

CHANGES IN GERMINABILITY AND ACTIVITIES OF POLYPHENOL OXIDASE AND PEROXIDASE IN SEEDS OF *Pentaclethra 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.

INTRODUCTION

Pentaclethramacrophylla Benth also known as African oil bean or Congo acacia, belongs to the family Leguminosae (Mimosaceae). The seeds of *Pentaclethramacrophylla* 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 (1). Another factor is the extreme drying out of seeds causing the embryo to reduce their viability in the field (2).

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 mouthwatering sauce, (3). Medicinally, the bark decoction is used in healing sores, wounds and cuts. Some antihelmintic properties have been ascribed to the bark (3).

Activities of peroxidase, polyphenol oxidase, hydroperoxide and lipid contents of *Irvingiagabonensis* seeds during desiccation have been reported by (4). 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 (5), (6). This work was undertaken to assess activities of Polyphenol oxidase and peroxidase in seeds of *Pentaclethramacrophylla* following exposure to low temperature.

MATERIALS AND METHODS

Seed Collection and Germination Studies

Fresh fruits of *Pentaclethramacropylla*Benth were collected from a local cultivar in Itam in Uyo Local Government Area of Akwalbom 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°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°C – 30°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 *Pentaclethramacropylla* 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°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°C was added to 500µl of enzyme preparation and 0.1ml of dihydroxyphenylalanine (DOPA) and the reaction started with the addition of . 50µl of 50% stock H₂O₂ in 10ml of distilled H₂O. The mixture was incubated for one minute and 30 seconds at 30°C and the absorbance measured spectrophotometrically at 470nm against a blank of water. Activities of PPO are expressed as mmolquinone products sec⁻¹L⁻¹ and calculated using an extinction coefficient of 1433m⁻¹cm⁻¹ (7).

(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 H₂O₂. The absorbance of the resulting mixture was measured spectrophotometrically after 60 secs. at 436nm. POD activity expressed as mmolascorbate product oxidized sec⁻¹ was calculated using an extinction co-efficient of 6.39 mol⁻¹cm⁻¹ (8).

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 *Pentaclethramacrophylla*(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 *Pentaclethramacrophylla* (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 (9),(10). In this study, activities of peroxidase (POD) increased initially in seeds of *Pentaclethramacrophylla* 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 (11) that desiccation injury, loss of viability and membrane, enzyme or transport disruption may occur at low moisture. This also agrees with the works of (4) who reported that activities of POD and PPO increased initially in *Irvingiagabonensis* 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 or reduced transports or accessibility of substrates as a result of prolonged low temperatures. Therefore, seeds of *Pentaclethramacrophylla* 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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REFERENCES

1. Esenowo, G. J.; Ibok, G. N. and Sam, S. M (2006). The use of *Aspergillusniger* in breaking the seed dormancy of *Mucunaurens* and *Pentaclethramacrophylla*. *Journal of Scientific and Industrial studies*, Vol.4 No. 3 pp 57 - 59
2. Nat, J. D. (1956). *Large Scale Germination of palm*. The palm Bulletin 40: 130
3. Etukudo, I. (2003). *Ethnobotany: Conventional and traditional uses of plants*. The verdict press, Uyo, p. 80.
4. Nya, P. J.; Omokano, D. N. and Nkang, A. E. (2003). Changes in activities of polyphenol Oxidase, Abscorbate peroxidase, hydroperoxide and lipid levels during desiccation of *Irvingiagabonesis*(variety Excelsa) seeds. *Global Journal of pure and Applied Sciences* Vol. 9 No. 2 pp 165 – 169.
5. Vaughan, K. C. and Duke, S. O. (1984). Functions of polyphenol Oxidase in higher plants. *Plant physiology* 60: 106 – 112.
6. Nkang, A. and Chandler, C. (1986). Changes during embryogenesis in rain forest seeds with orthodox and recalcitrant, viability characteristics. *Journal of plant physiology* 126: 243 – 256.
7. Jimenaz, M and Garcia- Carmona, F. (1995). pH-induced Hystercs of latent Broad Bean polyphenol oxidase. *Phytochemistry* 40: pp 373 – 376.
8. Putter, J. (1974) *Peroxidase*: In: Methods of enzymatic Analysis (ed. H. U. Bergmey). Verlary chemic Meinhein, pp 685 – 690.
9. Gasper, T. H.; Penel, G.; Thorpe, T. and Gripin, H. (1982). Peroxidase: 1970, 1980. *A survey of their biochemical and physiological role in higher plants*, University of Geneva.
10. Asins, M. J., Benito, C. and Perezde La Vaga, (1983). A comparative study of the changes of peroxidase patterns during wheat, rice triticale germination. *Canadian Journal of Botany* 61; 3393 – 3398.

11. Harrington, I. F. and Roberts, E. H. (1972). *Seed storage and longevity*. In: *sec Biology vol.3*. (T. T. kozlowiski): New York pp. 145 – 245.