Vascular plants are frequently exposed to drying period. In the life cycle of the plants, seeds are bestowed with all the inherent attributes that are essentially required for tolerating extreme dehydration. The desiccation tolerant seeds can survive prolonged dry storage conditions for extremely long period that ranges from decades to centuries and even millennia [1] by shifting to metabolic state of suspended animation [2]. Desiccation tolerance an inherent and exclusive property of the seed permits their long-term ex-situ storage [3]. Until 1970 seeds of all plant species were categorized as orthodox. The orthodox seeds are tolerant to extreme desiccation and their tolerance is increased in the specific and quantifiable manner with the decrease in the storage temperature and moisture content. The property of desiccation tolerance in the orthodox seeds offer them longer longevity and storability during dry storage (from years to centuries). Generally, under cool and dry storage conditions all orthodox seeds have an ability to survive drying up to 5% moisture content or range of water content between 0.03 to 0.07 H$_2$O g$^{-1}$ dry mass basis [2, 4]. Roberts for the first in the year 1973 reported dichotomy in the seed storage physiology are unlike orthodox seeds are killed when they are dehydrated below relatively high water content [5] i.e. close to 30 to 80% moisture content or water content 0.43 to 4.0 g H$_2$O g$^{-1}$ FM [6, 7, 8, 9] very much close to wilting point of plant parts such as root, shoot, leaves, flowers etc. The non-orthodox seeds have been further categorised as recalcitrant [5] or intermediate [10, 11, 12] based up on the degree of tolerance to dehydration. The intermediate seed storage category is applicable to all those seeds which can be desiccated safely (with no loss of germinability), i.e. limited desiccation tolerance, to low water content than the recalcitrant seeds but sufficiently higher than the orthodox seeds [13, 14, 15]. Additionally, most of the recalcitrant seeds are also sensitive to low temperature whereas the intermediate seeds are tolerant to specific range of low temperature thus offer a provision of ex-situ storage (Royal Botanic Gardens Kew 2015). In contrast to recalcitrant seeds which are desiccation and temperature sensitive hence poses problems in their short and long-term storage [16] the limited desiccation and temperature tolerance of intermediate seeds offers scope for dry ex-situ storage. Therefore, it is imperative to assume that detailed study on the intermediate storage behaviour of seeds will reveal the possible mechanism (s) for limited desiccation tolerance and short longevity.

The desiccation tolerance referred to the seeds capability to tolerate the withdrawal of free water during dry storage without any sign of cellular damage and resuming all the metabolic activities up on rehydration. In desiccation tolerant orthodox seeds the removal of cellular water mediated cellular modification is completely reversible up on rehydration. The seed longevity and storability during dry storage is mostly depends up on the degree of desiccation tolerance of seeds. The physiological and
molecular basis of variation in the seed longevity ranging from days in *Shorea robusta* [17, 18, 19], *Madhuca indica* [20] and *Avicenna marina* [8] to months in *Azadirachta indica* [21, 22], *Buchanania lanzan* [23], *Gmelina arborea* [23], *Diospyros melanoxylon* [23], citrus [13], coffee [24, 14], *Pritchardia remota* [15], papaya [11] and *Elaeis guineensis* [11] to decades (Crop seeds) is a subject of extensive investigation [25, 26, 27, 28, 29].

In seeds, the acquisition of desiccation tolerance is accomplished during maturation phase in the seed development. The seed dehydration occurs during maturation phase works as a double way switch in critically regulating the transition of seed development mode to germination mode (quiescent state) [30]. In developing seeds the drying mediates distinct and specific changes in the transcriptional, translational and metabolome patterns during maturation phase that offers adequate protection to survive under range of environmental stress [30]. Extensive changes, nearly 30% of the genome (6963 genes), during drying of seed development of *Arabidopsis thaliana* have been observed [31]. Surprisingly, the transcriptome analysis conducted in the dehydrating *Arabidopsis thaliana* seeds during maturation revealed up regulation of several genes that are associated with many cellular activities not directly related to desiccation [32, 33]. The pivotal role of dehydration in punctuating the mode of seed developmental and commencing the mode of germination has been revealed by detecting accumulation of abundant seed germination related mRNA in the developing seeds [34, 35, 26, 36]. One of the most outstanding biological importance of seed desiccation is to ensure the wide dispersal and species survival by providing longevity and storability. Therefore, the role of dehydration and period of maturation has been critically evaluated in explaining the variation in the seed storage physiology. The period of maturation is highest in the orthodox seeds and involves essentially completion of dehydration mediate cellular and metabolic changes that offer sensitivity of cell membrane to hormones, desiccation tolerance for longer storability, dormancy, germination potential and adequate protection to the seed for successful survival under harsh environmental conditions. In contrast, the non-orthodox seeds are desiccation sensitive and characterized by short longevity and poor storability perhaps due to abbreviated maturation phase that permits incomplete drying. The incomplete drying (basis for higher water content at the time of shedding) signal during maturation in the non-orthodox seeds is not enough to initiate dormancy in these seeds but certainly adequate to initiate the germination related events. The fresh and undried non-orthodox seeds showed quick germination initially but the germinability is sacrificed severely especially when dehydrated below relatively high water content that is referred as critical water content [37, 38] or lowest safe moisture content [39]. The list of non-orthodox seeds is expanding since the first work reported by Roberts [5] and according to the study of Tweddle et al. [40] nearly 48% of the plant species in the tropical region produces non-orthodox seeds.
The fresh and mature non-orthodox seeds with high water content and incomplete drying during maturation make them desiccation sensitive thus unsuitable for short or long term storage. Amongst several techniques employed for improving the storability of non-orthodox seeds the rate of drying; slow and rapid are widely used. Slow drying is mostly favourable for the safe removal of water in the immature orthodox seeds or somatic embryos [41, 42, 43] or inducing tolerance in the seeds exposed to high drying temperature [44]. Unlike orthodox seeds the rate of drying exhibits remarkable effect on the survival of non-orthodox seeds [18]. The survival of non-orthodox seeds varies remarkably on the rate of drying during storage [45, 46, 47] as most of these seeds during development experience abbreviated maturation. Therefore, in plant producing non-orthodox seeds, inhabiting tropics or sub-tropics environments, the rapid removal of water which allow retention of higher viability even at sufficiently low water content is effective rather than the slow drying where the removal of water in these seeds mediates deleterious effects. The critical water content especially in the desiccation sensitive or non-orthodox may vary because of the seed size, period of maturity, the deposition of various protectants and the drying rate [48, 46, 49, 50, 51, 18]. Further, the limit of desiccation tolerance especially in the non-orthodox seeds may vary within the species depending upon the time of post-harvest (different maturation period) and post-harvest handling (storage temperature and RH) [46, 52]. In non-orthodox seeds, rapid and earlier desiccation following maturation is the most suitable and successful technique for drying the seeds or axes to lower water content without losing vigour and viability [50, 53]. The fast drying alleviated desiccation tolerance in the non-orthodox seeds perhaps by permitting less time at intermediate water contents that may cause hydration specific cellular damages [54]. The slow drying of intermediate seeds favours adequate time for seeds to remain at intermediate water contents that allows aqueous-based deleterious reactions promoting seed deterioration in the absence of protective system [55, 56, 57, 18, 58]. The rapid drying has been reported to improve desiccation tolerance and thus survival in several non-orthodox seeds Landolphia kirkii Dyer [59], Araucaria hunsteinii [60], Hevea brasiliensis [61], Castanospermum australe [62], Scadoxus membranaceus [63], Avicennia marina [64] Camellia sinensis [47], Trichilia dregena, Castanospermum australe [65], Chinese wampee [66], and Dipcadi saxorum [67]. Although the mechanism of rapid drying induced alleviation of DT is unclear several assumptions has been put forward. Broadly, the drying rate influences the DT by regulating the physical process of desiccation that affects the metabolic pathways associated with the seed longevity. During slow drying, the intermediate seeds may undergo mechanical stress due to uneven withdrawal of water that leads desiccation stress mediated volumetric changes in the well-organized seeds tissues or the cells of the axes and cotyledons [68]. On the contrary, the higher drying rate that promotes dehydration time exponentially reduces the mechanical forces by decreasing the dehydration stress and its related cellular damage [69,
Despite of rapid desiccation mediated lowering of water limit irreversible cellular damages because of the prolonged dehydration of non-orthodox seeds below critical water content in the seeds is inevitable and attributed to withdrawal of bound water from membranes or biomolecules like proteins [53]. Thus, determining critical water content at which seed viability is reduced in reference to non-orthodox seeds may be variable at least when the seeds are exposed to different drying rates [54, 70]. The aforementioned literature on differential rate of drying clearly indicates the beneficial effects of rapid drying although its underlying mechanism is far from clear hence needs urgent attention as its understanding will have way to develop promising ex-situ storage protocols for seeds exhibiting intermediate storage behaviour.

In non-orthodox seeds the dehydration induced pathway that triggers the loss of germinability viability and vigour is still far from clear. The most generalized understanding proposed so far indicates that the removal of water below a critical threshold which can’t be tolerated by the seeds thus initiating deteriorating biochemical reaction because of uncontrolled cellular metabolism [62, 54, 48, 71, 53, 70, 68]. Plethora of literature on uncontrolled metabolism advocates that “free radical theory” is one of the central features in mediating cellular and biomolecules damage that ultimately is responsible for the loss of seed viability. Collectively, all active oxygen species mainly singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radical [72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82] are involved in the oxidative stress mediated cell injury [83, 84, 85, 86, 76, 77, 78, 87, 79, 80, 81, 82]. Oxidative injury can be defined as an over accumulation of free radicals resulting imbalance between generation and removal of AOS [88, 89, 90]. Free radical mediated chain of reaction can be divided into three separate processes namely; initiation, extension and termination. The reactive species are produced from normal metabolic processes or generated after stimulation from exogenous cues like ultraviolet light, ionizing radiations and chemical reactions, biotic and abiotic stresses, etc. [91, 74, 92, 93, 94, 76, 77, 78 79, 80, 81, 95, 82]. Actually, it is the concentration and kind of AOS that determine [96, 97, 94, 98, 82] the type of cellular responses [99, 100]; signalling or damaging. The low or sub-lethal concentrations of AOS is known to regulate signalling network related to induce defense genes, adaptive responses and PCD [94,101, 96, 97, 98, 82] whereas, excess or relatively higher doses are associated with oxidative stress induced cell damage.

The \( \text{O}_2^- \) produced intracellularly or extracellularly is short lived (1\( \mu \)s), impermeable membranes and rapidly dismutated to \( \text{H}_2\text{O}_2 \) either spontaneously at low pH or enzymatically [102]. Superoxide being highly reactive free radical can react with very well-known signaling and free radical Nitric oxide (NO*).
to produce peroxy nitrite (OOONO⁻). In aqueous solutions protonation of superoxide that give rise to \( \text{HO}_2^* \) is permeable to membrane thus able to oxidize polyunsaturated fatty acids (PUFAs) by removing H-atoms and lipid hydroperoxides finally triggering the autocatalytic peroxidation of lipids [103]. Unlike superoxide, the \( \text{H}_2\text{O}_2 \) is moderately reactive and has a relatively long life (half-life 1ms) thus can diffuse across the membrane readily from the production site [102]. The enzymes containing thiol groups are sensitive to attack by \( \text{H}_2\text{O}_2 \). The relatively long shelf-life and ability of \( \text{H}_2\text{O}_2 \) to diffuse longer distance especially across the biomembranes makes it highly suitable and potential molecule as a messenger in signal transduction [104, 105, 94, 106, 107, 108, 109]. \( \text{H}_2\text{O}_2 \) has been shown to work as a sensor in several adaptive responses of the plant and is potential in the regulation of genes associated with the heat shock (proteins and transcriptional factors), calcium signaling (calmodulin), redox reactions (blue-copper binding protein), uncoupling proteins of mitochondria, \text{myb}-related transcriptional factors, cross talk with hormonal signaling etc. The last species which can be produced is \( \text{OH}^- \), the extremely reactive with a half-life of <1\( \mu \text{s} \) [110]. The \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) are precursors for the production of \( \text{OH}^- \) catalyzed by Iron (Fenton reaction) or Copper ions reaction [111]. This reaction can be catalyzed enzymatically by peroxidases [112]. The \( \text{OH}^- \) radical is extremely aggressive, short-lived oxidant and not scavenged enzymatically thus potential radical in disintegrating all kinds of organic molecules such as pigments, lipids, proteins and DNA [103, 113]. Hydroxyl radical has not so far been reported as signaling molecule [107, 108] although its derived products are considered important in eliciting the signaling cascade such as PCD [102, 114, 80]. Literature is accumulating [115, 116, 117] on the antioxidant capacity of several soluble sugars such as raffinose, stachyose and verbascose collectively named as “Raffinose Family Oligosaccahrides (RFOs) in efficiently removing the hydroxyl radical. Remarkably, the detoxifying ability of the RFOs depends up on the total number of OH-groups [115].

AOS plays a significant role in loss of cellular; structural and biomolecular integrity following loss in seed viability as suggested by an extensive literature in seeds [17, 118, 119, 120, 121, 22, 122, 123, 124]. Free radical of oxygen [17, 121] and its derivatives (\( \text{H}_2\text{O}_2 \)) have been implicated in oxidative damage and accumulation of these oxidative products has been shown to be positively correlated with the loss of seed viability [123]. Desiccation induced rapid accumulation of extracellular AOS in the cotyledon and axis of \textit{Castanea sativa}, a recalcitrant seeds, has been correlated with the sharp loss of viability and vigour by changing the AOS metabolism [123]. Several lines of evidence [125, 17, 118, 119, 121, 22] suggest that AOS mediated chages during ageing is associated with the degradation of membrane lipids that is the prime cause of severe membrane perturbations. Free radicals and lipid-peroxidized products are widely considered to be major contributors to seed deterioration [126, 17, 118, 119, 121, 22]. AOS mediated damage to transport proteins, receptors and ion channels and that leads to extensive cellular dysfunction.
is considered one of the major pathways for loss of seed viability and vigour. Literature is scant on the role of AOS in the oxidation of protein, lipid and nucleic acid in seeds during longevity and storability.

The AOS mediated oxidative attack on lipids is the most widely discussed and acclaimed pathway for explaining the deteriorative changes in seed ageing. The oil rich seeds are usually reputed for short longevity. Prolonged storage conditions promote the deterioration of seed quality by degrading the oil and protein content [127]. Adverse storage conditions of lipid rich seeds is frequently associated with the loss of total oil content and increased free fatty acids, key determinant of oil sensitivity to oxidation [128] that together leads to loss of germinability. In seeds, storage lipids are mainly consists of triacylglycerols and that are accumulated during maturation phase of seed development. Storage lipids represent up to 80% of the dry mass of storage tissues. A marked decrease in lipid concentration was detected during ageing/desiccation, indicating the importance of adequate lipid reserves during developmental stage [129]. Oily seeds are always exposed to risk of auto-oxidation encompass oleic (18:1), linoleic (18:2) and linolenic (18:3) fatty acid chains [130, 131]. During desiccation, decline in stored lipids have been correlated with reduced viability of somatic embryos [132]. Losses in lipid fraction was also observed during accelerated ageing in *Vicia faba*, *Oenothera caespitosa* and *Daucus carota* seeds and are correlated well with poor seed vigor [133]. In the ageing seeds, lipase plays a vital role in degrading the lipids and releasing substantial amounts of free fatty acids (FFA) [134] that accelerates further the seed deterioration by undergoing peroxidation [135, 136]. The FFA, well established membrane destabilizing agents [137, 138], are used as biochemical markers for assessing the seed quality and ageing [139, 140]. FFA also acts as a detergent and can damage lipid bilayers, especially of mitochondria, leading to reduced energy production [141]. The degradation of lipids in senescing membranes and the release of FFAs initiate oxidative deterioration by providing substrate for lipoxygenase (LOX) [142]. In ageing seeds, the lipoxygenase (LOX) are activated in the degradation of lipid and generating intermediates such as lipid hydroperoxides (LOOH), conjugated dienes (CD) and volatile aldehydes [143, 144, 145]. Activity of LOX rises considerably during storage or desiccation of seeds [146,147, 148, 149]. Lipid peroxidation is one of the foremost and widely accepted molecular mechanisms that is advanced for explaining the oxidative cellular damage due to accumulation of variety of toxicity products both in plants and animals. The lipid peroxidation occurs essentially in the presence of oxygen and involves generation and propagation of lipid radicals that initiates the degradation of PUFA of membrane and source of several lipid peroxidized products like ketones, alcohols, alkanes, aldehydes, ethers etc. [150]. Hydroxyl radical (OH) is one the highly reactive species in promoting peroxidation PUFA (Poly Unsaturated Fatty Acids). First-chain initiation involves the removal of H-atom by the attack of OH on the methylene (-CH2-)
group of PUFA finally leaving the carbon (-CH-) with an unpaired electron. Several molecular species such as hydroxyl (•OH), alkoxyl (RO•), peroxyl (ROO•) and HO2• [151] are potentially active in the abstraction of first hydrogen atom in the initiation phase of lipid peroxidation. This unpaired electron also referred as carbon free radical initiates series of H-atom abstraction especially from the double bonds in the free fatty acid chain ultimately resulting in the formation of conjugated dienes, one of the important intermediates of lipid peroxidation. The conjugated dienes can be a good source of peroxyl radical generation in the presence of O2. As lipid peroxidation is an autocatalytic reaction which continues till the last molecule of PUFA exists it is the peroxyl radical which propagate the process of oxidation by oxidizing adjacent PUFA. The peroxyl radical has an ability to produce another equally important intermediate is hydroperoxide by reacting readily with the hydrogen. These hydroperoxides are often reduced to their corresponding alcohol enzymatically by glutathione peroxidase or degraded non-enzymatically in the presence of Fe2+ [152] to yield carbonyl compounds, mostly aldehydes and hydroxy-alkenals [153, 154]. The commonly occurring cytotoxic alkenals produced as a result of lipid peroxidations are 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexanal (HHE) [155, 108]. HNE formed by oxidation of N-6 unsaturated fatty acid is considered a secondary toxic messenger [156, 157]. The SH-group of cysteine, the e-amino group of lysine and the imidazole group of histidine forms adduct with HNE [158, 159, 160, 161]. Some of the other biological events associated with HNE are inhibition of DNA and protein synthesis, inactivation of enzymes, modification of low density lipoprotein and regulation of gene expression [158]. Mitochondrial enzymes like 2-oxoglutarate dehydrogenase, pyruvate dehydrogenase, cytochrome c oxidase are primary targets of HNE to form adducts [162]. The accumulation of peroxidative products of lipid [17, 163, 8, 124, 149] and rate of seed deterioration is exhaustively used as a molecular marker for monitoring ageing and predicting survival of seeds [22, 8, 124]. Enhanced rates of lipid peroxidation has been proposed as a prominent cause for viability loss in ageing seeds of Shorea robusta [17], Coffea Arabica [164], Azadirachta indica [21, 22], Trichilia dregeana [58] during storage. Higher rates of lipid peroxidation in deteriorating seeds consequently resulted in massive loss of membrane phospholipids [165]. The amounts of MDA (malonaldehyde), a final product of lipid peroxidation, is closely linked with the seed ageing in Shorea robusta [17, 118, 149], Prunus dulcis [147], Azadirachta indica [121, 22], Theobroma cacao [164], Ammopiptanthus [124] and Trichilia dregeana [58] and Telfaria occidentalis [166] during storage. Detailed study conducted by [149] detected increased amounts of most of the intermediates of lipid peroxidation such as conjugated diene, lipid hydroperoxide, malondialdehyde and 4-hydroxy-2-nonenal during loss of viability in the dehydrating seeds of Shorea robusta. Several other intermediates like lipid hydroperoxide [LOOH] and conjugated dienes [CD] are reputed as early indicators of lipid peroxidation [167, 166, 168, 169, 170]. The cell membranes are primary targets of AOS attack by easily available free radicals in the cell. Due to
lipid peroxide there is impairment of membrane fluidity and/or plasticity that leads to irreversible damage of the cell’s integrity [171]. Among different lipid components of the cell, PUFAs exhibit the highest sensitivity to AOS attack during seed deterioration [172, 173, 126, 174]. A reduced ability of reorganization of membrane is accompanied with higher losses of solutes in the incubation medium and is an indicator of poor seed germination and vigour [175, 121 22, 176]. Increasing leachate conductivity was well correlated with declining seed germination and vigor [17, 177, 178, 179]. In several seed species like Zygophyllum xanthoxylon [180], Garcinia kola [181], Oryza sativa [182], Gossypium hirsutum seeds [183], a direct correlation between electrical conductivity and seed viability has been reported. Seeds with low germination suffer with higher rates of electrolyte leakage [184, 185, 186, 118]. Decline in viability [18, 8, 22] and vigour [187, 188] of seeds of several plant species is positively correlated with increased leakage loss during imbibition, principally because of membrane perturbation [121, 22, 18].

Detailed proteomic study conducted on natural and accelerated aged seeds of Arabidopsis thaliana revealed detection of several proteins that are associated with high vigour and viable seeds and are lost with the seed deterioration during ageing [189, 26, 190]. Loss of protein content may occur due to reduced synthesis or enhanced proteolytic activity [191] or protein oxidation or combination of all in ageing seeds. Rapid loss of soluble and total protein with loss of viability was reported in Azadirachta indica [192] and Shorea robusta [119] seeds. Marked qualitative change in the stage specific proteins was suggested as a consequence of suppression of the synthesis (more or less permanently) in seeds of Phaseolus vulgaris and Ricinus communis seeds [193, 194]. Net loss of protein during ageing is a key feature in several crop and tree seeds [195, 196, 197, 119]. An increased activity of protease in response to desiccation and ageing leads to loss of viability was endorsed for decline in protein content [119]. Hundreds of proteases are coded in plant genome, but till now the roles are still unresolved in plants. In general, most of the deteriorative processes in plants as well as in seeds involve active protease activity [198, 199]. The roles of serine proteases, cysteine proteases, aspartic proteases and caspase-like proteases are associated with programmed cell death (PCD) in plants [198, 200]. In seeds, protease plays important part during seed germination, especially in mobilization of stored reserve which is required for growing embryo. Decline in the protein content in desiccation mediated loss of viability may be a cumulative effect of high protease activity and biosynthesis of protein [119]. The enhanced protease activity may result in the concomitant decline in both storage and enzymatic protein [201, 202]. Severe loss in cellular protein was also observed in desiccating Shorea robusta [119], Gossypium hirsutum [203], Daucus carota [204] and Glycine max [205] seeds. The AOS are also involved in the protein oxidation [206, 207, 108], which may occur through different mechanisms, viz., carbonylation of selected amino acids, conversion of sulphydryl groups [208, 220] to form cross links of disulphide [209, 210] and glycoxidation adduct
[211] of nitration of tyrosine residues [212]. Moreover, selection of targets amino acid residue is very site specific for AOS [83, 212]. Amino acid residues of several proteins and free amino acids are preferred targets of AOS attack and such oxidative modifications of these molecules has been suggested as one of the pathways for seed ageing [213, 214, 215]. Aromatic amino acid residues of protein such as histidine, cysteine and methionine are the leading sites of AOS attack [216, 217, 218]. The AOS induced oxidation occurs mostly by the hydroxyl radical catalyzes cleavage of peptide bond [219]. Protein oxidation is very often used as a diagnostic marker for evaluating oxidative stress in plant and animal tissues [220, 122, 221, 222]. The accumulation of carbonylated protein enhanced in the axes and cotyledons of desiccating seeds of *Glycine max* and *Arabidopsis* seeds [223, 215, 26]. Carbonylation is a widely used marker of protein oxidation which results in loss of properties of protein [224, 225, 226, 227]. AOS mediated oxidation of protein leads to modification of enzymatic and binding protein properties, increased susceptibility to proteolytic degradation, unfolding and protein aggregation and also lead to diverse functional changes and selective degradation [226, 228, 212, 229, 230, 231]. The oxidized proteins formed during protein oxidation are highly hydrophobic in nature thus considered better substrates for proteolytic degradation [232, 233, 234, 235, 236, 237]. Any modification in enzymatic and binding properties or decline in amounts of protein severely affects diverse cellular metabolism [238] as it has several biological functions. About 10-50% of antioxidant potential of seed is due to scavenging enzyme proteins [239].

The degraded or oxidized protein can readily converted into protein hydroperoxide (PrOOH) which tends to play an important role in ageing due to their ability to react with critical biological components [240, 241]. The PrOOH have ability to react with the biomolecules such as; metals, antioxidants and DNA [242, 243, 240, 241] and act as secondary free radicals [244, 245, 246]. The hydroxylated derivatives of some amino acids are known to be highly susceptible to peroxidation [247].

The end products of lipid peroxidation, both MDA and HNE [159, 248, 249] have an ability to react with Lysine, Cysteine and/or Histidine molecules, and form stable adducts. Both have various cytopathological effects such as; inhibition of enzymes and protein, disruption of DNA and RNA synthesis, and cell cycle arrest [250]. The intermediates like MDA and HNE of peroxidized lipids reacts spontaneously with protein and generates cytotoxic and genotoxic adducts i.e. MDA-protein and HNE-protein. These adduct of modified proteins act as potential inhibitors of proteasome and are resistant to proteolytic degradation [251].
The amino group of carbonylated proteins can be further modified into glycated proteins by actively reacting non-enzymatically with the reducing sugars accumulated during seed ageing [252]. These modifications are detected in the form of advanced glycation end products (AGEs) [223, 252]. Glycation of proteins can occur by two distinct routes namely; Amadori and Maillard reactions [223, 253]. The non-enzymatic modifications of protein through both Amadori and Maillard reactions play an important role in the loss of seed viability during natural ageing [172, 254, 223, 253]. The Maillard reactions along with free radical oxidation are known to be major factors in seed ageing [255, 252, 19]. Conflicting evidences have been reported on the significance of the Maillard reaction in the loss of seed viability. Quantitative estimation of Maillard products in the axes of *Glycine max* promoted with the reduced ability of germination [254] whereas, no consistent correlation between seed viability and the Maillard reaction products was discernible in either naturally or artificially aged seeds of *Daucus carota, Allium cepa, Lycopersicon esculentum* and *Brassica oleracea* [256].

The degraded proteins can be efficiently removed by the proteasomes. Proteasomes are large complexes and regulate many metabolic pathways by degrading proteins in the cytosol and nucleus of eukaryotic cells [257]. Lysosomal and ubiquitin-proteasome system are two major proteolytic systems responsible for most of the intracellular protein turnover [258, 259]. Before discovery of the ubiquitin-proteasome pathway in the early 1980’s degradation of protein was originally thought as an un-regulated event but now it was described as one of the highly regulated mechanisms [257, 260]. The proteasomes acts selectively upon degraded or oxidized proteins that are tagged with ubiquitin. The attachment of ubiquitin to degraded protein is ATP-dependent/or independent [261] and involves three specific groups of enzymes namely E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme) and E3 (ubiquitin ligases) [262]. Degradation of oxidatively damaged protein is of utmost importance for the regulation of the steady-state level of oxidized protein in the cell [263]. The proteasome, which is the main non-lysosomal system for intracellular degradation of protein has also been implicated in the degradation of altered protein particularly oxidized ones, that are generally more sensitive to proteolytic activity [264]. Moreover, it has been recently confirmed that the proteasome is the main proteolytic system involved in the removal of oxidatively damaged protein after oxidative injury [265, 266]. Thus, investigations have centered on a possible decline in the proteasome activity with age that would explain the age-related accumulation of oxidized protein.

Maintenance of genetic integrity is of utmost requisite for the survival of seed during seed desiccation and ageing [267]. The genome integrity that results into DNA damage is threatened due to several environmental (ionizing radiation, heavy metals, pollutants, UV-light, heat and drought) and oxidative
stresses (AOS) [268, 91, 269, 270, 271]. Loss of DNA levels with reduced transcriptional activity has been reported in ageing seeds [272]. The extensive DNA oxidation leads to DNA damage [273]. During oxidative stress, DNA lesions may occur due to lipid peroxidation generated electrophiles that are involved in the production of DNA adducts [274, 275, 276]. The oxidized products of both lipid and protein formed intracellularly during ageing readily react with DNA and form adducts and is important biomarkers [277, 278, 279, 280]. AOS induced oxidative damage to nuclear and mitochondrial DNA is considered one of the major pathways causing irreversible genotoxicity during seed ageing [281, 282, 299, 283]. Extensive DNA oxidation results in the increase amounts of oxidised DNA and DNA laddering during ageing and or programmed cell death (PCD) [284, 285] in deteriorating seeds. The altered transcription and translations of mtDNA due to oxidative damage leads to increased uncoupling in the electron transport chain resulting in enhanced production of AOS [281, 286, 287]. Under normal growth conditions, AOS leads to a low level of both mtDNA and nuclear DNA (nDNA) damage, which is rapidly repaired, and most of these oxidative DNA lesions are repaired by the base excision repair pathway [283, 91]. When cells are exposed to biotic or abiotic stresses they can either die passively/accidentally or can self-destruct, depending on the type of stress and its severity [288]. The developmentally regulated self-destruction is categorized as PCD. The PCD in ageing seeds have been evaluated by observing increase DNA fragmentation in the agarose gels as a result of DNA cleavage at inter-nucleosomal sites by nucleases [289, 290, 291]. Fragmentation of DNA has been reported in desiccating recalcitrant seeds of Quercus robur, Trichilia dregeana and Inga vera [292, 293, 294]. Single and double strand breaks of DNA accumulate [295] in ageing seeds. In general, all events like diminished ability of DNA synthesis, DNA repair and decline in nucleic acid content are associated with fall in germinability and vigor.

The neem tree (Azadirachta indica A. Juss.), a member of Meliaceae family, is an evergreen tropical tree of Indian sub-continent [192]. A series of publications from our laboratory established that the neem seeds exhibits intermediate seed storage behaviour [21, 296, 121, 22] as they showed LSMC close to 11.8% which is far below than the true recalcitrant seeds like Shorea robusta [17, 118] (32-40%MC), Madhuca indica [297] (40%MC), H. odorata (45%MC), H. parviflora (41%MC), S. almon (41%MC) [298] and Avicennia marina [7] (54%MC). The short viability (140 to 160 days) reported in the intermediate storage behaviour of neem seeds is associated with the enhanced formation of superoxide with reduced seed viability and vigour. The dehydration induced loss of germinability was closely related to impairment of scavenging enzymes like superoxide dismutase, catalase and ascorbate peroxidise. Our previous study clearly indicated the dehydration induced loss of seed germinability which is associated with the excessive accumulation of superoxide during prolonged storage of neem seeds [21, 296, 121, 22]. Hence, the present investigation was designed to address 1- the effect of rate of dehydration and 2-
the underlying biochemical mechanism associated with the rate of dehydration in neem seeds during storage.

The objectives of the present study were to evaluate, in detail, the variants of AOS and its role in oxidative modification of lipid, protein and DNA during ageing in slow (natural) and rapidly dried neem (*Azadirachta indica*) seeds showing non-orthodox seed storage behaviour. The following parameters were studied in differentially (slow and rapid) desiccated neem seeds during storage:

- Monitored the critical moisture content, seed germination and viability.
- Determined changes in various forms of AOS.
- Estimated changes in lipids and its peroxidized products {(MDA, conjugated diens, hydroperoxides and HNE) - indicator of seed deterioration *vis-à-vis* lipid peroxidation} and its catabolic enzymes like lipase and lipoxygenase.
- Evaluated changes in soluble proteins, carbonylated and glycosylated proteins and proteasome activity during ageing and storage of seeds.
- Assessed changes in the levels of DNA and its degradation during storage of seeds.