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Membrane Perturbations in ageing Jamun (*Syzygium cuminii*) Seeds

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ABSTRACT

Recalcitrant seeds of *Syzygium cuminii* (Jamun) exhibit 100% viability upto 3 dh after maturity. The rapid loss of viability after 3dh is associated with reduction in moisture content below lowest safe moisture content (47.71% MC). Seed became nonviable after slow drying to 20% MC within a very short period of 27 dh during storage at ambient condition. Decline of percent germination in dehydrating Jamun seeds was strongly and negatively correlated with increased leakage loss of electrolyte and MDA accumulation during drying. Jamun seed of low vigor or quality can be recognized as increased leakage loss and MDA content. Thus the result suggests that Jamun seeds deterioration during natural ageing is closely related to lipid peroxidation which results in the accumulation of MDA.

KEY WORDS: Recalcitrant seeds, *Syzygium cuminii*, Membrane perturbation, Lipid peroxidation.

ABBREVIATIONS: Moisture content (MC), Malondialdehyde (MDA), CWC (Critical Water Content), dh (days after harvesting)

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1. INTRODUCTION

Jamun (*Syzygium cuminii*) seeds are categorized as tropical recalcitrant as they are shed with high water content (0.93 g H_2O g^{-1} DM), have a short viability (27 days), high CWC(0.86 g H_2O g^{-1} DM), and desiccation sensitive. In most if not all, membrane are the primary target of desiccation injury and play a key role in maintaining seed viability and vigor[1,2,3,4,5]. Seed imbibition invariably accompanies solute leakage during the process of membrane reorganization following rehydration in the dry seeds. The rate of leakage is directly correlated with the cell damage and repair in response to ageing[6, 7]. Enhanced electrolyte leakage loss estimated in the damaged seeds of *Lotus corniculatus*[8], *Artocarpus heterophyllus*[9], *Euterpe edulis*[10], *Fagus sylvatica*[11] and *Hopea ponga*[12] was considered an indicator of loss in membrane integrity as a result of dehydration and is accountable for reduced germination and vigor of seeds. Initial viability loss has been associated with loss of cellular constituents mainly due to oxidation of protein and lipid; major components of membrane[13, 14, 15]. The lipid peroxidation plays an important role in initiating and mediating ageing process as the lipids are major component of semi permeable membrane and probably the first easy target of free radicals. During seed imbibition, leakage loss of solutes as a result of altered membrane integrity is closely associated with the accumulation of malondialdehyde (MDA), a final product of lipid peroxidation [16, 17, 18].

The objective of this research was to follow the changes in membrane integrity in desiccating recalcitrant Jamun seeds. The relative significance and contribution of axis and cotyledon was also determined during loss of viability by monitoring the amount of MDA formation in these tissues.

2. MATERIALS AND METHODS

2.1 *Site of Fruit Collection*

Jamun (*Syzygium cuminii* L.) fruits were collected from avenue trees nearby Bhilai (Chhattisgarh), India. Nearly 80 plus trees were marked for collection of fruits.

2.2 *Fruit Collection*

Freshly mature fruits of Jamun were plucked manually during June-July. The collected fruits were transported to the laboratory within one hour of collection. The healthy fruits were sorted out. The fruit is a drupe, variable in size, oblong or sub-globose, crowned with a persistent truncated first pink, then black with pink juicy mesocarp.

2.3 *Seed Extraction*

Seeds were extracted by rubbing the fruit with sand to remove pulp. Three replicates of 50 seeds each were used for determination of moisture content. Remaining pulp free seeds were then washed thoroughly with tap water to remove traces of pulp and allowed to air-dry to initial moisture contents. Each fruit contains one seed which is 1-2 cm long, oblong in shape green or brown in colour.

2.4 *Seed Drying and Storage*

The mature Jamun seeds collected were for conducting experiments related to slow (natural drying at laboratory conditions RH 30% and Temperature (27-30^{o}C). The seed was subjected to slow drying by spreading them in one layer in a perforated basket at ambient conditions (27-30^{o}C at RH 30%).

2.5 *Moisture Content and Estimation of Water Content*

Moisture contents of slow dried seeds were determined by oven drying the seeds for 72 hours at 103°C [19]. Five replicates with 10 seeds each were used to determine the moisture content, on a fresh weight basis. The seed moisture content was determined by the following formula and was expressed in percentage:

$$\% \text{ Moisture Content} = \frac{\text{Seed Fresh Weight - Seed Dry Weight}}{\text{Seed Fresh Weight}} \times 100$$

Water content of slow dried seeds was determined by the method given by [20].

$$\text{Water Content} = \frac{\text{Seed Fresh Weight - Seed Dry Weight}}{\text{Seed Dry Weight}} \times \text{g } H_2O \text{ g}^{-1}\text{DM}$$

2.6 *Leachate Conductivity*

Solute leakage loss was measured in terms of electrolytic conductivity by [21]. The leachates were collected after 24 hours imbibition of seed in distilled water and the specific conductivity was estimated using a conductivity meter (Elico). Results were expressed as mS seed^{-1}.

2.7 *Lipid Peroxidation*

Lipid peroxidation was measured as the concentration of thiobarbituric acid (TBA) reactive products equated with malondialdehyde (MDA), the end product of lipid peroxidation. The method given by[22] was followed to determine lipid peroxidation. To 0.05 g of axis and 0.1 g of cotyledons add 3 ml of 0.5% TBA prepared in 20% TCA and 1 ml of BHT. The reaction mixture was incubated in boiling water bath (97^{o}C) for 30 minutes to permit formation of pink colour of the product when the lipid peroxidation products (i.e. MDA) react with TBA. The reaction tubes were plunged into the pail of ice to stop the reaction. The reaction mixture was than centrifuged at 10,000 rpm for 30 minutes, to precipitate all the suspending particles. The clear supernatant was collected and the absorbance was recorded at 440, 532, and 660 nm using spectrophotometer. The following formula given by [23] was used to calculate Lipid peroxidation and expressed as Δ A_{540}/gm FW of axis or cotyledon:

$$\text{Lipid peroxidation} = \frac{[A_{532} - A_{660}] - [(A_{440} - A_{660}) \times 0.0571] \times 10}{157000}$$

3. RESULTS

3.1 *Desiccation and Loss of Viability*

Absolute germination (100%) was recorded in Jamun seeds during slow drying of seed water content from 0.93 to 0.86 g H_2O g^{-1} DM. Thereafter, with quick decline in water content, a corresponding decline in %germination was also registered, i.e. 95% germination was recorded at water content 0.81 g H_2O g^{-1} DM and 80% at water content 0.42 g H_2O g^{-1} DM. Complete loss of germination was noticed at the time when seed was desiccated to 0.23 g H_2O g^{-1} DM. Along with the per cent germination, the germination index of the seed also decreased considerably with loss of water content during seed storage.

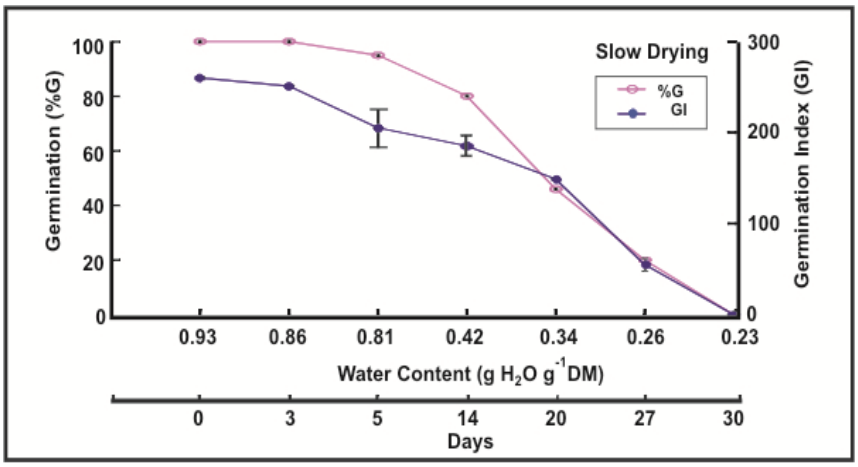

Figure 1. The Decline in Percentage of Germination and Germination Index of *Syzygium cuminii* Seeds During Storage (Slow Drying) Under Ambient Conditions.

3.2 *Electrolyte Leakage*

The specific conductivity of the seed leachates estimated in the imbibing seeds showed gradual promotion with the loss of seed water content during slow drying. Drying of seeds initially from 0.93 to 0.26 g H_2O g^{-1} DM, resulted in rapid increase in electrolyte leakage from 0.12 to 0.44 mS $seed^{-1}$ but further loss of seed water content to 0.23 g H_2O g^{-1} DM did not much alter the specific conductivity

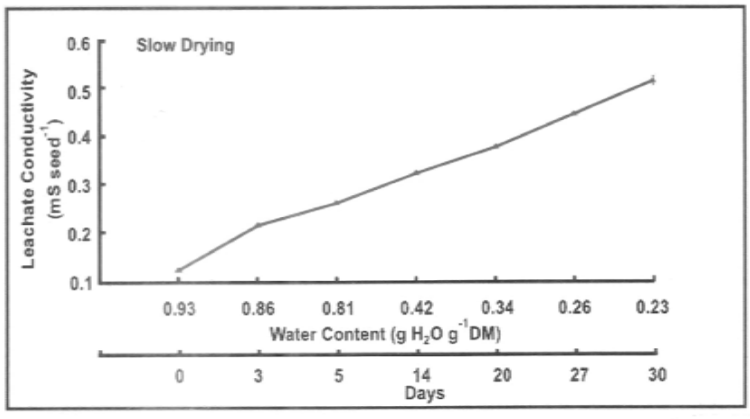

Figure 2. Loss of Electrolytes with Decline in Water Content of *Syzygium cuminii* Seeds During Storage Under Ambient Conditions (Expressed as Specific Conductivity of Leachates).

3.3 *Lipid Peroxidation*

The lipid peroxidation product MDA (also called as Thiobarbituric Acid Reactive Substances-TBARS) increased in the embryonic axis and cotyledon of Jamun seeds with the decrease in water content during slow drying at ambient storage condition. The TBARS levels remained significantly low when the water content of the seed declined from 0.93 to 0.86 g H_2O g^{-1} DM during slow drying both in axis and cotyledon. Slight promotion in MDA content from 2.22 to 2.62 A_{540} g^{-1} FW was observed in the axis, whereas it increased sharply in the cotyledon from 2.53 to 5.73 A_{540} g^{-1} FW (more than 2-fold) in response to loss of seed water content from 0.86 to 0.81 g H_2O g^{-1} DM. Further, slow drying of seeds from 0.81 to 0.23 g H_2O g^{-1} DM resulted in sharp increase in MDA levels both in axis (2.62 to 4.20 A_{540} g^{-1} FW) and cotyledon (5.73 to 6.06 A_{540} g^{-1} FW). In general, the levels of MDA were comparatively higher in the cotyledons than the axis throughout the period of analysis.

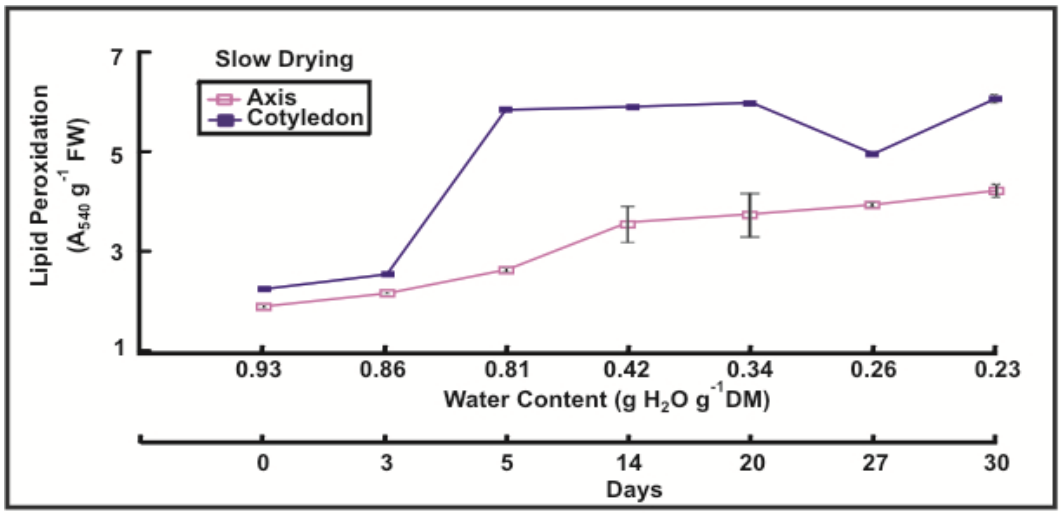

Figure 3. Accumulation of TBA Reactive Substances in the Embryonic Axis and Cotyledon of *Syzygium cuminii* Seeds with Decline in Water Content during Slow Drying.

4. DISCUSSION

The present study has confirmed that drying of seeds during maturation phase of seed development and later during storage generally leads to structural and functional alteration in membrane and its components. The integrity of membranes must be repaired during early phase of imbibition[24, 25]. The reorganization and permeability of the membrane is monitored by measuring the specific conductivity of solutes leaked from a dry seed upon rehydration or imbibition. Dehydration, induced loss of percent germination in recalcitrant Jamun seeds was accompanied by membrane deterioration as indicated by

increased rates of, **1**-electrolyte leakage from imbibing seeds and, **2**-MDA accumulation. Membrane damage as a result of increased peroxidation of membrane phospholipids caused loss of selective permeability [26] that, in turn, is responsible for increased leakage loss. Increased leakage that is invariably associated with ageing is due to enhanced membrane permeability[27, 28]. In the present study, loss of percent germination in dehydrating Jamun seeds was strongly and negatively correlated with increased leakage loss of electrolyte and MDA accumulation during slow drying.

Loss of germinability, viability and vigor during storage of orthodox and recalcitrant seeds is frequently related to potential damage of membranes due to uncontrolled peroxidation of membrane lipid[29, 30, 31, 32]. Lipid peroxidation estimated by recording MDA content, an end and volatile product of lipid peroxidation, in the cotyledon and axis indicated promotion in MDA accumulation in response to desiccation of Jamun seeds. Slow drying of fresh Jamun seeds from 0.93 to 0.23 g H_2O g^{-1} DM resulted in net increase in the estimated MDA by 2.6-fold in cotyledon and 2.2-fold in axis. Differential response of cotyledon and axis in terms of lipid peroxidation to drying perhaps implies the differential sensitivity of these tissues.

Taking together the results of leakage loss and MDA in dehydrating Jamun seeds, it is concluded that constant drying of recalcitrant Jamun seeds activated the autocatalytic reaction of lipid peroxidation resulting in the accumulation of MDA. MDA accumulation, an indicator of membrane damage, is consequently responsible for membrane perturbations in the dehydrating Jamun seeds that exhibited severe leakage loss. Leaky membranes due to extensive lipid peroxidation in desiccating Jamun seeds may be a prime cause of reduced germination and vigor (GI). Enhanced MDA contents mediated by organic radicals and peroxides that are produced during lipid peroxidation of polyunsaturated fatty acids (PUFA) are considered to be one of the likely explanations for lipid peroxidation [33,34,35,36] in desiccating seeds during germination.

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