UNIVERSITI PUTRA MALAYSIA

STORAGE OF COCOA (THEOBROMA CACAO L.) SEEDS AND CHANGES ASSOCIATED WITH THEIR DETERIORATION

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STORAGE OF COCOA (*THEOBROMA CACAO L.*) SEEDS AND CHANGES ASSOCIATED WITH THEIR DETERIORATION

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LIST OF ABBREVIATIONS

The following abbreviations were used in the text :

ABA	=	Abscisic acid
PEG	=	Polyethylene glycol
LSD	=	Least significant difference
DMRT	=	Duncan's multiple range test
RH	=	Relative humidity
ER	=	endoplasmic reticulum
ETOH	=	ethyl alcohol
TBA	=	tertiary butyl alcohol
w/w	=	weight by weight
v/v	=	volume by colume
wb	=	fresh weight basis
db	=	dry weight basis
h	=	hour
min	=	minute
^oC	=	degree centigrade
cm	=	centimetre
mm	=	millimeter
u	=	micron, micrometre
kg	=	kilogram
g	=	gram
mg	=	milligram
ug	=	microgram
l	=	litre
ml	=	millilitre
ul	=	microlitre
M	=	molar
mM	=	millimolar
uCi	=	microcurie

ABSTRACT

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philisophy.

STORAGE OF COCOA *(THEOBROMA CACAO L.)*
SEEDS AND CHANGES ASSOCIATED WITH THEIR DETERIORATION

by

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Cocoa *(Theobroma cacao L.)* seeds are recalcitrant and rapidly lose their viability during preparation and within two to three weeks of storage. To improve their storability, the responses of a cultivar of cocoa seeds (NA 33) to different drying methods and storage factors including seed moisture, storage temperature and fungi were investigated. Physiological, biochemical and structural changes associated with seed death caused by dehydration in the air-conditioned room ($22^{o}C$, 55% RH) and chilling at $10^{o}C$ were also monitored.

Freshly harvested seeds were best dried in the air-conditioned room. The critical seed moisture content was 26% to 27%, but for storage a moisture content of 33.5% to 35% was optimal. Temperatures below 15°C were lethal and storage in the air-conditioned room at 22°C is recommended. Seed dusting with 0.2% w/w of an equal benlate-thiram mixture was essential since untreated seeds were rapidly killed by storage fungi such as *Penicillium* spp., *Aspergillus* spp. and *Botryodiplodia theobromae*. Treated seeds maintained germination for at least six weeks and retreatment with fresh fungicides either as a dust or a soak did not prevent rapid seed death after this period. For optimal storage, cocoa seeds at 33.5% to 35% moisture and dusted with 0.2% w/w of an equal benlate-thiram mixture should be stored in thin perforated polythene bags in loosely closed plastic boxes in the air-conditioned room. Germination of at least 80% could be maintained for two to three months.

Changes associated with dehydration damage and chill injury of cocoa seeds were different. Although germination and seedling growth were rapidly decreased in both cases, axial respiration and protein synthesis were unaffected by the chill treatment, but were reduced significantly in axes moderately damaged by dehydration. Loss of membrane integrity as evidenced by increased leacheate conductivity also occurred in seeds moderately damaged by dehydration, but was not detected in chilled seeds until they were totally killed. Progressive damages to cell organelles including cell membranes, mitochondria, ribosomes and nucleii were observed with increasing dehydration damage. Conversely, organelles were essentially unchanged in chilled seeds except for severe derangement of the plasmalemma and tonoplast. The data suggest that death caused by dehydration is progressive and involves damage to many biological processes including respiration, protein synthesis and function of cell organelles; culminating finally in total cell collapse. Death caused by low temperature is more abrupt and may be triggered by only a few vital processes resulting mainly in severe degeneration of cell membranes and their related functions. Respiration, protein synthesis and other cell organelles were essentially unaffected.

1. INTRODUCTION

Cocoa, *Theobroma cacao*, is one of several species belonging to the family *Sterculiaceae*. It originates from the tropical rain forest of Central America where its seeds were used for concocting a drink popular with the Mayas and Aztecs. Today the seed is used for manufacturing a wide range of beverages and confectioneries. This was supported by a world production of 1.67 million tonnes of cocoa beans in 1981 (FAO, 1982).

The main producers of cocoa are localised in the tropical Americas and Africas. These include Brazil, Ghana, Nigeria and the Ivory Coast. There is less emphasis on the crop in tropical Asia, probably because of the pre-eminence of other plantation crops such as rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*). In Malaysia, small areas of the crop were grown, but owing to disease, poor management and lower profitability, it did not expand as rapidly as rubber and oil palm. However, in the early 1970's crop diversification was emphasised and this together with higher price for cocoa resulted in an expansion of the crop. This is evident from the increased area from which the crop is harvested, from 9,000 hectares in 1970 to 72,000 hectares in 1981 (FAO, 1982).

With the expansion of the crop, the demand for planting materials has also increased. In Malaysia, the crop is largely propagated by seed although vegetative propagation is possible. The demand for planting materials exists throughout the year, but seed production is bimodal and generally peaks during the months of February to March and October to November. Such seasonal supply in the face of continual demand emphasises the need for a suitable method of seed conservation during the peak seasons.

However, cocoa seeds are recalcitrant in that they do not withstand dehydration and low temperature (Swarbrick 1965, Barton, 1965). They deteriorate rapidly when exposed to humid tropical conditions. The maximum period of storage reported is only approximately three to four weeks. Even under such short storage period, a high percentage of the seeds was already pregerminated (Evans, 1950; Swarbrick, 1965). If handled like orthodox seeds, they lose their viability even faster in a matter of two to three days. Because of this, much planting materials have been lost as a result of poor handling and storage before the seeds were planted.

The recalcitrant nature of the seed has also made it difficult to conserve the genetic resources of cocoa. As very short term storage is only currently available, the main method for conserving cocoa germplasm is by the planting out method. With the current rate of depletion of our world natural reserves, the loss of germplasm materials of cocoa and other recalcitrant seeds is very real.

The need to improve the storability of cocoa seeds has prompted the first objective of this study, which is to investigate the effects of various seed and environmental factors on the storability of cocoa. It is hoped that with a clearer understanding of the behaviour of cocoa seeds under various conditions, an improved method of storage may be devised to prolong their viability.

The second objective of the study is a follow up of the first, and involves investigations into the physiological, biochemical and structural changes associated with the deterioration of cocoa seeds. It is aimed at elucidating the changes involved as seed viability decreases during storage. It is hoped that these studies will provide a better understanding of seed deterioration and point out venues for improving further the storability of cocoa seeds.

2. REVIEW OF LITERATURE

2.1. STORAGE OF COCOA SEEDS

Based on the behaviour of seeds during storage, Roberts (1973 a) categorised them as orthodox or recalcitrant. Orthodox seeds are those which can be dried to a relatively low moisture of 4% (Harrington, 1970) without loss of viability. On the other hand, recalcitrant seeds are unable to withstand excessive dehydration and rapidly lose their viability at relatively high moisture levels of 12% - 31% (Roberts, 1973 a). Many recalcitrant seeds also do not tolerate low temperature and are often injured at temperatures of less than 10°C - 15°C. Because of their susceptibility to drying and low temperature, cocoa seeds are also considered to be recalcitrant.

Maintenance of the viability of recalcitrant seeds is more difficult since they have no inherent quiescent stage and are not storable at low moisture and temperature (Tang and Tamari, 1973; Chacko and Singh, 1971; Chin 1975; Teng 1977 b; Ang, 1976; and Chin *et al*, 1983). A review of the literature on the storage of cocoa seeds illustrates clearly the problems involved in storing these seeds, especially when they are unable to withstand cold conditions. Such studies began in the 1930's, but initial works were concentrated mainly on pod storage. Subsequent studies shifted the emphasis to the storage of the extracted seeds.