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REVIEW ARTICLE

Beyond the surface: Unveiling the impact of acid delinting on seed coat integrity and performance in cotton

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Abstract

Delinting is widely used process aimed at removing residual lint from cotton seeds after ginning to enhance the efficiency of mechanical planting and improve germination rates. However, concerns have been raised regarding its potential impact on seed coat integrity and overall seed vigor. The seed coat serves as a crucial protective barrier that shields seeds from microbial infections, regulates water uptake during imbibition and plays a significant role in maintaining seed longevity. Damage to this protective layer can reduce germination rates, lead to abnormal seedling development and increase susceptibility to pathogens, ultimately compromising the seed quality. This review synthesizes current research on the effects of acid delinting on seed coat integrity and its implications for seed vigor. Various factors, including water absorption dynamics, electrical conductivity, seed viability and changes in mineral and metabolite composition, are analyzed to understand the extent of damage caused by delinting. Furthermore, this review examines various methodologies and testing protocols used to assess seed vigor in relation to seed coat integrity. This work aims to enhance our understanding of strategies that balance improved seed germination with the preservation of seed health. The insights from existing studies can help develop better testing methods to spot cotton seed lots with weaker seed coats. This can also support efforts to keep seeds viable during storage and ultimately improve productivity.

Keywords: acid delinting; cotton; seed coat integrity; viability

Introduction

Cotton (*Gossypium* spp.) is one of the most economically significant crops globally, valued for its versatility and wide range of uses. It produces high-quality lint for the textile industry, which employs millions and contributes substantially to export earnings in countries like India (1). In addition, cotton provides raw materials for renewable energy and seeds rich in nutrients, which are essential for oil extraction and livestock feed production (2). Beyond textiles and agriculture, cotton also supports a range of other sectors such as pharmaceuticals and alternative medicine (3). By products like cotton seed oil and meal further enhance its value across both industrial and agricultural domains (4). Cultivated in more than 75 countries and spanning over 30 million hectares, cotton supports the livelihoods of approximately 250 million people worldwide. Leading producers of cotton seed include China, India, the United States and Pakistan (5). During the 2022-2023 season, global cotton seed production was about 42.47 million metric tons (MMT), with a projected decline to 41.46 MMT in 2023–2024. In 2020, India alone contributed 11.6 MMT (6). Often referred to as “white gold”, as cotton plays a vital role in the global economy, with an estimated impact of over $600 billion (7). The cotton seed coat yields cellulose-rich fibers, while the embryo is a valuable source of proteins and oils, highlighting the dual economic contributions of both maternal and filial tissues (8). Having evolved from wild perennials to herbaceous annuals, *Gossypium* comprises around 50 species, including both diploid (2n = 2x = 26) and tetraploid (2n = 4x = 52) forms (2).

The first post-harvest step for cotton seeds is ginning, after which residual fibers, known as linter, still cling to the seeds. This linter can complicate mechanized planting and encourages pathogen growth that obstruct germination and normal seedling development which is essential for uniform field stand encourage, delinting is critical for ensuring seed quality (9). Cotton seed delinting is generally classified into three types: mechanical, acid and gas delinting. Mechanical delinting typically leaves behind 1 % to 2 % of residual lint on the seed surface. Although it is a widely practiced method, it often results in physical injury to the seed coat, leading to embryo necrosis and adversely affecting seed viability and storage longevity. Mechanical abrasions and fractures also increase the risk of infection, as soil-borne pathogens can easily penetrate the compromised seed coat. Gas delinting, the second method, involves the use of compressed anhydrous hydrochloric acid

(AHCL) to eliminate lint. While this approach is less corrosive to machinery, it requires low humidity conditions for successful operation. Furthermore, if seeds are already physically compromised, exposure to the gas may result in loss of viability or complete seed mortality (10).

Acid delinting, most performed using sulfuric acid, effectively removes all lint while also acting as a disinfectant. Its widespread adoption is primarily attributed to its ability to enhance sowing efficiency by improving seed flowability and promoting uniform germination, while also facilitating planting and reducing microbial contamination and insect damage during storage (11, 12). While acid delinting is widely used for its efficiency in removing lint from cotton seeds, it also comes with several concerns. These include potential damage to the seeds, difficulties in safely handling and disposing of the acid and rinse water, corrosion of processing equipment and an increased vulnerability of seeds to environmental stress. Since this method involves a direct chemical reaction on the seed surface, the outer and sub-epidermal layers of the seed coat are particularly at risk of being compromised. Although neutralization is commonly done to stop the acid's reaction and protect seed quality, the corrosive nature of sulfuric acid can still leave lasting effects on the seed coat (13, 14).

The structural vulnerability caused by acid delinting highlights the need for a deeper understanding of its effects on seed coat integrity. More than just a protective barrier, the seed coat plays a crucial role in safeguarding the embryo, regulating water uptake and preserving seed vigor during storage. Despite these critical functions, the impact of acid delinting on the inner seed coat layers and seed physiology remains underexplored. Given that the success of cotton cultivation largely depends on seed quality, this review examines how acid delinting influences key physiological parameters such as water uptake (imbibition), electrical conductivity, viability and the seed's mineral and metabolite profile.

Cotton seed structure

Cotton seeds are complex structures composed of several key components, including the cotyledon, epicotyl, hypocotyl, radicle and seed coat as shown in Fig. 1 (15). Among these seed coat plays a vital role in protecting the developing embryo, endosperm and perisperm, each contributing to the seed's

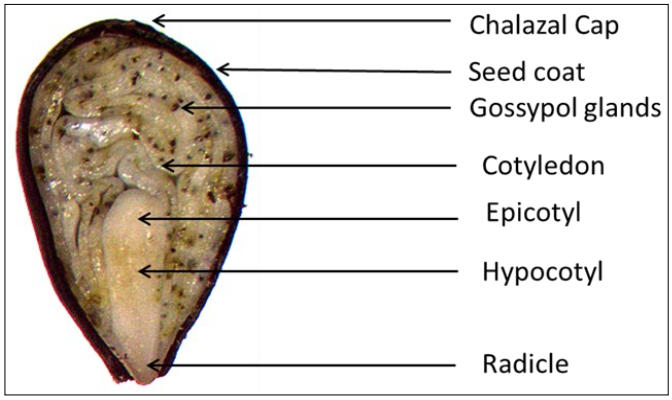

Fig. 1. Structure of cotton seed coat.

development and nutrient composition and influencing traits critical to cotton yield and quality, such as seed size and shape (16). As illustrated in Fig. 2, the cotton seed coat is a multilayered structure primarily derived from the inner integument. It consists of anatomically and chemically distinct layers: the epidermis, outer pigment layer, colorless layer, palisade layer and inner pigment layer (16, 17). As shown in Fig. 3, these layers are composed of a variety of compounds including cellulose, lignocellulose, waxes, uronate anions, tannins and lignin-type aromatics. Each layer performs specific structural and protective functions vital to seed integrity (17). As shown in Fig. 3 using advanced techniques like FT-IR microspectroscopy, researchers have discovered that these layers contain a wide variety of compounds. These include aromatic compounds, lignin-related polyphenols, uronate anions, carbonyl compounds, tannins and different types of waxes (16). Chemical analysis in the previous study also confirms the presence of these compounds (18). The epidermal layer, the outermost portion of the seed coat, is composed of cutin, wax, cellulose and pectin, forming a robust barrier against environmental stress. Beneath it lies the outer pigment layer, rich in lignin, which enhances the seed coat's rigidity and contributes to its coloration. The colorless layer serves as a transitional zone between the outer and inner layers. The palisade layer, composed of pectin, hemicellulose and lignin, provides additional mechanical support. Innermost pigment layer also primarily consists of lignin, further reinforcing the structural integrity of the seed coat (19). These components not only determine the physical properties of the seed coat but also play a vital role in maintaining seed viability and supporting

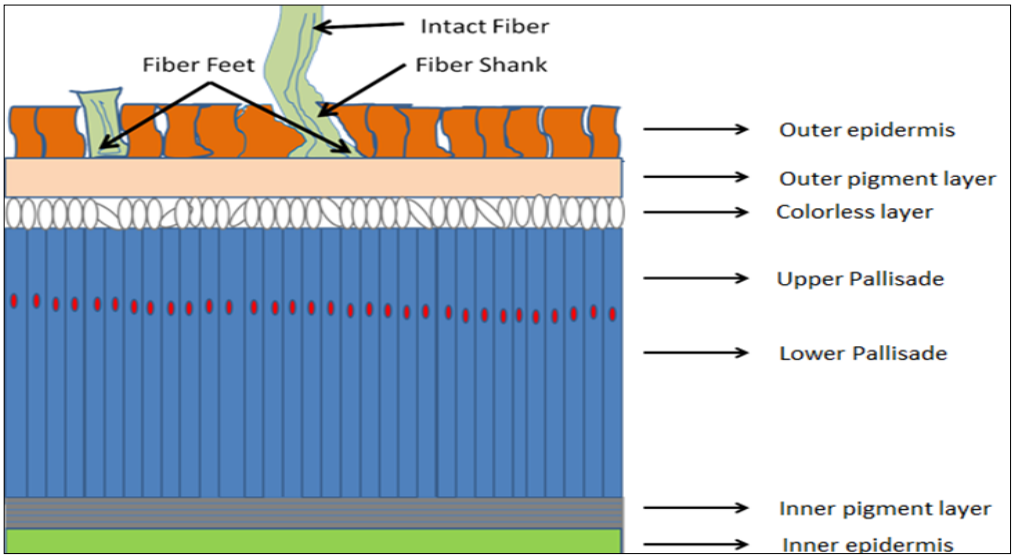

Fig. 2. Schematic of cross-section of cotton seed coat showing anatomical components.

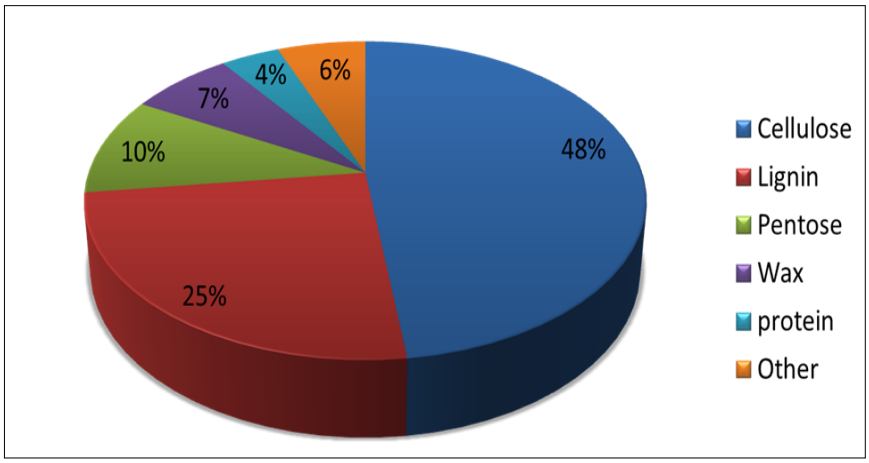

Fig. 3. Components of seed coat.

germination, as the seed coat's structure is crucial for shielding the embryo and regulating water and solute movement, directly affects physiological performance (20). Cotton fibers originate from epidermal cells with their bases anchored in the outer pigment layer and the fiber shafts encased by the cells of the outer epidermis. Detachment of the fibers from the seed generally occurs at or just above the outer epidermal surface as is explained in Fig. 4. Significant differences in seed coat structure and composition have been observed between diploid and tetraploid cotton species, such as *Gossypium herbaceum* (diploid) and *Gossypium hirsutum* (tetraploid), which influence traits like seed hardness, early maturity and fiber cleanliness (20). Wild cotton species typically exhibit tougher seed coats, which are advantageous for breeding programs aimed at improving fiber quality and reducing contamination (20).

The embryo is predominantly composed of oil and protein, while the endosperm contains varying amounts of starch, oil and protein (17). As the seed matures, oil content increases rapidly after around 35 days, whereas carbohydrate levels decline (21). During seed development, starch initially accumulates in the integuments but dissipates before the seed reaches full maturity (17). The cell wall composition also varies, with cellulose being the main component in the embryo and endosperm and lignification occurring in some of the outer layers of the seed coat (19, 22).

The intricate structure and chemical makeup of the cotton seed coat play a crucial role in seed protection, development and performance. Understanding these characteristics is essential for maintaining the viability of cotton seeds during storage.

Seed coat integrity studies

Seed coat integrity is essential for seed longevity and germination, as fractures in the coat can significantly diminish seed lifespan (23). The physical structure of the seed coat plays a critical role in protecting seeds from deterioration by preventing fungal and microbial attacks (24). However, this same structure can make it harder for seeds to germinate by preventing them from absorbing enough water (25).

Most studies have treated the entire seed as a single unit, even though the seed kernel and seed coat have distinct roles. Seed kernel contains nutrient reserves, including the embryo and either the endosperm or perisperm. It comprises the embryo along with the endosperm or perisperm, plays a structural role by housing the key developmental tissues and a physiological role by serving as a storage reservoir for nutrients and metabolic energy required during germination and seedling establishment (26).

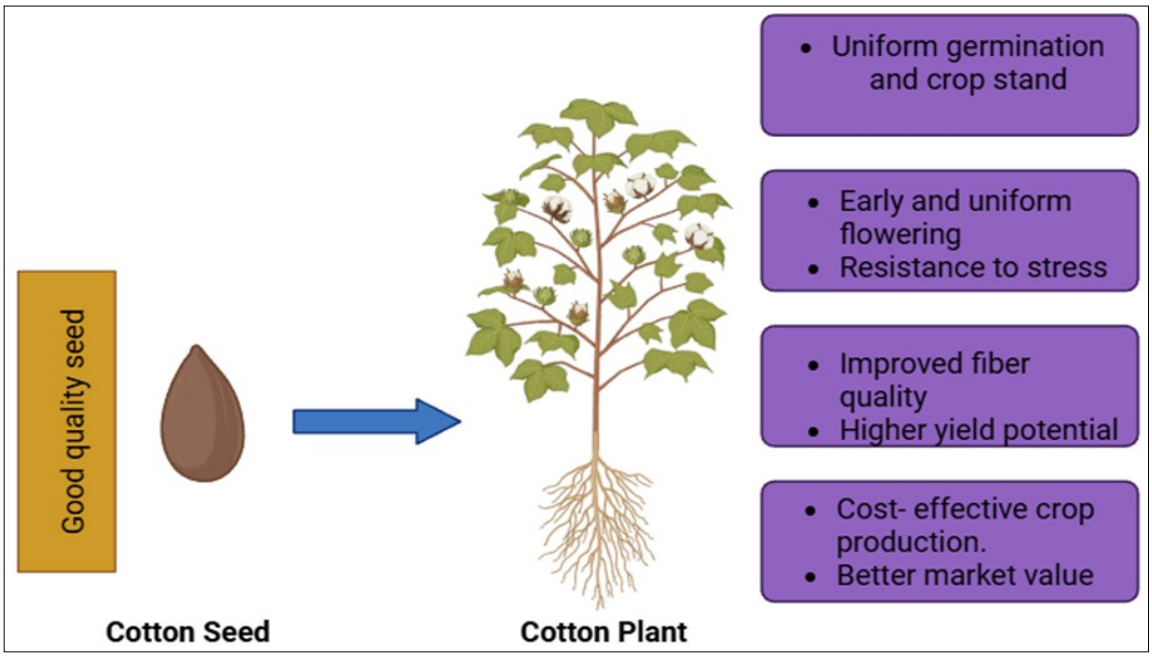

Fig. 4. Effect of seed vigor on overall plant development.

The seed coat is the protective tissue surrounding the seed kernal, sometimes including an endocarp and the testa (27). Structurally, it serves as a rigid barrier that physically protects internal tissues from mechanical damage and microbial invasion. The physiological role of it includes regulating water uptake, gas exchange and interacting with environmental signals that influence dormancy and germination (28, 29). The seed coat ratio (SCR) is defined as the mass of seed coat divided by the total seed mass and it varies significantly between species (30). Higher SCR is strongly correlated with desiccation tolerance, indicating greater storage potential (31, 32). In soybean, genotypes with denser or blacker seed coats, which typically have higher SCR show prolonged storage longevity compared to pale, thin-coated cultivars (33). A research study found that smaller seeds invest proportionally more biomass in protective tissues than larger seeds do (28).

The seed coat not only influences longevity but also affects dormancy and protection against deterioration (34, 35). Factors such as the physical structure, secondary metabolite concentrations and chlorophyll levels within the seed coat are closely linked to seed longevity (34). For instance, GmHs1^{-1} transgenic soybean lines exhibit significantly higher calcium levels in their seed coats compared to wild-type seeds, resulting in “hard-seededness”, which reduces germination but enhances longevity (36). Seed longevity is influenced by several internal factors, such as the amount of chlorophyll, the structure of the seed coat, the balance of plant hormones and the integrity of nucleic acids and proteins, as well as the systems that help repair them (34). Additionally, the seed coat plays a vital role in nutrient supply to the embryo during seed development (37). Maintaining genomic integrity is crucial for seed quality and longevity, particularly through effective DNA repair mechanisms during early imbibition (38). Seed deterioration often correlates with cellular damage to macromolecules such as DNA, proteins and lipids. The reduction in seed viability during storage is closely associated with loss of membrane integrity, indicated by increased electrolyte leakage (39). The seed coat’s lignin content and its presence in the pod wall contribute to enhanced resistance against mechanical damage, environmental stress and overall seed quality (40). However, there are conflicting insights on SCR and seed longevity. Although a higher SCR is frequently associated with improved seed longevity and dormancy due to enhanced physical protection, several studies have shown that, in certain cultivated species, a moderately lower SCR may promote faster and more uniform germination. This is particularly advantageous in agricultural systems where rapid and synchronized emergence is desired. Therefore, SCR represents a physiological and structural trade-off between ecological strategies favoring survival and dormancy and agronomic objectives emphasizing efficient crop establishment (41). Factors like genetics, environmental conditions and crop management practices such as delayed harvesting, uneven irrigation, excessive nitrogen application and planting in low-temperature-prone environments have been reported to influence seed coat cracking and the occurrence of green seeds in soybeans, ultimately impacting germination and vigor (42). Lignins and phenolic polymers within the seed coat are thought to enhance compressive strength and resistance to microbial degradation. They also affect water permeability within the polysaccharide-protein matrix of the cell wall (43). The seed coat is a critical determinant of seed germination, vigor and overall longevity potential (44). Gossypol, the predominant terpenoid found in flower petal glands and seed kernels, also plays a role in these dynamics (45). Cotton seed morphology significantly influences germination, growth and the formation of quality traits. Recent advancements in micro-CT technology have enabled detailed analyses of seed structure, revealing correlations between seed size and kernel-to-coat volume ratios (46). Moreover, cotton seed development and fiber formation are sensitive to environmental conditions, with abiotic stresses affecting source-sink relationships and fiber quality (47). Notably, over time, Chinese cotton varieties have shown increases in seed volume, surface area and coat thickness, while kernel volume and fullness have decreased (46).

Recent research highlights significant conflicts in our understanding of seed coat function in legumes. In soybean, Ranathunge et al. (2010) used cuticular analysis to show that seed permeability correlates more closely with cuticle cracking rather than the traditionally emphasized micropyle: permeability increased abruptly once cracks formed in the palisade cuticle (48). In contrast, the permeable seeds of mung bean and black soybean, water entry occurs primarily via an open micropyle, indicating that micropyle structure no surface cracking can govern imbibition in certain genotypes has been observed in previous study (49).

Acid delinting - methodology and its effect on seed quality

The ginning process marks the initial step in preparing standardized cotton seeds by separating the fiber from the seed (9, 50). Cotton seeds left with linters, commonly referred to as “fuzzy seeds”, present several challenges. Their fibrous coating makes it difficult to distinguish between viable, broken, or damaged seeds, complicating quality assessment before planting. This often results in lower germination rates and reduced crop yields. Additionally, fuzzy seeds tend to retain moisture, making them more susceptible to insect infestations during storage. When sown, they also contribute to irregular plant spacing, leading to uneven field density and compromised crop uniformity. The application of diluted sulfuric acid for delinting has been shown to effectively address these issues, complete removal of linters, improving seed quality, storability and overall field performance (12, 13). Acid delinting with 98 % sulfuric acid has been found most effective, significantly increasing germination percentage (51) and reduce susceptibility of seed to diseases like damping-off (52). Concentration, quantity of acid and duration of treatment may vary as presented in Table 1. Delinted cotton seeds have various advantages, including the ability to be mechanized, minimize seed consumption per unit area, germinate faster in the soil and emerge seedlings more quickly in the field (56). Studies showed that when compared to seeds with lint, delinted cotton seeds exhibited a higher germination percentage of 90 %, whereas seeds with lint had a lower percentage of 82 % (56, 57).

Table 1. Different methods of acid delinting in correlation with time required

Concentration	Time duration	References
100 mL	10 min	(53)
H_2SO_4:Seed	10 min (short staple) 30 min (long staple)	(54)
98 % H_2SO_4	2-3 min	(52)
100 mL/kg os seeds	2-3 min	(55)

Despite its effectiveness, acid delinting presents several drawbacks. One of the primary concerns is its potential to reduce germination rates and increase the proportion of abnormal seedlings (56, 58). These negative effects are largely attributed to the corrosive nature of concentrated sulfuric acid, which can damage seed coat integrity if not carefully managed. To mitigate these risks, neutralization with agents such as quick lime, hydrated lime, or filler lime is commonly employed. This step is essential for halting residual acid activity and preserving the physiological quality of the treated seeds. However, incomplete or improperly conducted neutralization can still compromise seed viability and lead to uneven field performance (55). Beyond seed quality concerns, acid delinting also raises significant environmental issues. The use of strong acids and the subsequent disposal of rinse water contribute to soil and water contamination and can pose occupational hazards to workers handling the chemicals. Recent studies highlighted the environmental burden associated with the improper management of neutralization waste, noting its potential to alter soil pH, disrupt beneficial microbial activity and degrade long-term soil fertility (51, 59). To address these challenges, recent studies recommend several strategies. These include optimizing acid concentration, standardizing treatment duration and seed-to-acid ratios and improving neutralization protocols to ensure both safety and efficacy. Additionally, sustainable alternatives such as enzymatic or biological delinting methods are being actively explored. Although still in the developmental phase, these approaches show promise for reducing the environmental footprint of delinting operations while maintaining seed quality (51).

Since the dilute sulfuric acid delinting remains prevalent it is also important to consider the genotypic variability in response to delinting treatments. The cellulose content of fibers, which differs among cotton varieties, influences the speed and effectiveness of lint removal. This variability, coupled with the lack of a universal standard for acid concentration and treatment duration, further underscores the need for tailored protocols that balance efficacy, safety and environmental responsibility (12, 13, 51, 58).

Changes occurring during seed deterioration

Seed deterioration is a progressive, multi-phase process involving interconnected structural, biochemical and molecular disruptions. It begins with the disintegration of cellular membranes, particularly the plasma and mitochondrial membranes, driven by lipid peroxidation. Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide radicals and hydroxyl radicals, accumulate when antioxidant defense systems such as superoxide dismutase, catalase and glutathione peroxidase are overwhelmed (60). This oxidative stress increases malondialdehyde (MDA) levels and electrolyte leakage, both of which are widely used as indicators of seed vigor loss (61). As deterioration advances, mitochondrial structures degrade. The cristae collapse, ATP synthesis declines and antioxidant enzyme activities fall, contributing to impaired respiration and possibly activating programmed cell death (60). Ribosomal dysfunction follows, marked by the breakdown of polyribosomes and a reduced capacity for protein synthesis, which severely affects germination. At the genetic level, ROS cause oxidative damage to DNA and RNA, including base modifications and strand breaks. DNA repair systems weaken, resulting in mutations and disrupted protein production. Additionally, ROS by-products like MDA form adducts with proteins and nucleic acids, further impairing cellular function (62). Lipidomic profiling of aged soybean seeds revealed a shift from phospholipids to lysophospholipids and glycerolipids, indicating membrane disintegration and greater solute leakage (63). Energy depletion also plays a key role. During storage, respiration continues to consume stored reserves. Prolonged storage leads to a shortage of readily available soluble sugars, limiting the energy required for germination and seedling growth. Excessive oxidation of unsaturated fatty acids and sugars, derived from triglycerides, contributes to this decline (56, 57).

These cumulative disruptions manifest as delayed germination, weakened seedling vigor and a higher incidence of abnormal seedlings or seed death. In cotton, compromised seed coat integrity, such as from acid delinting, can accelerate these internal deterioration processes. A weakened coat allows easier entry of oxygen and water, enhancing oxidative stress and reducing storability. Thus, preserving seed coat integrity is critical to maintaining seed viability during storage.

Seed vigour

Maintaining high yields will require the successful and uniform establishment of plants in the field under altered environmental conditions. Seed vigor, a complex agronomic trait that includes seed longevity, germination speed, seedling growth and early stress tolerance, determines the duration and success of this establishment period (64). Seed vigour is the sum of seed properties that determine the ability of viable seeds to germinate fast and uniform and to produce healthy seedlings with rapid and uniform emergence under both optimal suboptimal environmental conditions (65). One important method for assessing seed vigor is the first germination count test. It is typically conducted as part of the standard germination test, with the first count occurring on the third or fourth day after test initiation. This test can be performed using various substrates, including paper towel rolls, cloth rolls, or sand, with a constant temperature of 25 °C recommended (66). This test is a reliable way to assess seed quality, as seeds that have deteriorated usually take longer to germinate and fewer of them germinate in the initial count. In contrast, healthier seeds tend to germinate more quickly than those that are less vigorous (Fig. 4) (67).

Vigor tests have limitations in predicting cotton field performance. For example, six upland cotton cultivars and found no significant correlation between field emergence and seed vigor index, indicating that traditional vigor metrics may fail to predict field establishment under variable environmental conditions was evaluated in the former research (68). Seed vigor can significantly influence initial plant development and yield; however, its effects are highly contingent on environmental conditions. Seed vigor impacts both early emergence and final fiber and seed yield in cotton, albeit the strength of these relationships varies markedly across growing seasons and site-specific was demonstrated in the previous studies (69). The relationship between laboratory vigor tests and field performance is complex, as other factors like soil crusting can overshadow seed quality impacts (63). The standard germination test done in the lab does not always reflect how well seeds will perform in the field. That's why a vigor test is also needed to confirm the germination results and see if seeds that germinate well in the lab will also emerge effectively in the field.

Evaluating the vigor index helps improve the performance of seed lots during storage (70). Seeds that germinate successfully are considered viable, while those that grow into seedlings with healthy shoots and roots under certain conditions are classified as vigorous. Seed performance in producing strong seedlings can be grouped into three levels: high vigor, medium vigor and low vigor (71). While seed germination provides a clear indication of seed deterioration, it is the seed's performance under challenging conditions that more accurately reflects its vigor. Vigor testing helps evaluate a seed's ability to grow into a healthy seedling, even under challenging conditions (72). According to previous study the seedling traits, such as germination percentage, seedling length and dry weight decreased as the storage period increased (73). In contrast, electrical conductivity, seed moisture percentage and the seed index increased with longer storage periods, from 0 to 6 months and then to 18 months. This happens because high relative humidity around the seeds causes them to absorb more moisture through their membranes, increasing the seed index weight. Unfortunately, this rise in moisture content accelerates seed deterioration over time. The decline in seed quality is linked to biochemical changes in oil crop seeds. As storage time increases, the aging process in seeds intensifies. Prolonging the storage period from 0 to 6 months and then to 18 months significantly reduced the average crude protein content to 24.0 %, 23.0 % and 22.1 %, respectively and oil content to 23.3 %, 22.5 % and 21.9 %. Meanwhile, acidity and free fatty acid levels increased as the storage period extended. Free fatty acids, which are harmful to most cells, are absent in healthy seed tissues. However, when seeds deteriorate, the levels of these acids increase significantly. This indicates that free fatty acids contribute to the breakdown of seeds by damaging and rupturing cellular membranes, which disrupts the seed's structural integrity and overall viability. These findings align closely with those reported previously (60, 74, 75).

Faster methods of seed vigour testing

Electric conductivity test

The electrical conductivity (EC) test is a fast and convenient method for assessing seed vigor. It works by measuring the loss of integrity in the seed's cell membrane system, which is one of the earliest signs of seed deterioration. As seeds degrade, their membranes become less stable, allowing more ions and other substances to leak out, which the EC test detects (61). As aging stress on seeds increased, a corresponding rise in electrolyte leakage was observed (62). This increase in leakage indicates a breakdown in the integrity of the cell membranes, which is a clear sign of seed deterioration. The greater the leakage, the more evident the reduction in seed quality, highlighting the negative effects of prolonged aging on the seed's viability and multiple seed health parameters, including germination percentage, seedling vigor, seedling length, enzymatic activity (such as dehydrogenase and peroxidase) and viability (76). In cotton, increased EC in leachates was first linked to reduced viability in aging seed lots (77). A recent storage trial confirmed that cotton seeds aged under high humidity exhibited significantly higher EC and reduced germination, reinforcing EC as a reliable vigor metric. A study on chickpea seeds demonstrated that the electrical conductivity test is an effective method for assessing their physiological potential, yielding results comparable to earlier vigor tests. The optimal outcomes were achieved by soaking 50 seeds in 150 mL of water for 24 hr at a temperature of 25 °C (63). The results indicated that longer seed imbibition times and higher temperatures resulted in increased electrical conductivity. Notably, temperature significantly influenced conductivity values, with the highest readings observed between 25 and 30 °C. The EC test is a promising tool for quality control programs. Although the required soaking time may vary by species, results can typically be obtained in under 24 hr (78, 79) facilitating rapid decision-making regarding the seed lot.

Tetrazolium test

Tetrazolium chloride (TZ) is commonly used in the cotton seed industry to assess seed viability. TZ reacts with the oxygen used during the seed's respiration process, resulting in characteristic red staining of viable tissues. To perform the test, the seed is cut about one-third from the tip and then placed in a 1.0 % TZ solution at 30 °C for 18 hr. The staining on the embryo area reflects the seed's respiration activity and overall vitality. For evaluation, the embryo is carefully removed from the seed coat and the stained or unstained areas are examined to determine the seed's health and viability (66).

The tetrazolium test works by reducing colorless tetrazolium salts to a red or deep purple compound called formazan in living tissues. This reaction indicates the presence of active cellular respiration, which is a marker of viability (80). The previous study used a different method for testing seed coat integrity in accelerated aged soybean seeds by avoiding removal of seed coat (81). Later the seeds cut open and examined and total dehydrogenase activity was measured at 480 nm. It found that the staining increased as the seed aged for longer duration than control. It is possible to develop such a method of tetrazolium test for cotton also. As the seed coat is impermeable to tetrazolium, seeds having high seed coat integrity will remain unstained. At the other end, seeds with low seed coat integrity will get stained (Fig. 5). Therefore, we used the uptake of tetrazolium salts by the embryo to assess the permeability of the testa. The embryo and the aleurone layer stain red upon entry of the tetrazolium solution into the viable seed, but stay whitish when the dye does not penetrate (82).

SEM

Common microscopic techniques that rely on taking serial sections include traditional transmission electron microscopy (TEM) and scanning electron microscopy (SEM). These methods give a detailed view of the internal structure of seeds, enabling detailed observation of cell morphology and distribution (83). The permeability of seed coats to water is a critical factor influencing seed germination. SEM studies have shown that the seed coat structure plays a significant role in water uptake. For example, the seed coat of *Tilia miqueliana* was found to be a barrier to water due to its complex multilayer structure, which also protects the embryo and controls water uptake (84, 85). In *Sapium sebiferum*, SEM revealed that cracked seeds absorbed water more quickly than intact seeds, indicating that the seed coat delays water uptake but is permeable (86). Similarly, *Gleditsia sinensis* seeds have a water-impermeable seed coat and SEM images showed that hot water treatment could break this barrier, facilitating water entry through the micropyle (87). The study identified various chemical components, including cellulosic material and phenolic compounds, in different layers of the seed coat. In another study, the mercerized *Moringa*

Fig. 5. Unstained seed.

oleifera seed coat was characterized using SEM and FTIR, showing that the adsorption of Congo red dye occurred through filled holes and changes in functional groups on the seed coat surface (88).

Gas chromatography mass spectrometry (GC-MS)

GC-MS is suitable for identifying and measuring individual components in complex mixtures, both qualitatively and quantitatively. Peak heights or areas under the peaks indicate the amount of each component (89). GC-MS has played a key role in analyzing metabolites in various cotton varieties. An analysis using untargeted GC-TOF/MS found 263 metabolites among 705 peaks in a study comparing the metabolomes of three different cotton varieties. Notable variances were observed in the levels of amino acids, carbohydrates, organic acids, flavonoids and lipids across the different cultivars. Remarkably, the levels of catechin showed an inverse relationship with different fatty acids, indicating an intricate biochemical connection in cotton seed (90). GC-MS analysis of cotton seed oil found 25 chemical compounds, some of which exhibit antibacterial, anticancer and anti-inflammatory effects. The oil's physicochemical parameters fell within acceptable ranges, suggesting it is suitable for both consumption and a variety of industrial uses (91). GC-MS studies in cotton have provided valuable insights into metabolite profiling, volatile organic compound analysis and cotton seed oil composition. The previous study conducted and analyzed phytochemicals, revealing that (Z,Z,Z)-9,12,15-Octadecatrienoic acid has the highest peak area percentage (92). Palmitic acid and linoleic acid ethyl ester were also identified. The study also found c-Sitosterol, c -Tocopherol and trace amounts of Vitamin E.

Inductively coupled plasma mass spectrometry (ICP-MS)

A study using ICP-MS and ICP-AES to examine the contents of heavy metals and microelements in transgenic cotton seeds discovered that most microelements, including B, Na, Si, P, K, Ca, Mn, Co, Ni, Zn, Se and Mo, were found in lower concentrations in transgenic cotton seeds than in regular cotton seeds non-GM counterparts. However, transgenic seeds contained more magnesium, iron and copper. Furthermore, heavy metals such as Al, As, Cd, Sb, Tl, Pb and Bi were identified in greater amounts in transgenic seeds, except for Cr and Hg, which were lower (93). Laser ablation ICP-MS (LA-ICP-MS) was employed to study the uptake and accumulation of Cd, Cu, Fe and Mn in sunflower seeds. The study highlighted the translocation of Cd to the seeds, particularly in the cotyledons and its impact on the homeostasis of essential micronutrients. This method proved effective in bioimaging and quantifying metal distribution, emphasizing the importance of evaluating metal translocation in plants grown in contaminated soils (94). A previous research conducted and used ICP-MS to determine the concentration of 15 elements in sesame seeds from different countries (95). Multivariate analysis successfully discriminates the origin of the seeds, demonstrating the method's effectiveness in provenance determination. These methods are summarised in Table 2.

Conclusion

Acid delinting continues to be the most widely used method in commercial cotton seed processing, primarily due to its effectiveness in removing lint, improving seed flowability and facilitating uniform planting. In most cases, its use is unavoidable despite the potential side effects. Although post-treatment washing and neutralization are commonly practiced, structural and biochemical alterations to the seed coat often persist. Existing literature confirms that acid delinting compromises both the physical integrity and chemical makeup of the seed coat, leading to measurable effects on imbibition patterns, germination and early seedling performance. However, while adverse outcomes such as abnormal seedling development have been observed, the direct causal link between these physiological issues and specific types of seed coat damage has not been fully established. This gap highlights the need for more targeted research to understand how chemical delinting affects individual seed coat layers and overall seed quality.

Advanced analytical techniques such as Scanning Electron Microscopy (SEM) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) have played a crucial role in characterizing these seed coat changes at microscopic and molecular levels. Among these, SEM has proven especially

Table 2. Comparative overview of rapid seed vigor testing methods

Method	Cost	Speed	Accuracy	Accessibility	Key Notes	References
EC (Electrical Conductivity)	Low	~24 hr	Moderate	High	Ideal for bulk testing; reflects membrane integrity loss	(77, 96)
TZ (Tetrazolium)	Moderate	~18 hr	High (viability)	Medium	Indicates seed viability; interpretation requires expertise	(71, 96)
SEM (Scanning Electron Microscopy)	High	Few hours	Very High	Low	Visualizes seed coat microstructure; requires lab facilities	(97)
GC-MS (Gas Chromatography-	Very High	Few days	Very High	Low	Identifies seed metabolites and biochemical changes	(98)
ICP-MS (Inductively Coupled-Plasma MS)	Very High	Few days	Very High	Low	Detects mineral and heavy metal profiles; expensive	(99)

effective for examining the microstructural features of the seed coat and assessing potential damage caused by the corrosive nature of acid treatment. SEM provides valuable insights into how such damage may influence water absorption and physiological performance. However, the high cost of SEM restricts its routine use to well-equipped research institutions and seed industries.

The extent of damage caused by acid delinting is influenced by factors such as acid concentration, exposure duration and cotton genotype. These findings carry important implications for seed producers, who must carefully balance delinting efficacy with seed quality preservation. Going forward, the development of cost-effective and reliable vigor assessment tools supported by advanced imaging and chemical analysis techniques is essential for maintaining seed quality during storage and improving crop establishment and productivity in the field.

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Authors' contributions

The conceptualization was performed by ARO and VV. The draft of the manuscript was written by ARO and VV. Review and editing were performed by UR, SN and PB. RJ and JR supervised the review process. All authors read and approved the final manuscript.

Compliance with ethical standards

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