Seed Res. 50 (2): 91-99, December, 2022

Seed Deterioration: An Overview

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(Received August 2022; Revised October 2022; Accepted October 2022)

ABSTRACT: Seed deterioration represents an undesirable facet of seed life, characterized by degenerative changes over time that heighten a seed's susceptibility to external stressors while diminishing its capacity to endure external and internal stresses. This phenomenon is distinct from seed expansion and germination, manifested during field weathering, harvesting, and storage. The intricacies of seed physiology and diverse conditions during the lifespan of the seed contribute to this deterioration. Investigations into seed deterioration reveal profound consequences on various physiological aspects within a normally functioning cell. Lipid peroxidation, membrane disruption, encompassing the degradation of DNA and RNA, impairment of transcription and translation processes, increased permeability of the cellular membranes, alterations in carbohydrate reserves, and antioxidant shifts contribute to the overall seed deterioration. Predominant evidences implicate free radical peroxidative assaults on membrane lipids as the instigator of many of these deteriorative events. Evaluation of seed deterioration conventionally relies on vigor and viability tests, such as germination, electrical conductivity (EC), performance tests, and ethanol assays. Modern technologies offer precise, or non-destructive and expeditious alternatives for assessing seed deterioration and quality. These include advanced spectroscopic techniques like FT-NIR and Raman spectroscopy, digital imaging, soft X-Ray imaging, and molecular marker-based tests.

Keywords: Deterioration, Germination, degradation of DNA, Molecular mechanism, Lipid peroxidation, Spectroscopy, Digital imaging

INTRODUCTION

Seed deterioration is an unwelcome and detrimental attribute in agriculture, characterized by degenerative alterations over time that increase a seed's vulnerability to external challenges, diminishing its ability to survive [1]. This process, distinct from seed development and germination, leads to a loss of seed vitality over time. Seed deterioration involves cytological, physiological, biochemical, and physical changes that reduce viability and eventually result in the death of the seed [2]. Irreversible degenerative changes in seed quality after reaching peak levels define seed deterioration [3]. Losses in seed quality, viability, and vigor from adverse environmental factors [1,3]. Field weathering, occurring during post-maturation and pre-harvest periods with high relative humidity and temperature, is a significant contributor to seed deterioration [1].

Harvest and post-harvest processes, including threshing, machinery handling, and drying, also exert a considerable impact on seed quality [4]. Mechanical damage, especially to very dry seeds, can lead to fractures or injuries, making seeds susceptible to fungal attacks during storage [5]. Storage, influenced by genetic factors, seed quality at the time of storage, pre-storage history, moisture content, temperature, and duration, is a critical factor in seed storability [4]. Despite inevitable damage during storage, controlling environmental conditions can prolong seed viability [6]. Lower temperature and humidity conditions slow the seed deteriorative process, extending the period of viability [1,7].

Where Does Seed Deterioration Occur?

Does not occur uniformly in seed.

Monocots: Deterioration begins in root tip and causes radicle extension to be reduced more than coleoptile extension.

Dicots: Deterioration begins in growing points (shoot and root) of embryonic axis. Seed deterioration occurs first in the meristematic tissues of seed

TYPES OF SEED DETERIORATION

Field Weathering

The deterioration of seed quality, vigor and viability, due to high relative humidity and high temperature during the post-maturation and preharvest period is referred to as field weathering. Weathering occurs in the period between the attainment of physiological maturity till harvesting in the field [8]. Exposure to hot and humid conditions, rainfall, photoperiod after ripening are pre-harvest factors, cause seed quality loss following physiological maturity [4]. Adverse environmental conditions during seed filling and maturation result in forced seed maturation, which is associated with low yields, leading to a significant decrease in quality and an extensive reduction in the crop productivity [1]. After physiological maturity if the seeds are retained on mother plant seeds will deteriorate, physiological changes in seed may lead to formation of rigid seeds or off-color seeds in pulse crops [8]. Harvest delays beyond optimum maturity extend field exposure and intensify deterioration.

Harvest and Post-Harvest Deterioration

Seed quality is highly affected by harvesting and handling methods. Harvest and postharvest deterioration comprise threshing, processing machinery, seed collection, handling, transporting and drying [9] Mechanical damage is one of the major causes of seed deterioration during storage. Very dry seeds are prone to mechanical damage and injuries [4,9]. Such damage may result in physical damage or fracturing of essential seed parts; broken seed coats permit early entry and easy access for microflora, make the seed vulnerable to fungal attack and reduce storage potential [5]. In its severest form, physical seed damage is exhibited by splitting of the cotyledon, shattered and broken seeds. Large seeded varieties are more sensitive to mechanical damage than small seeds.

Storage

Storability of seeds is mainly a genetically regulated character and is influenced by quality of the seed at the time of storage, pre-storage history of seed (environmental factors during pre and post-harvest stages), moisture content of seed or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents [3,4,9]. Storage life of seeds doubled with each 1% reduction in seed moisture or each 5°C reduction in temperature [10]. Damage of seed during storage is inevitable [11]. These environmental conditions are very difficult to maintain during storage [2]. The seed storage environment highly influences the period of seed survival. After planting of deteriorate seeds, seedling emergence may be poor and transmission of pathogens to the new crop may occur. Lower temperature and humidity result in delayed seed deteriorative process and thereby leads to prolonged viability period.

Characteristics of Seed Deterioration

Seed deterioration, an inexorable and irreversible process inherent to all living organisms, underscores the inevitable cycle of life, where death remains an inescapable outcome. However, through meticulous adherence to optimal storage practices, the pace of deterioration can be ameliorated. Once seed deterioration has commenced, this metabolic degradation becomes irreversible, emphasizing that low-quality seeds are incapable of transformation into high-quality counterparts. The rate of deterioration exhibits considerable variation among diverse seed types. Orthodox seeds undergo a gradual decline over several years, while recalcitrant seeds experience a drastic loss of viability once their moisture levels plummet below the critical threshold of 30%. At the point of seed maturation, deterioration is at its nadir, as seeds attain optimal quality. Subsequently, the seed deterioration process is set in motion.

Intriguingly, the rate of deterioration not only varies among different seed types but also within seed lots of the same kind stored under identical conditions. The complex sequence of events during seed deterioration, though not fully comprehended, is speculated to follow a probable model. Delouche and Baskin's (1963) hypothetical framework, encompassing impaired energy and synthesis mechanisms, heightened membrane degradation, respiration, and biosynthesis, coupled with diminished germination rate, growth and development, plant resistance, and field emergence, sheds light on major parameters used in measuring seed vigor. Vigor tests, being more sensitive than standard germination tests, underscore the significance of monitoring membrane integrity early in the deterioration process. Following the decline in respiration and biosynthetic capacity, the germination rate dwindles, leading to a loss of uniformity within seed lots. Additional repercussions of deterioration include diminished storability, reduced resistance to disease infection, and compromised field emergence under biological and environmental stresses, with implications for final crop yields. The ultimate parameter in seed deterioration is seed germination, highlighting the necessity for vigor tests to complement routine laboratory germination assessments.

SYMPTOMS OF SEED DETERIORATION

Morphological Changes: Manifestations of seed deterioration are evident in morphological changes, particularly in the seed coat color of legumes. Darkening of the seed coat, attributed to oxidative reactions accelerated under conditions of elevated temperature and relative humidity, signifies the progressive deterioration of seeds [12]. Beyond seed coat effects, other morphological transformations, such as necrotic lesions in cotyledons and yellowing in lentils after prolonged storage, are indicative of deteriorating seeds.

Ultrastructural Changes: Ultrastructural changes observed through electron microscopy reveal coalescence of lipid bodies and plasmalemma withdrawal associated with deterioration, impacting cell membrane integrity [7]. The decline in phospholipids, attributed to phospholipase enzyme activity or lipid peroxidation, plays a crucial role in disrupting cell membranes. example:In lettuce, this coalescence of lipid bodies has been detected in the embryonicaxis but not the cotyledons [13]. Withdrawal of the plasmalemma has also been detected in these species as well as in rye. It is significant that both of these events influence cell membrane integrity.

Cell Membrane: Cell membrane disruptions during seed deterioration lead to decreased capability in retaining cellular constituents, with a decline in phospholipids linked to phospho lipase enzyme activity or lipid peroxidation [12]. Notably, accelerated aging conditions may intensify the decline in phospholipids, potentially expediting the rate of seed deterioration compared to dry seed storage. In this context, lipid peroxidation emerges as a significant contributor to observed declines in membrane integrity.

Loss of Enzyme Activity: The most sensitive tests for gauging incipient seed deterioration involve measuring enzyme activity associated with the breakdown of food reserves or biosynthesis during germination [5]. Enzymes such as amylases, proteinases, cytochrome c oxidase, and glyceraldehyde phosphate dehydrogenase are key indicators of the deteriorative process [11]. Biochemical tests, including the tetrazolium test (dehydrogenase) and the glutamic acid decarboxylase (GADA) activity test, offer insights into enzyme activity loss [5]. Oxidases such as catalase, peroxidase, amylase, and cytochrome oxidase are additional enzymes correlated with seed deterioration.

Reduced Respiratory Activity: As seeds deteriorate, respiration weakens progressively, ultimately leading to a loss of germination. A respiration quotient of 1.5 or more serves as an indicator of deteriorating seeds [11]. Deterioration may result from an excess uptake of CO_2, reduction in O_2 uptake, or a combination of both processes [9].

Increase in Seed Leachates: A notable symptom of deteriorated seeds is the increased leachate content observed when soaked in water [9]. The degree of deterioration is linked to the concentration of seed exudates found in the steep solution [7]. Measurement through electrical conductance methods or determining the soluble sugar content of the leachate provides insights into the extent of membrane degradation associated with seed deterioration [1].

Increase in Free Fatty Acid Content: The hydrolysis of phospholipids precipitates the liberation of glycerol and fatty acids, a reaction that gains momentum with escalating seed moisture content [7]. The incessant accrual of free fatty acids leads to a reduction in cellular pH, proving detrimental to normal cellular metabolism. Moreover, it induces the denaturation of enzymes, resulting in a loss of activity [9]. Seeds harboring 1% or more of free fatty acids typically exhibit compromised germination [9].

Performance Symptoms: The eventual degradation of seeds becomes apparent in their diminished performance during germination. Initial signs include delayed seedling emergence, succeeded by a decelerated rate of seedling growth and development, accompanied by a decline in overall germination efficiency. As seeds deteriorate, the range of environmental conditions conducive to germination narrows, a symptom that manifests early in the deterioration process. Loss of field emergence potential is a frequently observed indication of seed deterioration, and hence taken as a status of vigour. Furthermore, deteriorated seeds exhibit reduced resistance to environmental stresses during germination and early seedling growth, translating into diminished yield potential. This susceptibility may be evident even in the absence of more overt symptoms accompanying germination and seedling establishment.

CAUSES AND MECHANISMS OF SEED DETERIORATION

Lipid Peroxidation: This process involves the loss of electrons in lipids, giving rise to peroxyl radicals as intermediate molecules. It unfolds through enzymatic and autoxidation methods, occurring in initiation, elongation, and termination phases. The initiation phase involves the loss of hydrogen from lipid molecules, leading to the formation of lipid radicals. These radicals react with oxygen to produce lipid peroxyl radicals, initiating a chain reaction.

Degradation of Functional Structures: The integrity of lipid rafts in cell membranes diminishes during seed deterioration, affecting membrane permeability and resulting in the leaching of seed constituents. Lipid rafts comprise proteins, carbohydrates, and other substances suspended in a sea of lipids. Cellular membranes, as deterioration progresses, lose their selective permeability, enabling cytoplasmic metabolites to leach into intercellular spaces. This membrane degradation arises from the hydrolysis of phospholipids by phospholipase and phospholipid autoxidation, representing an outcome of aging.

Inability of Ribosomes to Dissociate: Polyribosomes must dissociate before preformed mRNA can attach, initiating protein synthesis in germinating seedlings. In nonviable seeds, ribosomes fail to dissociate, impeding protein synthesis and serving as a measurable symptom of aging [9]. There is also evidence that long-lived mRNA is lost during extended seed storage.

Enzyme Degradation and Inactivation: Deteriorating seeds exhibit decreased activity of enzymes such as catalase, dehydrogenase, and glutamic acid decarboxylase [14]. The overall decline in enzyme activity reduces the respiratory potential of the seed, diminishing both energy (ATP) and food supply during germination [11]. Changes in enzyme macromolecular structure contribute to their reduced effectiveness, involving compositional alterations, oxidation of sulfhydryl groups, and configuration changes [15].

Formation and Activation of Hydrolytic Enzymes during Germination: As seed moisture content approaches levels necessary for germination, hydrolytic enzymes are activated [16]. However, if moisture levels remain high or increase further, the seed deteriorates due to energy expenditure or accumulation of breakdown products [17] Phospholipases, a related group of enzymes, hydrolyze phospholipids, destroying the membrane structure [15,18]. Activation of phosphatase enzymes converts ATP to ADP, causing an energy loss accompanied by increased phosphate acidity. Other hydrolytic enzymes activated by high moisture levels include amylases and proteolases [14].

Breakdown in Mechanisms for Triggering Germination: Gibberellins and cytokinins typically trigger enzyme activity, leading to germination [19]. In aged seeds, germination can be enhanced by treating them with growth hormones, indicating a deficiency of hormones for germination and highlighting a breakdown in triggering mechanisms [20].

Genetic Degradation: Seed aging is associated with chromosomal aberrations, termed mutagenic effects, including fragmentation, bridges, fusion, ring formation of chromosomes, and variations in nuclear size [21]. Free radicals or reactive oxygen species (ROS) are implicated in genetic degradation, reacting with the hydrogen bonds of DNA during irradiation and resulting in chromosomal aberrations [15]. Supporting evidence includes the retardation of fresh seed germination by extracts from aged seeds, correlation between mutations and seed age, and the occurrence of spontaneous mutations in aging seeds [22].

FACTORS AFFECTING SEED DETERIORATION

Environmental and Biological factors: The rate of seed deterioration is significantly influenced by environmental factors such as temperature, relative humidity, and seed moisture content, as well as biological factors, including fungi that create conducive niches [23]. Seed longevity is determined by the interplay of genetic and environmental factors during seed maturation, harvesting, and storage.

Kind/Variety of the seed: The storability of seeds varies considerably based on their kind or variety which is influenced by genetic makeup [24]. Some seeds are inherently short-lived, while others, despite superficial similarities, differ in storability due to their genetic makeup [25].

Initial Seed Quality: High initial viability extends the storage life of seeds, with vigorous and undeteriorated seeds maintaining quality longer than compromised counterparts [26,27,28]. Seeds subjected to mechanical damage or environmental stress during development may deteriorate more rapidly.

Effect of temperature: Elevated temperatures accelerate biochemical processes, hastening deterioration in seeds with high moisture content [23,29]. The sensitivity of seeds to high temperatures depends on their water content, with quicker loss of viability as moisture content increases.

Effect of Moisture Content: Seeds stored at high moisture content experience increased respiration, heating, and fungal invasion, leading to reduced vigor and viability [26]. The rate of seed quality loss after physiological maturity depends on the surrounding environmental conditions [23,25,30]. Optimal moisture content for maximum longevity is around 6-8%, below which lipid autoxidation becomes damaging.

Effect of Organisms Associated with Seeds: Bacteria, fungi, mites, insects, and rodents associated with seeds (pathogenic or saprophytic) in storage can cause damage, leading to loss of vigor, viability, or complete seed loss [23,25,30].

Mitigation of Seed Deterioration

Pre-Storage Treatments: Measures such as seed conditioning, packing, sanitation, fumigation, halogenation, and antioxidant treatment are employed to mitigate seed deterioration before storage.

Hydration/Dehydration Treatments: A practical approach to repair lipid peroxidation-induced membrane damage involves hydration/dehydration treatments, addressing membrane integrity.

Mid-storage Treatments: Mid-storage seed treatments aim to reduce age-induced damages, restoring seed vigor and viability. Methods include hydration-dehydration, soaking-drying, dipping-drying, and spraying-drying.

DETECTION OF SEED DETERIORATION

General Vigor and Viability Tests

Morphological Test: Observe changes in seed coat color as an indicator of deterioration.

Germination Test: Assess germination rates, with less-deteriorated seeds showing higher germination.

Tetrazolium Test: Measure dehydrogenase activity, with deteriorated seeds exhibiting lower activity.

Electrical Conductivity Test: Irreversibly proportional to seed vigor, where less-vigorous seeds release more leachates.

Flow Cytometry Method: Measure endoreduplication, with deteriorated seeds showing less endoreduplication.

Vital Coloring Test: Use dyes to differentiate live and dead tissues, particularly useful for tree seeds.

Enzyme Activities: Measuring enzyme activities (hydrolytic, respiratory, anti-oxidative etc.) as an indication of seed viability.

NEW APPROACHES

Marker based testing: Usually Marker assisted selection or marker aided selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself. Here the study of various biomolecules such as proteins, various enzymes and nucleic acids could be used to differentiate the deteriorated and non-deteriorated seeds.

In a study with differentially stored rapeseeds, highest germination percentage was seen in the seeds from 2016 stored under 4ºC and was followed by seeds stored under 23ºC, 2013 seeds stored under 4ºC and 23ºC at 120 hours after germination and the seeds from17 hours 21 hours after germination was taken and the excised embryo was subjected under flow cytometry in which the seeds from 2016 stored under 4ºC were more vigorous and had highest 8C % in all dry, 17 and 21 hours germinated seeds and least was recorded in 2013 seeds at 23ºC. The gene *BnRSH1, BnRSH2* and *BnRSH3* were studied against the housekeeping gene *BnUBQ* and *Bn*5S rRNA, here the house keeping gene showed no denaturation while the*BnRSH1* and *BnRSH3* showed gradual degradation in their content were it was least in 2013 seeds stored at 23ºC and was highest in the seed stored at 4ºC and *BnRSH2* showed no changes. As seed deteriorates many changes occurs in the cell and the low seed vigor is characterized by low intensity of DNA synthesis (measured with flow cytometry), less germination percentage and lesser gene expression [31].

The untreated seeds recorded 100 % germination and the lowest germination recorded in seeds after controlled deteriorated for 7 days with 37% and even the shoot and root length followed the same trend showing that untreated seeds was non-deteriorated. Hydrogen peroxide is one of the ROS or free radicle which causes lipid peroxidation and other damages to the biomolecules, as the seed deteriorates its content increases in the cell and it is evident by increase in the MDA (Malondialdehyde is one of the final product of the lipid peroxidation and oxidative injury caused by environmental stress) where the MDA was very much less in controlled seeds and was recorded highest in seeds subjected for 7 days' deterioration.

Out of 1626 non-redundant proteins found, 352 proteins were completely degraded making it impossible to measure in the aged/deteriorated seeds and 146 proteins showed changes in their abundances in which 5 were increased, rest 141 were decreased abundance. 141

proteins which showed changes were subjected to 2-Dimensional gelelectrophoresis to know the increase and decrease in their abundances, here majorly the increase of some stress related proteins PM34 and Rossmann-fold proteins were recorded and decrease in the formate dehydrogenase enzyme and translation elongation factor in which the former is responsible for the formation of tertiary fold in the RNA and the latter in converting mRNA to proteins is impaired leading to deterioration and also late embryogenesis abundant proteins (LEA) was decreased. Also there was decrease in the ROS/free radicle scavenging enzymes such as DHAR (De hydro ascorbate reductase), APX (Ascorbate peroxidase), SOD (Superoxide dismutase), MDHR (Mono dehydro ascorbate reductase) resulting in higher rate of lipid peroxidation. Revealed that Controlled deterioration results in increase in ROS/Free radicles which leads to loss of vigor and viability, Controlled deterioration increases the abundance of seed maturation protein and decreases LEA and few others, controlled deterioration decreases the SOD, APX and others scavenging enzymes [32].

In the *Arabidopsis thaliana* expression of genes (B16, B20) was highest at 100 % germination and gradually decreased along with decrease in the germination percentage. The trend of denaturation in the genes and their expression was almost similar in both naturally aged (NA) seeds and artificially aged (AA) seeds. The rate of degradation was directly proportional with the length of the long-lived mRNA, were the mRNA with more base pair or length degraded firstly and the one with less base pair degraded slowly. When the base pairs are more, then the genetic material is prone to more damage and is left with only fewer genetic material and in turn requires more cycle threshold level(ct cycle). When the basepairs are less, then the genetic material is less prone to damage and is left with more genetic material and in turn requires less cycle thresholds to make it quantifiable in PCR.29 genes from both NA and AA seeds were used to get mRNA and the mRNA concentration from deteriorated seeds were subjected for PCR and came to a conclusion that the genes with highest base pair required more ÄCt value (difference between the ct cycle of aged and untreated/controlled seeds). 2 genes B16 and B20 with basepair of about 2500 and 2000 basepair respectively and recorded that the B16 required more ÄCt (7) value than the B20 (5) in case of NA seeds and in case of AA seeds it was 5 and 3 respectively when compared with the untreated/dry seeds. ÄCt values for six different fragments of B16 genes of AA when compared with the aging time recorded highest Ct cycles at 20 days of AA and minimum at 0 days AA and the same trend was seen in B20 genes [33].

When the fragment length of 2500 bp from b16 genes was studied against the 250 bp fragment as reference the gene with 2500 bp (effective base pair of 2500-250=2250) required highest cycles and the aging time had also a positive impact i.e., as the aging days increased the cycles also increased and was least recorded in the 500bp (effective 250bp). And same trend was observed in the B20 genes too. The long-lived mRNA and came to know that the mRNA with more basepair degrades firstly. Here the B16 gene having length of 2500bp was studied for five different fragment 2500 bp, 2000 bp, 1500 bp, 1000 bp and 500 bp against the 250 bp as reference leading it to the effective 2250, 1750, 1250, 750 an d250 bp respectively and B20 genes having length of 2000 was studied for effective 1750, 1250,750 and 250 bp and the breaks per nucleotide per day (β) was more in all the fragment of B16 than the B20 and overall breaks per nucleotide per day incase of B16 was 1.22±0.03. Found that as seed deterioration takes place the long-lived mRNA also degrades. Degradation and length/bp of long-lived mRNA are correlated. Breaks per nucleotide per day increase with increase in the length of the long-lived mRNA [33].

Spectroscopy technique: The term "spectroscopy" defines a large number of techniques that use radiation to obtain information on the structure and properties of matter. The basic principle shared by all spectroscopic techniques is to shine a beam of electromagnetic radiation on to a sample, and observe how it responds to such a stimulus. The response is usually recorded as a function of radiation wavelength. A plot of the response as a function of wavelength is referred to as a spectrum.

Based on the wavelength used by spectroscope, they have been classified into Near-Infrared spectroscopy (NIR) (780 nm-2500nm), Mid-infrared spectroscopy (MIR) (2500nm- 25000nm), Fourier transform infrared spectroscopy (FT-NIR) (700nm- 25ìm), Raman-spectroscopy (785nm), Hyperspectral imaging spectroscopy (NIR+digitalimaging), X-rayimaging.

Near-Infrared spectroscopy (NIR) (780 nm-2500nm): It is based on the absorption of electromagnetic radiation at wavelengths in the range of 780–2500 nm. While the radiation penetrates the sample, their spectral characteristics change through wavelength dependent scattering and absorption processes. Thet issue structures of products made up of the cells and intra/ extra cellular environments are responsible for the scattering. The absorption is mainly caused by C-H, O-H, and N-H bonds of the main compounds (water, sugars, chlorophylls, carotenoids, etc.). NIR spectra comprise broad wave bands arising from over lapping absorptions corresponding mainly to overtones and combinations of these chemical bonds, making it feasible to detect organic and biological materials.

Fourier transform infrared spectroscopy (FT-NIR) (700nm-25µm): It is necessary to preprocess the spectral data to remove any irrelevant information and improve calibration model performance. Spectral pretreatment methods such as spectral filtering, smoothing, normalization, mean centering, auto scaling, baseline offset cor-rection (BOC), first and second derivative, Fourier transform (FT), wavelet transform(WT), orthogonal signal correction (OSC), standard normal variate (SNV), and multiplicative scatter correction(MSC) are commonly utilized. In case of FT the electromagnetic radiation from light source is not directly projected on the sample but it is made to pass through a beam splitter and then the radiations move perpendicular to each other and then meets once again at beam splitter after hitting the fixed and movable mirrors in their way and then falls on the seed sample, later the absorption and reflectance spectrum are given by the detector.

Hyper-spectral imaging: It combines the properties of both NIR and digital imaging, we have discussed the former one the latter one digital imaging uses the property of seed coat color and composition of the seed were the intensity of the seed color is divided into red, green, blue fractions and compared with the healthy seeds.

X-Ray imaging: X-ray computed tomography (CT), is electromagnetic radiation with the wave-length range of 0.01–10 nm. The photon energy of an X-ray is in the range of 0.1–120keV which leads to a strong penetrability. X-ray, similar to other electromagnetic waves, can show the following phenomena: reflection, refraction, scattering, interference, diffraction, polarization and absorption. Through which internal structures can be seen for damages and other things.

Raman spectroscopy: Raman spectroscopy (RS) is a form of analytical spectroscopy based on Raman scattering, which was discovered by C.V. Raman in 1928. According to the principle that vibrational frequencies are specific to molecule's chemical bonds and symmetry, Raman peaks are typically obvious in most cases, which provide fingerprints to identify molecules and make the analysis of chemical compositions more precise. For example: Characteristic peaks associated with the basic constituents of pepper seeds could be observed around 1520 cm^{-1} (C=C), 1440 cm^{-1} (=CH2), 1263 cm^{-1} (=CH),1154 cm^{-1} (C-C), and 1090 cm^{-1} (C-O).

The absorption spectrum of both FT-NIR and RS showed that the absorption was more in untreated seeds and less in treated seeds and the absorption spectrum in case of FT-NIR was clear and distinct and was not distinct in case of RS. Here the principal component analysis was made for the absorption spectrum and incase of FT-NIR the 3 principal components accounted for the 99.3 % and rest several components accounted for 0.7% the principal loadings was based on the variable weights of the seed, absorption spectrum and principal component loadings when scattered on the graph the points were very distinct and clear for both untreated and treated seeds. In case of RS the two principal loadings PC1and PC2 accounted for 84.6% and 14.7% respectively and the points scattered on graph was not distinctive and were overlapping. When the spectra of both FT-NIR and RS was subjected to PLA-DA the absorption at a particular wavelength was observed and it was related to particular bonds in the food reserve of the seed, in case of FT-NIR of maize seed, the absorption at 1180nm was due to C-H bond, 1420 nm for CH_3 functional group, 1918 nm for carbohydrates, 2035 nm for C=O and the peaks at 2058 nm and 2275 nm corresponded for N-H and O-H stretching and the FT-NIR spectrum was 100% accuracy with the predictability of greater than 95%.

In case of PLA-Dao f RS the absorption spectrum between 1580-1640 cm^{-1} was correlated with the germination ability of the seeds, coming to the other absorptions at 700 and 950cm^{-1} was due to C-H band, 1280-1444 cm^{-1} was due to lipid content, here the RS was 98.9 % accurate with predictability of 93-100%. Found that FT-NIR was found to be more accurate and precise method for interpreting the data on viability and non-viability of corn seeds. Aged corn seeds have reduced amounts of starch and protein compared with non-aged/new kernels, and these components are easily detectable in the longer wave band [34].

High vigour seed lot showed lower reflectance and more absorption because of high amount of healthy tissue and more food reserve materials. Low vigour seed lot showed high reflectance and less absorption because of high amount of deteriorated tissue and less food reserve food materials. Found that multispectaral and x-ray images have a strong relationship with seed physiological potential and reflectance. X-ray data individually or with others showed >96% of accuracy to predict seed quality.

CONCLUSION

Seed deterioration refers to the decline in seed vigor and viability, resulting in reduced germination and seedling establishment. Losses in seed quality can occur during pre-harvest (field weathering), harvest (handling), and post-harvest (storage) stages. To investigate seed deterioration mechanisms, accelerated aging techniques are employed in laboratory settings. Various controlled seed hydration methods, such as seed priming, are utilized to enhance seed quality and germination potential. Recent advancements in detecting seed deterioration using sophisticated techniques, including biochemical assays, spectroscopy, and X-ray imaging, offer enhanced accuracy and precision. However, implementing these methods requires significant financial resources and specialized expertise. A deep understanding of the factors, mechanisms, and remedies for seed deterioration is crucial. This knowledge enables us to prolong the storage life of seeds while maintaining their quality. Creating optimal conditions to slow down deterioration is vital for producing high-quality seeds and providing sound guidance on secure storage practices to ensure their long-term viability.

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