* | | | |
| 2 | 2-3 | 9.68 | 160 | 36 | 18.4 | 0.43 |
| 4 | 3-4 | 6.22 | 180 | 42 | 30.7 | 0.39 |
| 6 | 5-6 | 4.15 | 200 | 44 | 36.8 | 0.35 |
| 7 | 6-7 | 2.07 | 200 | 44 | 32.2 | 0.35 |
| 8 | 7-8 | 1.38 | 210 | 50 | 14.0 | 0.27 |
| | | | *Boerhaavia diffusa* | | | |
| 2 | 2-3 | 76.03 | 220 | 56 | 22.6 | 0.37 |
| 4 | 3-4 | 82.26 | 230 | 60 | 26.1 | 0.36 |
| 5 | 5-6 | 71.89 | 240 | 62 | 26.1 | 0.33 |
| 6 | 7-8 | 69.12 | 240 | 62 | 35.3 | 0.43 |
| 7 | 8-9 | 68.43 | 270 | 66 | 42.9 | 0.43 |
| | | | *Chenopodium album* | | | |
| 2 | 3-4 | 77.45 | 170 | 28 | 59.8 | 0.51 |
| 4 | 6-7 | 79.49 | 180 | 28 | 73.6 | 0.40 |
| 6 | 7-8 | 76.04 | 200 | 26 | 65.9 | 0.40 |
| 7 | 8-9 | 51.15 | 165 | 14 | 53.7 | 0.21 |
| 8 | 9-10 | 37.33 | 80 | 24 | 48.3 | 0.17 |
| | | | *Crotolaria striata* | | | |
| 3 | 4-5 | 28.34 | 560 | 84 | 61.4 | 0.43 |
| 5 | 5-6 | 20.74 | 860 | 84 | 70.6 | 0.39 |
| 7 | 6-7 | 14.52 | 880 | 88 | 58.3 | 0.35 |
| 8 | 7-8 | 18.66 | 880 | 90 | 62.9 | 0.35 |
| 9 | 9-10 | 13.13 | 740 | 70 | 53.7 | 0.27 |
| | | | *Hibiscus micranthus* | | | |
| 3 | 4-5 | 26.27 | 280 | 52 | 35.3 | 0.18 |
| 6 | 7-8 | 15.21 | 280 | 58 | 36.8 | 0.25 |
| 8 | 9-10 | 13.82 | 285 | 60 | 37.6 | 0.17 |
| 10 | 10-11 | 9 .68 | 350 | 76 | 29.9 | 0.10 |
| 12 | 11-12 | 8.29 | 360 | 90 | 27.6 | 0.04 |
| | | | *Nicotiana plumbaginifolia* | | | |
| 2 | 2-3 | 13.80 | 285 | 24 | 22.2 | 0.13 |
| 4 | 3-4 | 12.40 | 300 | 32 | 27.6 | 0.11 |
| 6 | 4-5 | 11.75 | 400 | 36 | 27.6 | 0.19 |
| 7 | 5-6 | 10.37 | 300 | 36 | 15.3 | 0.14 |
| 8 | 6-7 | 0.48 | 300 | 28 | 11.5 | 0.06 |
| | | | *Raphanus sativus* | | | |
| 2 | 2-3 | 26.27 | 85 | 30 | 21.5 | 0.18 |
| 4 | 4-5 | 26.27 | 90 | 28 | 23.0 | 0.25 |
| 6 | 6-7 | 23.50 | 90 | 22 | 20.7 | 0.17 |
| 8 | 7-8 | 11.75 | 90 | 26 | 9.2 | 0.10 |
| 10 | 9-10 | 7.60 | 100 | 36 | 6.1 | 0.04 |
| | | | *Tabernaemontana coronaria* | | | |
| 2 | 4-5 | 8.97 | 350 | 60 | 21.5 | 0.25 |
| 4 | 9-10 | 10.37 | 370 | 64 | 30.7 | 0.25 |
| 6 | 10-11 | 9.68 | 440 | 70 | 26.1 | 0.26 |
| 7 | 11-12 | 5.53 | 370 | 76 | 23.1 | 0.19 |
| 8 | 12-13 | 1.38 | 320 | 70 | 19.2 | 0.08 |

(7, 9) an increase in the activity of acid inorganic pyrophosphatase. The present investigation showed that the trend of change in acid inorganic pyrophosphatase is different for attached and detached leaves.

Senescence involves a predominance of catabolic process over anabolic process and, therefore, alkaline inorganic pyrophosphatase supposedly involved in biosynthetic pathways should decrease during senescence. On the other hand, acid inorganic pyrophosphatase supposedly involved in the catabolic process showed considerable variation in activity during senescence. In this context, a decline in the level of alkaline inorganic pyrophosphatase activity in the leaves can be taken as an indication of the onset of senescence.

Another significant aspect is that it added 16 species to the list of plants which have alkaline inorganic pyrophosphatase activity and six species (*B. diffusa, C. album, E. corocana, P. typhoideum, S. vulgare,* and *Z. mays*) capable of utilizing $C_4$ carboxylation pathway of photosynthesis had a higher alkaline inorganic pyrophosphatase activity.

Unlike alkaline inorganic pyrophosphatase activity, the distri-

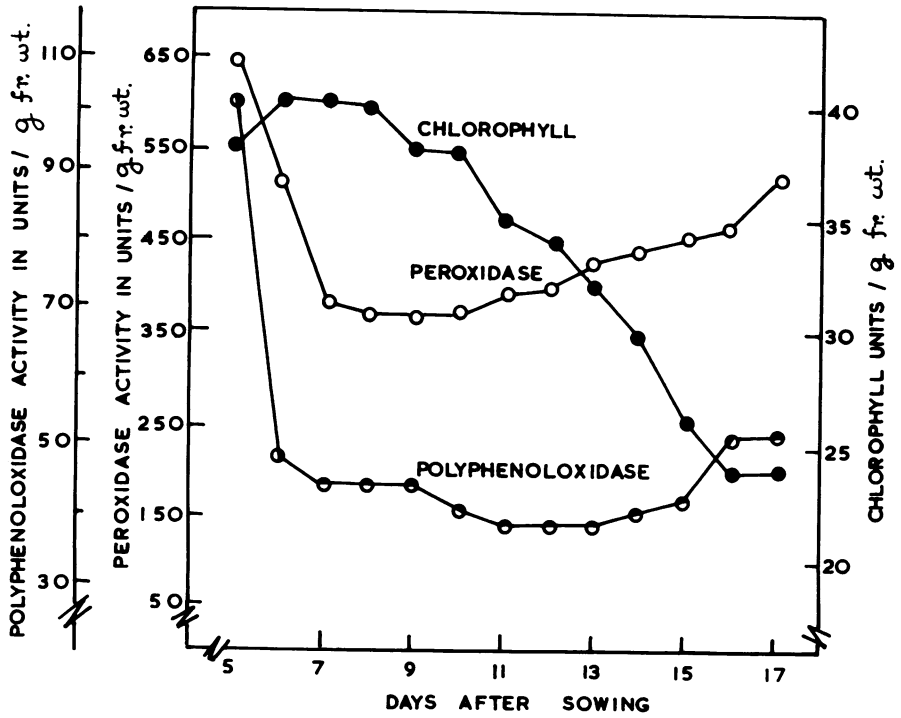


FIG. 1. Peroxidase and polyphenoloxidase activities and Chl contents during senescence of primary leaves of rice.

Table III. Changes in the Level of Inorganic Pyrophosphatase and Peroxidase and Polyphenoloxidase Activities and Protein and Chlorophyll Contents of Detached Leaves Floated on Water.

Values expressed as percentage of initial.

| Plant Species | Duration Floated (Hours) | Enzyme Activity | | | Protein | Chlorophyll |
|---|---|---|---|---|---|---|
| | | Alk PPase | Peroxidase | Polyphenol-oxidase | | |
| Eleusine coracana | 72 | 84.7 | 131.5 | 110.0 | 76.2 | 48.5 |
| | 144 | 62.7 | 128.9 | 110.0 | 69.8 | 36.4 |
| Hordeum vulgare | 72 | 58.1 | 141.8 | 191.4 | 40.9 | 58.1 |
| | 144 | 19.4 | 136.5 | 116.5 | 40.9 | 51.6 |
| Oryza sativa | 72 | 73.0 | 130.7 | 166.5 | 68.8 | 82.4 |
| | 144 | 50.0 | 207.6 | 344.1 | 57.8 | 73.5 |
| Pennisetum typhoideum | 72 | 100.0 | 153.8 | 140.0 | 77.3 | 100.0 |
| | 144 | 82.1 | 184.6 | 140.0 | 62.1 | 73.8 |
| Triticum vulgare | 72 | 73.2 | 154.8 | ND[1] | 77.7 | 77.3 |
| | 144 | 24.4 | 89.9 | ND | 73.0 | 75.0 |
| Sorghum vulgare | 72 | 100.0 | 199.8 | 199.4 | 92.2 | 100.0 |
| | 144 | 78.9 | 244.0 | 221.6 | 84.1 | 94.7 |

[1] ND stands for no activity detected.

bution of peroxidase and polyphenoloxidase activities in attached leaves of different physiological ages of 16 species varied to a considerable extent. Some species showed higher peroxidase and polyphenoloxidase activities toward the basal senescent leaves whereas other species showed high activities in the middle mature leaves or the topmost leaves. Increase in enzyme activities during detached leaf senescence determined whether the tissue was capable of *de novo* synthesis in all of the plant species studied (5). This rise in enzyme activity was probably due to enzyme activation following upon detachment (2, 3, 8, 22). If a rise in peroxidase and polyphenoloxidase activity during senescence can be taken as a criterion of senescence, all of the species did exhibit high activities in old leaves. But controversial reports suggest that the behavior of the enzymes is to increase during senescence in some species and to show a decreasing pattern in others.

Another experiment was designed to verify the changes in the enzymic levels during development and subsequent senescence of leaves which coincide with our previous studies on attached leaves of rice at different developmental stages (8). Decreased activity in senescing attached leaves of some species described above can be compared with that in the attached senescing cucumber cotyledons (5). Both increasing and decreasing tendencies of peroxidase and polyphenoloxidase activities during senescence may have some functional significance during development of leaves, and it may be that peroxidase and polyphenoloxidase activities during development and senescence of leaves of higher plants follow irregular

patterns and that the fluctuation of enzyme activities is species-specific.

Although some tendency of peroxidase and polyphenoloxidase activities to increase is noted during detached leaf senescence, their distribution is different in attached leaves; this clearly shows that peroxidase and polyphenoloxidase activities cannot be taken as indicators of leaf senescence.

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