STUDIES ON THE SEEDS OF *Lablab niger* Medic. (*Leguminosae, Papilionatae*)[1] /

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Resumen

Tres variedades de semillas, clasificadas con base en su color y grado de manchado, fueron identificadas en las vainas de Lablab niger, *un cultivar tropical común. Las variedades fueron designadas como ligeramente manchado pardo oscuro (SDB), fuertemente manchado pardo oscura (HDB) y ligeramente manchada pardo clara (SLB). Entre ellas, la característica SDB fue dominante, mientras que SLB se mostró como recesiva; la proporción en que se presentan todas ellas fue de 7:4:1. Las variantes de semillas recolectadas recientemente, así como aquellas atacadas por insectos, sometidas a varios estudios experimentales, mostraron un grado similar de germinación tanto a la luz como en la oscuridad, con una temperatura óptima de 30°C. La profundidad óptima para plantar las semillas fue de 2 cm.*

Su almacenamiento fue favorecido por temperaturas bajas, pues retienen un mayor porcentaje de viabilidad. Entre los diferentes métodos de almacenamiento, el uso de una película de aceite castor sobre las semillas dio los mejores resultados.

Introduction

Lablab niger Medic. is a climbing perennial herb which grows in the tropics. It is native to East Indies and has been observed to thrive very well in the West Coast of Africa (4). Purseglove (5) also reports that *L. niger* may likely have its origin in Asia. Although it occasionally grows wild, in Nigeria it is commonly cultivated for food. The fresh pods, which are used for food, are a good source of easily available plant protein.

The plant is cultivated at the beginning of the rainy season, March/April and it starts flowering in May/June. It continues to bear pods till the end of December and fruiting declines till the next season.

Although it is a rich source of plant protein, much attention has not been given to its large scale cultivation in Nigeria. This is probably because a large number of the population depends on animal protein and other sources of plant proteins which are now becoming more expensive.

The present investigation is undertaken with a view to carrying out a more elaborate study on the various aspects of the seeds in relation to viability, germination and storage.

Materials and methods

Healthy seedlings of *L. niger* were raised directly at the beginning of the rainy season (the first week of April, 1983) at a distance of 150 cm apart in single rows of raised ridges. Seedlings were raised in the University of Ilorin main campus (Latitude 08° 26'N, Longitude 04° 30'E). At full growth the climbing stems were supported with wooden stakes. Details of the climatic data are shown in Table 1. Seeds used for the present study were collected as a fresh harvest in July 1983. Dry seed pods were randomly collected from 50 plants and then pooled. Ten random samples of 30 pods each were taken from the pool, opened

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Table 1. Climatic data with regards to the locality during the growth of *Lablab niger* Medic.

Parameters	May	June	July	Aug.	Sept.	Oct.
Av Max Air Temperature (°C)	32.9	30 7	28 8	27 9	32 5	34 6
Av Min Air Temperature (°C)	22.8	21 9	21 6	21.1	21 4	21 1
Rainfall (mm)	156 2	131 1	126 2	78 7	42 4	0 8
Relative Humidity at 06 00 GMT (%)	91	90	86	81	77	70
Av Max Soil Temperature						
at 5 cm	30 8	29 8	28 4	27.2	31 8	33 1
30 cm (°C)	23 5	21 2	20 9	20 2	24 6	25.6
Av Min Soil Temperature at						
5 cm	21 9	20 4	20 2	20 0	20 0	20.9
30 cm (°C)	17 6	17 2	17 1	17 1	17 6	18 1

and the seed distribution was observed The seeds were also examined for morphological characters by using a hand lens On the basis of testa colour and degree of spotting, seeds were classified in three distinct types, consistently present in all the pods The occurrence and ratio of each variant in each sample of 30 pods were recorded A high percentage of the freshly harvested seeds was found to be attacked by the beetle *Callosobruchus phaseoli* Gyll Since a high percentage of the seeds is attacked by insects similar experiments performed on normal seeds were also carried out on insect-attacked seeds to determine whether they would deviate from the behaviour of normal seeds. All the insect-attacked seeds used had insect-bored holes of 1 to 4.

Imbibition studies

This experiment was carried out to determine the water absorption capacity of the seeds A batch of 50 seeds from each variant (10 seeds per replicate) was weighed on a Mettler balance and soaked in sterile distilled water for 12 hours The seeds were then blotted dry and weighed to determine the increase in weight due to imbibition (3)

Effect of temperature on seed germination

Preliminary experiments have shown that the seeds of *L. niger* germinate equally well in the dark or in the light The effects of temperature on the different seed variants were carried out at 20°C, 25°C, 30°C, 35°C and 40°C Ten seeds of each variant were plated aseptically in 9 0 cm petri dishes containing sterile filter papers moistened with 10 ml of sterile distilled water Petri dishes were incubated under a light intensity of 200 lux for ten days and germination counts were recorded daily The experiment was replicated ten times

Effect of planting depth on seedling emergence

Ten seeds of each variant were planted in pasteurised loamy soil placed in cocoa bags at depths of 2, 4, 6 8 and 10 cm Ten bags containing the seeds were then placed in open plastic bowls (34 cm diameter) Each plastic bowl contained sufficient water to keep the soil moist Water ascended each bag through the perforations in the basal region by capillary force, thus minimising soil crusting and compaction The initial water level in the bowls was maintained constant throughout the experimental period The experiment was replicated ten times Emergence counts were recorded daily for 15 days. In order to determine the effect of planting depth on hypocotyl length all the seedlings were carefully uprooted and the measurements of the hypocotyl were recorded on the 15th day from the date of emergence The stem length above the cotyledons, as well as the number of leaves including cotyledonary leaves, were recorded

Seed storage and viability

Various temperatures and cultural methods were tried for storage and viability of only normal seeds. Seed storage was performed in order to identify the best and effective method that will prevent pest attack during storage without the seeds losing their viability The temperatures tried were 5°C, 15°C, 27 ± 3°C, 40°C and 55°C, while the cultural methods were (i) addition of dried fruits of *Capsicum annum* to seeds, (ii) thin smearing of seeds with previously heated castor oil (10 ml of castor oil/kg of dried seeds), (iii) admixture of benzene hexachloride (BHC) or DDT dust with seeds, (iv) addition of naphthalene ($C_{10}H_8$) crystals to seeds kept in a closed container to prevent vaporisation of the crystals and odour, (v) addition of fresh neem leaves, *Azadirachta indica* to seeds. All the cultural methods were maintained at room temperature after treatment. Seeds were put in portable sacks before they were stored for a maximum period of 6 months during which periodic germination tests were performed every month at 30°C. Germination tests were performed as previously described. Storage effectiveness was also assessed by calculating percentages and viability of insect-free seeds under each storage condition.

Results and discussion

Pods of *L. niger* have been observed to contain three seed variants which are morphologically different in terms of testa colour and spotting. These three seed variants have been designated as slightly spotted dark brown (SDB), heavily spotted dark brown (HDB) and slightly spotted light brown (SLB). Similar observation on seed variance has also been reported for *Parkia clappertoniana* (2). SDB and HDB seeds were observed to occur together in the same pods, with SDB seeds occuring in a higher number. SLB seeds, wherever they occur, were always confined to one pod only. The approximate average ratio of occurrence of SDB, HDB and SLB seeds in 10 random samples of 30 pods each was 7:4:1 respectively (Table 2). Generally the number of seeds per pod varied from 2 to 6.

The percentages of seeds attacked by the beetle *C. phaseoli* in freshly harvested pods were found to be approximately 38%, 19% and 3% for SDB, HDB and SLB seeds respectively (Table 2). These results suggest that early harvest after seed maturation is essential if seed wastage due to insect attack is to be avoided.

The average weight of normal and insect-attacked seeds varied from 340-380 mg and 330-360 mg respectively. These data differ markedly from those reported by Irvine (4) and Purseglove (5) who gave seed weight range of 250-500 mg. The loss of weight in insect-attacked seeds was due to the destruction of part of the cotyledons. No significant difference in weight was observed between normal SDB and HDB variants while normal SLB variant showed a significant difference from the former two variants (Table 3). The percentage increase in seed weight of normal SDB, HDB and SLB seeds after imbibition of water was 108, 105 and 68 respectively. Water imbibition by the seeds compared to the initial weight tends to be directly proportional to initial seed weight. Insect-attacked seeds showed a higher percentage of water imbibition than normal seeds probably due to water occupying the holes bored by the insects (Table 3). All the seed variants of *L. niger* were observed to germinate equally well both in the dark and in the light. The optimum temperature for germination of normal and insect-attacked seeds was 30°C for all variants (Table 4).

Although a satisfactory percentage of germination occurred for all the normal seed variants at 35°C, the insect-attacked seed variants showed only 25% germination for SDB while HDB and SLB showed no germination (Table 4). This is likely due to thermal effects on the embryos exposed to direct temperature through the holes bored or to embryo damage.

The poor germination at high temperatures such as 40°C suggests that seeds are not suitable for seedling propagation during the dry season except where irrigation facilities are available.

The most suitable planting depth for seedling emergence was 2 cm. No normal seeds emerged below 8 cm planting depth. A similar observation was made

Table 2. **Distribution of seed variants in randomly selected pods of *Lablab niger***

Samples of 30 pods	Total number of seeds in 30 pods	Average number of seeds per pod (± 1 SE)[1]	Number of SDB seeds in 30 pods	Number of HDB seeds in 30 pods	Number of SLB seeds in 30 pods	Ratio of occurrence of seed variants in 30 pod samples	Number of insect attacked seeds in 30 pods		
							SDB	HDB	SLB
A	117	3.9 ± 0.91	89	24	4	22:6:1	14	3	0
B	121	4.0 ± 0.63	83	29	9	9:3:1	15	4	1
C	114	3.8 ± 0.12	65	37	12	5:3:1	14	4	1
D	117	3.9 ± 0.58	68	46	3	23:15:1	12	10	0
E	114	3.8 ± 0.15	56	49	9	6:5:1	10	8	0
F	119	4.0 ± 0.63	69	39	11	6:4:1	12	6	2
G	116	3.9 ± 0.39	58	50	8	7:6:1	10	5	1
H	105	3.5 ± 0.25	53	42	10	5:4:1	8	5	1
I	112	3.7 ± 0.83	42	55	15	3:4:1	8	5	3
J	112	3.7 ± 0.15	66	32	14	5:2:1	12	6	1

SE = Standard Error

Table 3. Water Imbibition (mg) by seeds of *Lablab niger*

Measurements	Normal seeds			Insect-attacked seeds		
	SDB	HDB	SLB	SDB	HDB	SLB
Final weight	770	780	570	750	720	590
Initial weight	370	380	340	350	330	360
Increase in seed wt (%)	108	105	68	114	118	109

Table 4. Influence of temperature on seed germination of *Lablab niger* Germination data taken after 10 day incubation period.

Temp °C	Normal (% germination) ± ISE[1]			Insect attacked (% germination) ± ISE		
	SDB	HDB	SLB	SDB ± ISE	HDB ± ISE	SLB ± ISE
20	11 ± 1 3	37 ± 1.8	26 ± 1 0	14 ± 0 8	26 ± 1.3	19 ± 0 4
25	82 ± 3 1	45 ± 1 7	64 ± 2.4	22 ± 1 1	43 ± 0.9	56 ± 1 8
30	89 ± 2 6	85 ± 2 2	100 ± 2 8	85 ± 1 5	64 ± 1.5	66 ± 1 8
35	36 ± 1 8	63 ± 2.6	100 ± 1 9	25 ± 0 9	0 ± 0	0 ± 0
40	9 ± 0.2	11 ± 0.3	13 ± 0 5	0 ± 0	0 ± 0	0 ± 0

1 ISE = Standard Error

for the insect-attacked seeds although they showed a lower percentage emergence (Table 5) This may be due to the less protection of the embryo of the insect-attacked seeds thus resulting in decay caused by microorganisms Examination of ungerminated and unemerged seeds showed that they had decayed. The planting depth had no impact on the hypocotyl length or stem length above the cotyledons of seedlings raised from normal and insect-attacked seeds The average total leaf number varied from 4 to 6 for all seedlings irrespective of the planting depth (Table 5)

Generally low temperatures (5-15°C) seem to be most favourable for seed storage and viability (Table 6) It was also observed that seeds stored under these temperatures were not affected by insects. Probably these low temperatures inhibit the development of the eggs of the insect pests Seeds stored at room temperature (27 ± 3°C) tended to be highly susceptible to insect attack that causes the destruction of the cotyledons Although the seeds under room temperature were prone to attack by insects, their viability was not severely affected Temperatures of 40°C and 55°C were found to be suitable for seed storage because they were relatively free from insect attack However, viability was highly reduced at 40°C and completely lost at 55°C It is probable that long storage under these high temperatures completely dehydrates the seeds and destroys the embryo due to high thermal effect These observations agree with those of Etejere (1)

Among the cultural practices used for seed storage, the castor oil smear was observed to be the most suitable Seeds were completely free from insect attack while still retaining a high percentage of viability (Table 7) The use of BHC/DDT dust or naphthalene crystals seems to be fairly effective for both seed storage and viability However, great care should be taken to get rid of these chemicals before they are used for animal or human consumption. Smartt (6) also reports that seeds meant for sowing can be protected against insect attack by an admixture of BHC or DDT dust The use of napthalene cystals, however, requires closed containers in order to prevent the evaporation of the crystals and the loss of the insect-inhibiting smell. The use of fresh leaves of *A indica* (neem) or dried fruits of *C annum* seems to be effective for storage for short periods since insects tend to develop with longer periods of storage due to the loss of the repelling strong smell of the neem leaves or *C annum* fruits

Table 5. Effect of planting depth on seedling emergence and development of *Lablab niger* raised from normal seeds. Data were obtained after 15 days of seedling emergence.

Planting depth (cm)	2			4			6			8			10		
Seed variants	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB
Seedling emergence at day 15 (%)	100	81	92	98	73	80	32	40	37	0	0	0	0	0	0
Av. hypocotyl length (cm)	11.5	15.3	11.8	17.0	15.1	12.0	14.0	15.2	10.0						
Av. stem length above cotyledons (cm)	59.6	65.3	56.0	65.0	52.0	67.6	63.0	64.0	74.0						
Av. total leaf number	5	6	5	5	6	6	5	5	6						

Table 6. Effect of planting depth on seedling emergence and development of *Lablab niger* raised from insect-attacked seeds. Data were obtained after 15 days of seedling emergence.

Planting depth (cm)	2			4			6			8			10		
Seed variants (insect-attacked seeds)	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB	SDB	HDB	SLB
Seedling emergence at day 15 (%)	66	30	51	33	12	18	6	8	0	0	0	0	0	0	0
Av. hypocotyl length (cm)	3.9	11.0	17.0	15.5	13.2	9.0	12.0	14.0							
Av. stem length above cotyledons (cm)	64.2	62.1	27.5	45.0	58.6	53.1	59.0	51.9	–	–	–	–	–	–	–
Av. total leaf number	6	5	4	6	5	6	6	6	–	–	–	–	–	–	–

Table 7. Seed storage and viability of *Lablab niger*. Data were collected after six months storage period.

Storage condition	Seeds free from insect attack (%)			Germination of seeds after 6 months storage (%)		
	SDB	HDB	SLB	SDB	HDB	SLB
5°C	100	100	100	92	82	98
15°C	100	96	97	90	86	92
27°C ± 3°C (Room Temp.)	5	8	12	81	79	75
40°C	98	91	93	12	10	17
55°C	98	99	96	0	0	0
Addition of *C. annum* dried fruits	11	14	22	80	84	76
Castor oil smear	100	100	100	98	89	93
Admixture of naphthalene crystals	75	70	68	80	80	71
Admixture of BHC/DDT dust	69	71	77	86	73	68
Addition of neem leaves	9	13	18	68	61	75

Low temperature storage methods, though highly effective for seed storage and viability, are not feasible in developing countries due to their high cost. The most suitable and feasible method for storage of *L. niger* is the castor oil smear. This has the advantage of keeping the seeds free from insects for very long periods while also retaining a high percentage of viability. Other storage methods suggested by Smartt (6) could also be practised and these include the use of sealed and thermally insulated store and for small quantities of seeds, sealed drums or polythene bags is effective.

Summary

Three seed variants based on testa colour and degree of spotting have been identified in pods of *Lablab niger*, a common tropical crop. These variants have been designated as slightly spotted dark brown (SDB), heavily spotted dark brown (HDB) and slightly spotted light brown (SLB). Among the variants, SDB seeds were observed to be dominant while SLB seeds are recessive, all being in the occurrence ratio of 7:4:1. Freshly harvested normal and insect-attacked seed variants subjected to various

experimental studies showed that they germinate equally well in the light and dark with an optimum temperature of 30°C. The most suitable planting depth for the seeds was found to be 2 cm. Storage of seeds was most favoured by low temperatures which retained a high percentage of viability. Among the cultural methods of storage, the use of castor oil smear on seeds gave the best results.

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