# The changes in germinability and the activities of polyphenol oxidase (PPO) and peroxidase (POD) in seeds of P. macrophylla during low-temperature treatment.

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## ABSTRACT

Activities of peroxidase (POD) and polyphenol Oxidase (PPO) were investigated in seeds of *Pentaclethra macrophylla* during low temperature treatment. The seeds from the small-sized fruits (variety A) and those of the big-sized fruits (variety B) showed high germination, with maximum germination values ranging between 60 – 90%. Low temperature treatment did not significantly (P< 0.5) affect maximum germination values. Activities of POD and PPO increased initially (2-4 days) but declined with prolonged (6–8 days) low temperature treatment.

**Keywords**: Polyphenol Oxidase (PPO), Peroxidase (POD), Geminability, *Pentaclethra macrophylla*.

## INTRODUCTION

*Pentaclethra macrophylla* Benth also known as African oil bean or Congo acacia, belongs to the family Leguminosae (Mimosaceae). The seeds of *Pentaclethra macrophylla* are known to remain dormant in the soil from several months before germination, a situation which has not helped our agriculturists to develop these species as plantation crop (Esenowo *et al,* 2006). Another factor is the extreme drying out of seeds causing the embryo to reduce their viability in the field (Nat, 1956).

The seeds, after sustained boiling are palatable and either eaten alone or with sweet fresh palm wine or fermented and used in making a local, popular and mouth watering sauce, (Etukudo, 2003). Medicinally, the bark decoction is used in healing sores, wounds and cuts. Some antihelmintic properties have been ascribed to the bark (Etukudo, 2003).

Activities of peroxidase, polyphenol oxidase, hydroperoxide and lipid contents of *Irvingia gabonensis* seeds during desiccation have been reported by Nya *et.al.,* (2003). Their studies showed that POD and PPO increased initially but declined in the latter desiccation period.

Browning in most plant tissues is caused primarily by the activity of the enzyme polyphenol oxidase. The activity of polyphenol oxidase and the content of polyphenol are usually considered as the main factors contributing to the browning potential of tissues (Vaugham and Luke, 1984; Nkang and Chandler, 1986). This work was undertaken to assess activities of Polyphenol oxidase and peroxidase in seeds of *Pentaclethra macrophylla* following exposure to low temperature.

## MATERIALS AND METHODS

### Seed Collection and Germination Studies

Fresh fruits of *Pentaclethra macropylla* Benth were collected from a local cultivar in Itam in Uyo Local Government Area of Akwa Ibom State. Two seed sizes were used. The seeds from the small-sized fruits (variety A) and those of the Big-sized fruits (variety B). The seeds were kept under refrigeration (5$^{o}$C) until used. Seeds were removed at 2 day intervals for enzyme extraction and assessment of germinability. Ten seeds of each variety were set-up to germinate under ambient laboratory conditions (approx. 28$^{o}$C – 30$^{o}$C).

Germination counts were made daily. Seeds were also selected from both varieties following the low temperature treatment to determine the moisture content.

## EXTRACTION AND ASSAY OF POLYPHENOL OXIDASE AND PEROXIDASE

1. **Enzyme Extraction**: Enzyme extraction and assays were done in duplicate. One gram fresh weight of seed of *Pentaclethra macrophylla* from each variety A and B was ground into paste using a pestle and mortar in 10ml 25mM mixed phosphate of extraction buffer (pH 7.0 at 4$^{o}$C). The homogenate or mixture was centrifuged at 4000rpm for 4 minutes. The supernatant fraction was stored on ice and used as crude enzyme source for the assay of both peroxidase and polyphenol oxidase.

### 2. Enzyme Assay

### (a) Polyphenol Oxidase

To 2mls of assay buffer (mixed 10mM potassium phosphate buffer, pH 7.0) at 30$^{o}$C was added to 500$\mu$l of enzyme preparation and 0.1ml of dihydroxyphenylalanine (DOPA) and the reaction started

with the addition of . 50μl of 50% stock $H_2O_2$ in 10ml of distilled $H_2O$. The mixture was incubated for one minute and 30 seconds at 30°C and the absorbance measured spectrophtometrically at 470nm against a blank of water. Activities of PPO are expressed as mmol quinone products sec $^{-1}L^{-1}$ and calculated using an extinction coefficient of 1433m –$cm^{-1}$ (Jimenez and Garcia-Carmona, 1995).

**(b) Peroxidae**

To 2ml of assay buffer (10mM phosphate buffer, mixed potassium salts, pH 7.0 at 30°C) was added to 0.5ml of enzyme preparation. To this was added 0.1ml of Guaicol (100μl). The reaction was started with the addition of 100μl $H_2O_2$. The absorbance of the resulting mixture was measured spectrophotometrically after 60 secs. at 436nm. POD activity expressed as mmol ascorbate product oxidized sec $^{-1}$ was calculated using an extinction co-efficient of 6.39 $mol^{-1}cm^{-1}$ (Putter, 1974).

## RESULTS

Seeds of variety A and B showed high germination with maximum germination values ranging between 60-90% (Table 1). Activities of polyphenol oxidase (PPO) and peroxidase (POD) increased with initial low temperature treatment in both varieties but declined with prolonged low temperature treatments (6-8days) (Tables 2 & 3).

**Table 1: Maximum Germination (%) in Seeds of *Pentaclethra macrophylla* following low temperature treatment**

| DURATION OF LOW TEMPERATURE TREATMENT (DAYS) | VARIETY A | VARIETY B |
|---|---|---|
| No treatment | 70% | 60% |
| 2 | 85% | 75% |
| 4 | 80% | 80% |
| 6 | 75% | 80% |
| 8 | 60% | 70% |

**Table 2: Activities of POD and PPO in Seeds of *Pentaclethra macrophylla* (Variety A)**

| | Peroxidase | | | Polyphenol oxidase | |
|---|---|---|---|---|---|
| | **Time** | **Absorbance 436nm** | **Activity µ/L** | **Absorbance 470nm** | **Activity µ/L** |
| **No treatment** | 30sec | 1.260 | 2139.5 | 0.882 | 6.647.2 |
| | 1min | 1.352 | 11467 | 0.964 | 3.6297 |
| 2 | 30sec. | 1.275 | 2156.5 | 0.995 | 7.4838 |
| | 1min. | 1.364 | 1156.0 | 1130 | 4.253 |
| 4 | 30 sec | 1.301 | 2207.2 | 1.128 | 8.5087 |
| | 1min. | 1402 | 1186.4 | 1.141 | 4.3032 |
| 6 | 30 sec. | 1.270 | 2146.5 | 0.962 | 7.2577 |
| | 1min. | 1.351 | 1140.7 | 1.123 | 4.228 |
| 8 | 30 sec. | 1320 | 2082.1 | 0.783 | 5.9765 |
| | 1min. | 1295 | 1090.1 | 0.772 | 2.9882 |

**Table 3: Activities of POD and PPO in seeds of *Pentaclethra macrophylla* (variety B)**

| | Peroxidase | | | Polyphenol oxidase | |
|---|---|---|---|---|---|
| | **Time** | **Absorbance 436nm** | **Activity µ/L** | **Absorbance 470nm** | **Activity µ/L** |
| **No treatment** | 30sec | 0.902 | 1522.6 | 0.681 | 5.1398 |
| | 1min | 0.885 | 758.01 | 0715 | 2.6905 |
| 2 | 30 sec. | 0.983 | 1664.7 | 1.034 | 7.7852 |
| | 1min. | 1.365 | 878.75 | 1.080 | 4.0697 |
| 4 | 30sec. | 1.295 | 2192.2 | 1.083 | 8.1545 |
| | 1min. | 1.295 | 1096.2 | 1.091 | 4.1151 |
| 6 | 30 sec. | 1.013 | 1712.3 | 1.033 | 7.854 |
| | 1min. | 1.012 | 856.05 | 1.035 | 3.9041 |
| 8 | 30 sec. | 0.842 | 1424.5 | 0.631 | 4.7635 |
| | 1min. | 0.823 | 694.5 | 0.682 | 2.5736 |

## DISCUSSION

Activities of peroxidase have been implicated and associated with various physiological processes including germination (Gasper *et al,* 1972; Asins *et al,* 1984). In this study, activities of peroxidase (POD) increased initially in seeds of *Pentaclethra macrophylla* with low temperature treatment but declined with prolonged low temperature exposure (6-8 days). The pattern of polyphenol oxidase (PPO) activity was similar to that of POD, increasing initially and thereafter declining also with germination. This agrees with a suggestion (Harrington, 1972) that desiccation injury, loss of viability and membrane, enzyme or transport disruption may occur at low moisture. This also agrees with the works of Nya *et al* (2002) who reported that activities of POD and PPO increased initially in *Irvingingia gabonensis* seeds but declined in the latter desiccation period.

The subsequent declines in the peroxidative activity occurred generally at 6 – 8 days and may be associated with some level of deterioration (stress damage).

The loss of enzyme activity with prolonged low temperature was apparent in all the treatments. This may be as result from the

depletion of substrates of reduced transports or accessibility of substrates as a result of prolonged low temperatures. Therefore, seeds of *Pentaclethra macrophylla* may show peroxidation at low temperature. The quality of the final product may be guaranteed if the seeds are dried at a higher temperature.

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