SUMMARY AND CONCLUSIONS

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The present study attempts to determine the physiological and biochemical changes of cocoa seeds (*Theobroma cacao* L.), known for the recalcitrant nature, in different dehydration regimes. The seeds were extracted from the fresh ripe pods, and stored in three different conditions viz open trays at room temperature, polyethylene bags at room temperature and polyethylene bags at 4°C in a refrigerator. Samples from seeds desiccated under different conditions were drawn daily up to 4 days and then at regular intervals of two days until the viability was found to be lost completely. The seeds were used for the estimation of moisture content, germination percentage, seed vigour index and electrical conductivity of the leachates.

The biochemical components of seed tissues of cocoa such as starch, total soluble sugars, reducing sugars, free amino acids, proline, proteins, lipids and total phenolics were extracted and estimated according to standard procedures. Analysis of sugars was also made using HPLC. Lipid peroxidation, and the activities of free radical scavenging enzymes like superoxide dismutase, peroxidase and catalase were assayed. Polyacrylamide gel electrophoresis was used for protein profiling of the seeds.

To determine the mobilization of the reserve materials, cocoa seeds germinated in dark were sampled at specific intervals and metabolites such as total soluble sugars, starch, lipids, proteins and total free amino acids were estimated.

Histochemical localization of important cellular macromolecules like starch and proteins was also carried out during desiccation and seed germination.
The following observations and conclusions were made from the present investigation.

Recalcitrant nature of cocoa seeds is established due to the following characters.

1. The seeds are fleshy, heavier; contain high moisture content (37%) and loss viability when desiccate beyond 20% MC.

2. Rapid germination of seeds immediately after shedding.

3. Short life span of seeds and loss of viability within two days.

4. Germination-associated metabolic changes in seeds under desiccation.

A close correlation is observed between moisture content, viability and seed vigour index during desiccation of cocoa seeds in different storage conditions.

Rapid increase in electrical conductivity of leachates of the seeds under desiccation indicates progressive membrane perturbation under storage and desiccation. The seed vigour index decreases gradually during desiccation, indicating a decline in vigour before the loss of viability.

A steady decrease in starch content during the early stages of desiccation shows the cocoa seeds are in a metabolically active state in the initial phases of desiccation. Further decline coincides with a loss of viability.

The decline in the total soluble sugar content in the later stages of desiccation is ascribed to its utilization during active metabolism. This may result in limited availability of respiratory substrate later on, leading to loss of seed vigour and viability.
The continued loss of seed viability in spite of an increase in sucrose content during desiccation indicates that sucrose alone is not a critical factor in imparting desiccation tolerance to cocoa seeds.

A steady decline in the raffinose content correlates with the desiccation induced loss of seed viability implying that sucrose imparts desiccation tolerance in conjunction with raffinose and that an optimal sucrose to raffinose ratio is critical for it.

An increase in the reducing sugar content is suggested to initiate the Amadori-Maillard reactions in desiccating seeds leading to the damage of cellular macromolecules like DNA and proteins beyond repair.

A decline in protein content with a corresponding increase in free amino acid content indicates progressive proteolysis in desiccating seeds, which results among other things in structural changes of cellular membranes, leading to increased permeability and eventually to the loss of seed viability.

The proline content, even though showing an increase during desiccation is found to be not related to any stress relieving mechanism in desiccating seeds.

A negative correlation can be drawn between seed viability and increasing phenolic content in cocoa seeds. The declining seed viability is attributed to inhibitory effects of increasing phenolic content on phytohormones and primary metabolism.

Lipids constitute 43% of the dry mass of seeds of cocoa. An increase in the lipid content in desiccating cocoa seeds remains as an enigma. Though a decrease in starch content implies the utilization of starch in lipid biosynthesis, further studies are warranted.
A substantial increase in malondialdehyde content is observed in cocoa seeds as desiccation progresses, indicating the extensive occurrence of lipid peroxidation with moisture loss. The enhanced lipid peroxidation leads to macromolecular damage and membrane perturbation, closely correlated with enhanced loss of seed vigour and viability.

A drastic decline in the activities of free radical scavenging enzymes like superoxide dismutase, peroxidase and catalase results in the failure to quench the superoxides, peroxides and hydroxyl radicals generated during the deranged metabolism of cocoa seeds during storage. This increases the likelihood of free radical attack resulting in reduced seed viability.

Even though germination in cocoa seeds starts immediately, as a continuum of development due to its recalcitrant nature, reserve mobilization is similar to that of orthodox seeds.

The dry weight of the cotyledons was found to decline drastically during germination and seedling growth coinciding with a depletion of soluble sugars, starch and lipids implying active mobilization of metabolites to the growing axis.

A significant reduction in protein content in the cotyledons of germinating seeds is found correlated with an increase in the amount of free amino acids implying active protein degradation and its mobilization to the growing axis.

Histochemical studies corroborate the rapid mobilization of starch and proteins in cocoa seeds during germination and seedling growth.

A parallelism is found to exist between the metabolic events occurring during earlier period of desiccation and germination indicating that germination-associated changes are occurring during desiccation. The
processes associated with desiccation are prematurely terminated and viability is lost when water becomes limiting. The unrestricted availability of water during germination permits the germination and reserve mobilization processes to proceed further.