Seed Germination and Peroxidase Analysis of Some Valuable Savanna Tree Seed Species.

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ABSTRACT

Seed germination of some selected tree species including *Tamarindus indica (L), Albizia lebbeck (L) Benth, Parkia Biglobossa (Jacq). R. Br. Don, and Prosopis africana (Guill*) *Perr* and Taub with seed germination problems were studied.

Determination of peroxidase and phenolic activities of the seeds were also examined.

Seeds were sorted out as small (Ss), medium (Ms), and large size (Ls) under visual observations. The maximum germination percentage for the seeds was 80-100% after scarification treatments. Oven dry heat and wet heat treatments at 20^{o}C, 40^{o}C, 60^{o}C, 80^{o}C, and 100^{o}C gave maximum percentage of 100%. Poor germination percentages were recorded for the control. The phenolic content in seeds was significantly ($P<0.05$) higher than Peroxidase and Polyphenolic contents. The sulfuric and oven dry heat treatment is recommended for effective germination of dormant seeds of *Tamarindus indica, Albizia lebbeck, Parkia biglobossa,* and *Prosopis africana*.

(Keywords: seeds, germination, peroxidase, trees)

INTRODUCTION

The regeneration of a crop plant requires the production of a viable seed and the germination of that seed to produce the next generation. The metabolic activity associated with these two processes is quite different, involving primarily the synthesis of storage materials during seed development and the degradation of storage materials to support the developing seedling during germination. Seed germination occurs when the seed coats rupture and the seedling emerges. Under soil conditions, germination of seed takes place below soil level.

Seed germination involves two cases: the one in which the cotyledon emerges from the seed and the one in which the cotyledon remains within the seed. In the first case, the seed swells to rupture the seed coat; the radicle elongates pulling the cotyledon upward as being found in cowpea. In the second case, elongation of the radicle suspending the cotyledon does not occur. Therefore, the cotyledon is buried beneath the ground level.

Germination in a real sense is defined as the physiological event that occurred from the beginning of seed imbibitions to the onset of radicle elongation. Seed germination is a prerequisite for maintaining an adequate sized population to ensure long term survival of species. The processes of producing seed and germinating seed are normally separated in time by desiccation of the seed which terminates the first phase. The relationship between the level of germination and the stage of development may vary in seeds. Changes in seed moisture content are important part of the germination process in mature seeds. The quality of a seed lot is determined by its germination capacity which is an expression of its viability.

The genus *Tamarindus* is monotypic; that is it has only single species. The specific name "*indica*" perpetuates illusion of Indian origin (Keay1989). It belongs to the family Leguminosae. It is a multipurpose tree species (MPTs) as vindicated by the activities of some Forest Research Institutes in Nigeria, International Centre for

under-utilized crop (ICUC) and other Forestry organization in the world (Gunasena, 2000).

Albizia lebeck L (Benth) (Leguminoceae) is a fast growing nitrogen fixing heavy shade tree recommended for afforestation and firewood plantation in the tropics (Alabi,1993). It is grown in the wild most especially in the Guinea Savanna regions of Nigeria. It is a deciduous tree which plays a vital role in savanna nutrient recycling and the prevention of soil erosion (Alabi, 1993).

Parkia biglobossa seed is commonly called "African locust bean". The plant is a legume slightly indented between the seeds at maturity (Aliero, 2000). The tree fixes atmospheric nitrogen to the soil, its seeds remain viable for along period of time and widely used as food additives.

Prosopis is a genus of 44 tropical species with only Africana common in Nigeria. The seeds are also used in food additive/flavor enhancer and used commonly with the people of Ighala, Tiv, and others who call it Ukpehi (Agboola, 1995).

Peroxidase activity and isoperoxidase profiles have been investigated in relation with the course of several development processes which include abscission, sex expression, fruit ripening, senescence, and seed germination (Lewerk, 1986; Venceet et al., 1986). Peroxidase changes have been found to be correlated with many physiological events (Gasper et al., 1991).

The present study showed various ways by which the seeds could be propagated locally in order to replace those that were felled recklessly without a corresponding afforestation and to determine the peroxidase content levels in the seeds.

MATERIALS AND METHODS

Seed Extraction and Processing

Ripe and fresh fruits of *Tamarindus indica*, *Albizia lebbeck,* and *Parkia biglobossa* were collected directly from parent stands after fruit fall, in Savanna parts of Nigeria. The seeds were removed, air-dried, and kept under ambient temperature ($30^{o}\pm20^{o}$C). Seeds were surfaced sterilized by stirring in 0.1% Mercuric Chloride for 1 min. and rinsed thoroughly thereafter in several changes of distilled water.

Morphological Studies

Seeds were grouped according to observable differences. Some were grouped and found to be lighter or darker while some were larger (1.5-1.6cm), medium (1.0-1.5cm), and smaller (0.6-0.8cm) in size. Some seeds are flat in shape while some are irregular. All these were noted under visual observation.

Dormancy Treatments

Seeds were exposed to various dormancy releasing methods which included: chemical, wet, and oven dry heat treatments. In the chemical treatment, seeds were divided into lots and immersed in conc. sulfuric acid (H_2SO_4) for the period of 5-15 mins., after which the seeds were rinsed in distilled water before fixing for germination.

Seeds were exposed to wet heat treatment by partial immersion of the seeds in water using a sterile white piece of cloth at 20^{o}C, 40^{o}C, 60^{o}C, and 80^{o}C for 10 mins. each. Treated seeds were immediately transferred into cold water before fixing for germination.

In the oven dry heat treatment, seeds were exposed to temperatures of 20^{o}C, 40^{o}C, 60^{o}C, and 80^{o}C for 5-10 mins. each. Treated seeds were allowed to cool at ambient temperature before fixing for germination.

In each case, after treatment, the seeds were fixed in 9cm diameter Petri dishes lined with filter paper; 10 seeds in each with 5 replicates for the different treatments. A control experiment was mounted containing untreated seeds. Treated and untreated seeds were allowed to imbibe distilled water under continuous light (750Lux) at bench level and at constant temperature under laboratory condition. Germination was recorded for 10 days.

Peroxidase Assay

About 2.5ml of assay buffer (20mM phosphate buffer containing 0.5g of KH_2P0_4, 0.7g of K_2HOP_4, pH 7.0) in a cuvette at 30^{o}C was added to 0.2ml of partially purified enzyme preparation and 0.1ml of guiacel (0.224ml in 20ml of distilled water). The reaction started with the addition of 0.05ml of H_2O_2 preparation (50% H_2O_2), 0.068ml in 50ml of

distilled water. The absorbance reading after 30 and 60 seconds were measured spectrophotometrically at 43.6nm with the assay buffer as blank. The peroxidase activity expressed was calculated using an extinction coefficient of $6.39mol^{-1}cm^{-1}$ for guiacol dehydrogenation product (Putter 1979).

PolyphenolOxidase Assay

A buffer of 2.5ml was mixed with 20mM potassium salts, pH 7.0, and was added to 0.1mlof hydroxyphenylalamine (20mM DOPA in distilled water) and 0.2ml of enzyme preparation. The reaction started with the addition of 0.2ml of 20mM H_2O_2 (7cmol H_2O_2) to a final volume of 3.0ml. The absorbance of the mixture was measured spectrophotometrically (30 and 60 seconds) at a wavelength of 475 (Kahn, 1983). The activity was calculated using an extraction coefficient of $1433mMcm^{-1}$ for the guinone product (Jimenez,1995).

Determination of Phenolic Content of the Seed

Approximately 4g of sample was weighed into a test tube and 3ml of 70% acetone was added in water to the test tube which was placed in water bath at 10^oC for one minute and stirred occasionally with a glass rod. It was then filtered through a 50-60 µm Gooch crucible into 50ml Erlenmeyer flask. The extraction was repeated 3 times. The test tubes was rinsed with the final 3ml portion of 70% acetone in water and emptied into the crucible. About 2ml of 0.1M Yb-acetate and 15 ml of 0.1M TEA was added into filtrate. The flask was closed and kept in cold storage (4^oC) overnight for complete precipitation. Hot empty crucible was hot weighed (W1) and hot weighed filter paper (W2) Yb-precipitated phenolic was filtered through the pre-weighed filter paper.

The precipitate was washed with 50ml of 70% acetate in water and 50ml of distilled water and finally with pure acetone until the filtrate becomes colorless and air dried. The filter paper with the precipitate was placed in the pre-weighed Gooch crucible. It was then oven dried overnight at 105^oC and weigh (W3); washed at 525^oC for 3 hours, and reweighed (W4). The loss of organic matter upon ash estimates the amount of 76 precipitated phenolic in sample. The organic matter precipitate was calculated as a percentage of the sample weight:

% Soluble Phenol = (W3-W2 (W4-W1) x 100/W x DM%

Where:
W-weight of sample
W1-weight of empty crucible
W2-weight of empty crucible + filtered paper
W3-weight of crucible +filter paper + precipitate
W4-Weight of crucible + ash

RESULTS AND DISCUSSION

Table 1: Effect of H_2SO_4 on the Percentage Seed Germination of *Albizia lebbeck.*

Seed size	5min	Control	10min	Control	15min	Control
Ss	100a	0.0e	80b	0.0e	100a	0.0e
Ms	80b	0.0e	60c	0.0e	100a	10.0d
Ls	100a	0.0e	100a	0.0e	100a	0.0e

Mean followed by the same letter are not significantly different at 5% level
Ss-small size, Ms-medium size, and Ls-large size

Table 2: Effect of H_2SO_4 on the Percentage Seed Germination of *Parkia biglobossa.*

Seed size	5min	Control	10min	Control	15min	Control
Ss	100a	0.0e	60b	0.0e	80b	0.0e
Ms	100a	0.0e	50c	0.0e	80b	0.0e
Ls	100a	0.0e	60b	0.0e	60c	0.0e

Mean followed by the same letter are not significantly different at 5% level
Ss-small size, Ms-medium size and Ls-large size

Table 3: Effect of H_2SO_4 on the Percentage Seed Germination of *Prosopis Africana.*

Seed size	5min	Control	10min	Control	15min	Control
Ss	80b	0.0e	80b	0.0e	100a	0.0e
Ms	100a	0.0e	100a	0.0e	100a	10.0d
Ls	100a	0.0e	100a	0.0e	100a	0.0e

Mean followed by the same letter are not significantly different at 5% level
Ss-small size, Ms-medium size and Ls-large size

Table 4: Effect of H_2SO_4 on the Percentage Seed Germination of *Tamarindus indica*.

Seed size	5min	control	10min	Control	15min	Control
Ss	100a	0.0e	100a	0.0e	100a	0.0e
Ms	100a	0.0e	100a	0.0e	100a	10.0d
Ls	100a	0.0e	100a	0.0e	100a	0.0e

Mean followed by the same letter are not significantly different at 5% level
Ss-small size, Ms-medium size and Ls-large size

Table 5: Effect of Oven Dry Heat Treatment on Percentage Seed Germination.

Seed type	20°C	40°C	60°C	80°C	100°C
T.*indica*	100	80	100	100	100
A.*lebbeck*	100	60	80	60	100
P.*biglobossa*	100	80	100	60	100
P.*africana*	80	80	80	100	100

Mean followed by the same letter are not significantly different at 5% level

Table 6: Effect of Wet Heat Treatment on Percentage Seed Germination.

Seed type	20°C	40°C	60°C	80°C	100°C
T.*indica*	100a	60c	80b	100a	100a
A.*lebbeck*	100a	60c	100a	70b	80b
P.*biglobossa*	100a	80b	100a	100a	100a
P.*africana*	50d	80b	100a	80b	80b

Mean followed by the same letter are not significantly different at 5% level

Table 7: Peroxidase Analysis of Tree Seed Species.

	T.*indica*	A.*lebbeck*	P.*biglobossa*	P.*africana*
Peroxidase(µg/ml)	0.8c	0.6c	0.8c	0.4d
Polyphenol(µg/ml)	1.4a	1.0b	1.2b	0.8c
Phenol (µg/ml)	2.5a	2.0a	2.5a	1.0b

Mean followed by the same letter are not significantly different at 5% level

DISCUSSION

Savanna tree seeds especially those used for the present study exhibit germination problems. Germination covers processes which are involved in the transformation of plant's embryo into an independent established seedling (Bryant 1985); certain seeds in the presence of all the conditions required for germination refuse to germinate hence the need for pre-germination treatments. Pre germination treatment of seeds is necessary in the case of *Tamarindus indica, Albizia lebbeck, Prosopis Africana, and Parkia biglobossa.* The seeds treated with H_2SO_4 for 5-10 mins gave maximum percentage 80-100%; wet and dry heat treatments showed maximum percentage germination of 100%, against 0% germination obtained in control experiments. This may be as a

result of hard integument of seeds or impermeability of the waxy seed coat to water and gases, or insensitivity to light. However, the nature of the seed coat is changed by the corrosive effect of acid, resulting in an inflow of water and gases and unrestricted expansion of the embryonic parts (Idu, 2002).

Wet and oven dry heat treatments at temperature of 20^{o}C, 40^{o}C, 60^{o}C, 80^{o}C, and 100^{o}C enhanced seeds germination. They gave highest percentage germination at 10 mins treatment. This might be as a result that the treatments helped in softening the seed coat thus giving rise to percentage germination. This study did not agree with the study of Idu, and Omonhinmi, (1999) on *Dchrostachys cinerea* seeds.

Higher temperature might tend to kill the essential food reserves in the seeds as the higher the temperature the higher the possibilities of destroying the embryo in preparation for germination as this may affect the output of germination; slightly dipping of seeds in boiling water or dry oven may lead to rupturing of the seed coat walls by allowing water to penetrate the seed tissues causing physiological changes and subsequent emergence of radical (Agboola 1991, Agboola 1998, and Sabongari 2001).

There were high significant differences ($P<0.05$) in activities of oxidative enzymes (peroxidase and polyphenol oxidase) and phenolic contents in the seeds of Parkia *Tamarindus, Albizia,* and *Prosopis*. The present study showed high peroxidase contents in the seeds especially in *Parkia* and *Tamarindus;* likewise in polyphenoloxidase. The study showed the higher phenolic content levels in the seeds reduce the polyphenol oxidase and peroxidase, and vice versa.

Phenolic content levels increased germination; as phenolic content interferes with water and oxygen uptake during germination (Gasper *et al.,* 1991). The association in activities of peroxidase and polyphenol with environmental stress is open for further research.

CONCLUSION

The research findings will assist in raising seedlings for afforestation most especially from seeds that are problematic or give erratic germination. It will also go a long way in accessing the level of peroxidase and the polyphenolic contents of the Savanna seeds.

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