

## Chapter 2

# Effect of Seed Traits on Seedling Establishment of Sorghum (*Sorghum bicolor L.*)

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## Abstract

Sorghum (*Sorghum bicolor L.*) is a major cereal crop of the semi-arid tropics where successful crop production strongly depends on seed quality and rapid, uniform seedling establishment. This review synthesizes current research on sorghum seed biology and early seedling development. Considerable genetic variability has been reported among sorghum genotypes with respect to seed morphological traits, biochemical composition, and vigour-related parameters. Seedling establishment is significantly influenced by abiotic stresses, particularly soil crusting, drought, salinity, and temperature extremes. Several early seedling traits have been identified as important contributors to stress tolerance and are increasingly used as selection criteria in breeding programmes. The need to identify, develop, and deploy stress-tolerant genotypes is highlighted as a key strategy for improving sorghum performance under resource-limited environments. The review also discusses agronomic and seed management practices that enhance germination, emergence, and stand establishment, thereby contributing to improved productivity in semi-arid regions.

**Keywords:** Sorghum, Seed traits, Seed chemical composition, Genetic improvement, Seedling establishment, Seed germination.

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## Introduction

Sorghum is an important food grain in the arid and semi-arid regions of the world and is widely utilized for various food preparations such as bread, beverages, biscuits, and other products. The productivity of sorghum depends strongly on agronomic practices. A sowing rate of 200,000–250,000 seeds  $\text{ha}^{-1}$  has been reported to be effective for weed suppression; however, seed germination tends to decrease with increasing sowing rates. Optimal sowing density improves germination and enhances a crop's competitive ability against weeds.

## Reviews under study

Grigorov (2000) reported that nitrogen application did not significantly affect the number of seeds per panicle, although substantial yield increases were obtained with nitrogen application up to 100 and 150 kg  $\text{N ha}^{-1}$  for May and June sowings, respectively. A study on *Sorghum bicolor* cv. CO 26 during the post-rainy season of 1996–97 at Coimbatore, Tamil Nadu, indicated that a spacing of 45 × 20 cm produced the highest yield per unit area (7002 kg  $\text{ha}^{-1}$ ). Spacings of 45 × 15 and 45 × 20 cm recorded the highest germination percentages (87% and 86%, respectively) and vigour index values (2407 and 2955, respectively) [1].

A healthy seed with strong genetic constitution and resistance to insects and diseases remains a primary objective for plant breeders. Seedling growth and vigour are inherent seed traits that largely determine crop establishment.

Recent studies continue to highlight the importance of planting density and spacing on sorghum productivity and seed quality under varying environmental conditions. Nutrient management—particularly nitrogen and micronutrients—also plays a critical role in enhancing grain yield and maintaining seed physiological quality [2]. The present paper summarises key advances in research on sorghum seeds and seedling establishment.

## Seed Characteristics

Maiti (1999) reviewed the inherent seed and seedling traits of grain sorghum related to adaptation in rainfed agriculture. A large extent of variability exists in seed morphology, including seed coat colour, seed shape, seed size, seed weight, and seed density. Considerable variation is also observed in the quantity of corneous and floury endosperm [3]. Seed vigour and germination are strongly influenced by several seed and seedling traits such as seed size [4], specific gravity, grain hardness, polysaccharide composition, kafirin compositional differences during endosperm development and storage conditions.

Using gas chromatography and mass spectrometry, [5] examined cuticular waxes in wild-type *Sorghum bicolor* and isogenic bloomless (bm) mutants. The amount of cuticular wax per seed dry weight increased significantly from the soft dough to the hard dough stage and then declined thereafter. Analysis of seeds from two leaf-wax mutants identified the first gene mutations affecting seed cuticular wax composition. Total cuticular wax quantities on bm2 and bm4 seed surfaces ranged between 37% and 75% of wild-type levels during development, with bm4 containing greater proportions of short-chain wax constituents throughout development.

Genetic diversity analysis using pools of simple sequence repeat (SSR) markers revealed exceptionally high diversity among 28 Eritrean sorghum landraces, both in the number and size range of SSR alleles. Individual landraces exhibited high within-population heterozygosity, while between-population diversity was also substantial. Most Eritrean landraces clustered separately from global sorghum accessions, although eight grouped with materials from Ethiopia/Sudan and India belonging to the durra and caudatum races.

Transmission electron microscopy of a sorghum mutant with high in vitro uncooked and cooked protein digestibility showed that alpha-, beta-, and gamma-kafirins were localized within irregularly shaped protein bodies containing numerous invaginations extending towards the central region. These structural features increased surface area for enzymatic action. Conversely, normal cultivars with low digestibility possessed spherical protein bodies lacking invaginations. The increased accessibility of digestive enzymes to alpha-kafirin in the mutant accounted for the higher digestibility. Anatomical studies of maize and sorghum further demonstrated that tube-like channels in the starch granules extended inward towards the hilum, with swelling significantly influencing granule permeability to dye molecules; under swelling, colloidal gold particles filled channels and cavities [6].

In a study on colour interactions, plant colour  $\times$  seed colour effects were non-significant; however, purple plant colour phenotypes exhibited higher cold germination, greater germination after accelerated ageing, and longer seedling elongation at 10 days than tan plant colour phenotypes. The authors concluded that white-seeded, tan-plant colour lines with performance comparable to red-seeded, purple-plant lines can be effectively selected from segregating populations.

Cluster analysis using agronomic descriptors grouped 94 sorghum accessions into four of the five major races, while RAPD fingerprinting of selected sorghum races revealed differences among accessions. No single clustering approach based on agronomic descriptors closely matched the grouping obtained by RAPD markers, indicating the need for integrated phenotypic and molecular characterization [7].

For long-term storage, Abdalla et al. (2002) reported that shallow pits, thick chaff-wall linings, and wide surface caps-traditional storage structures used by farmers-were highly effective in minimizing grain deterioration in sorghum.

## Seed Chemical Composition

During sorghum caryopsis development, sucrose is progressively converted to starch, a process coordinated by multiple phosphorylase and transferase enzymes that function at different stages of grain filling and maturation. Studies on chemical composition, dry matter digestibility, and protein quality of major sorghum cultivars from the southern Urals region of Russia revealed low crude protein levels (10.79% in 'Sakharne 32' to 12.2% in 'Kamyshinskoe 75'). Protein solubility was generally low (20–22%), although 'Kamyshinskoe 75' (33.98%) and 'Kamyshinskoe 8' (34.94%) exhibited higher solubility, which corresponded with improved grain quality and superior dry matter digestibility-attributes that support their suitability for animal feed formulations.

Maiti (1999) [8] reported significant variability in seed chemical traits, including total amino acids and proline during germination, soluble protein and esterase isoenzyme profiles in male-sterile sorghums, and hydrolytic degradation of endosperm protein bodies. Water-soluble sugars abundant in stems and leaves before anthesis are translocated to developing grains during filling.

Twenty-three sorghum cultivars-including hybrids, parental lines, and inbreds-were characterized using seed and seedling morphology, esterase isoenzymes, and SDS-PAGE profiles. Two diagnostic keys based on morphological and biochemical traits enabled precise varietal identification. High-CO<sub>2</sub> storage environments did not significantly affect germination or seed viability in soybean or sorghum under varying temperature and moisture conditions.

Water-unextractable solids (WUS) isolated from maize seeds contained 7% protein, 8% starch, and 57% non-starch polysaccharides (NSP), mainly glucose-xylose-arabinose-glucuronic acid polymers. Sequential Ba(OH)<sub>2</sub> extraction indicated highly substituted glucuronoarabinoxylans resistant to endoxylanase activity developed a free zone capillary electrophoresis (FZCE) method capable of separating hydrophobic storage proteins of maize and sorghum using acetonitrile-rich buffers at low pH, providing effective genotype differentiation.

Biochemical characterization and cloning of the  $\alpha$ -kafirin gene demonstrated that lysine-rich cultivars (IS 21702, CVS 365, G 1058, G 205, CVS 549) contain ~25% less kafirin and increased alcohol-insoluble glutelin fractions, without altering total protein content. SDS-PAGE revealed the absence of 25.3- and 25.9-kDa  $\alpha$ -kafirin proteins in these genotypes relative to the low-lysine cultivar White Martin. A genomic clone ( $\lambda$ GK5) encoding  $\alpha$ -kafirin was sequenced from White Martin.

Electrophoretic profiling (SDS-PAGE) of 12 forage sorghum varieties showed distinct banding patterns (5–70 kDa), enabling reliable varietal differentiation except in HC-171 and HC-308, which shared identical profiles [9]. A highly branched  $\alpha$ -D-glucan (PSa glucan) with an apparent molecular weight of  $6.85 \times 10^4$  was isolated from *Sorghum arundinaceum* seeds; structural analyses indicated extensive  $\alpha$ -(1 $\rightarrow$ 6) and  $\alpha$ -(1 $\rightarrow$ 3) branching, and the compound demonstrated anti-inflammatory activity in capillary permeability assays.

Recent studies confirm continued interest in sorghum grain chemistry. Modern genomic and metabolomic analyses have revealed significant diversity in kafirin structure and its association with grain hardness, protein digestibility, and food quality. Advances in seed proteomics also highlight the importance of stress-responsive proteins and antioxidants during germination under water-deficit and salinity conditions [10, 11]. These findings complement earlier biochemical studies and reinforce the complex regulation of sorghum seed composition.

## Seed Dormancy

Seed dormancy plays a critical role in regulating germination behaviour in sorghum. Fraga (1982) comprehensively described the causes and types of dormancy, the substances that promote or inhibit germination, and various methods for breaking dormancy in sorghum. Only limited research has been conducted on sorghum dormancy [12], with early studies evaluating dormancy-breaking techniques such as low-temperature treatment [13] and mechanical scarification [14].

The plant hormone abscisic acid (ABA) is known to play a central role in the induction and maintenance of seed dormancy across several species. In sorghum, the degree of dormancy in developing seeds is strongly associated with ABA-mediated inhibition, counterbalanced by the promotive effects of gibberellins (GAs). Genetic analyses using quantitative trait loci (QTLs) have indicated that the transcription factor Vp1 contributes to determining dormancy levels, similar to its role in maize. It has been shown that neither GA content nor embryo sensitivity to GAs differs significantly between high- and low-dormancy genotypes. This supports the hypothesis that variation in embryo sensitivity to ABA is a key regulatory point governing the dormancy status in different sorghum varieties. Additionally, other dormancy-related mechanisms independent of ABA may also contribute and require further investigation [15].

The Vp1 gene, which is seed-specific and involved in the regulation of dormancy and germination, has been studied in sorghum. The complete sequence of the sorghum Vp1 promoter and enhancer region has been characterized, revealing elements responsive to ABA and light [16].

Pre-harvest sprouting (PHS) constitutes a major constraint in humid environments, leading to grain deterioration and reduced quality. Extensive variation among sorghum genotypes has been documented for traits associated with PHS susceptibility, including germinability, water uptake, and electrical conductivity during seed development. These variations indicate significant potential for selecting PHS-resistant lines. A molecular linkage map developed from an  $F_2$  population derived from IS 9530 (PHS-resistant)  $\times$  Redland B2 (PHS-susceptible) using 112 markers identified two major QTLs associated with PHS resistance, with LOD scores of 8.77 and 4.39. The findings also suggest a putative relationship between PHS resistance in sorghum and the maize Vp1 gene [17].

PHS in sorghum is primarily attributed to insufficient dormancy during seed development and maturation. Using a PCR-based approach, *Sorghum bicolor* genomic and cDNA clones of vp1 were isolated from two contrasting genotypes differing in PHS response and ABA sensitivity. The predicted 699-amino-acid proteins differed at two positions-341 (Gly/Cys within the repression domain) and 448 (Pro/Ser)- and exhibited over 80%, 70%, and 60% similarity to maize, rice, and oat Vp1 proteins, respectively. Expression analysis revealed slightly higher levels of vp1 mRNA in embryos of PHS-susceptible lines than in resistant lines during embryogenesis, with temporal differences in expression patterns. Under favourable germination conditions and in the presence of fluridone (an inhibitor of ABA biosynthesis), vp1 mRNA levels were correlated with ABA sensitivity but not with ABA content or dormancy [16].

Teo-Sherrell and Mortensen (2000) evaluated seedling emergence, dormancy status, and survival of *Sorghum bicolor* seeds buried in soil in the Midwestern United States. They observed that many seeds displayed signs of cold damage; however, burial in soil reduced cold-induced mortality due to the protective buffering provided by soil.

## Seed germination

Seed germination in sorghum is influenced by a range of environmental and physiological factors. According to [8], both seed imbibition and germination are modulated by environmental conditions. Considerable variability among sorghum genotypes has been reported in terms of water uptake efficiency [18–20], largely dependent on the nature of seed–water contact [21]. Spacing also plays a significant role, with wider spacings producing higher germination percentages than closer spacings [22]. Recent studies further confirm that seed hydration kinetics and microenvironmental interactions remain important determinants of germination uniformity in cereals, including sorghum.

Gibberellins (GAs) play a central role in promoting germination, and it has been suggested that de novo GA synthesis is essential following imbibition to initiate germination. Increased GA biosynthesis is considered a key mechanism through which environmental stimuli, such as light, promote germination. During the GA biosynthetic pathway, oxidation at C-20-catalyzed by GA 20-oxidases is regarded as a regulatory step. A cDNA clone encoding GA 20-oxidase (SbGA20ox) was isolated from sorghum embryos, and its expression was detected early after imbibition in seeds with low dormancy. Changes in endogenous GA<sub>4</sub> levels were found to correlate closely with SbGA20ox mRNA abundance, suggesting that GA production may be regulated at the transcriptional level of this gene. Enhanced expression of SbGA20ox was detected in incubated embryos regardless of dormancy level, indicating that surrounding seed tissues exert physiological control on its expression. Furthermore, abscisic acid (ABA) was shown to suppress transcription of this gene [23]. Recent molecular investigations support the role of GA–ABA cross-talk in regulating dormancy release and germination in sorghum and other cereals.

Seed sanitation treatments can also influence germination performance. Hypochlorite exposure significantly reduced the germinative energy of grains, while treatment with 1% formaldehyde or sodium hypochlorite decreased diastatic power, free amino nitrogen, and reducing sugars. Emerging evidence likewise indicates that chemical sanitizers can alter enzyme activity and carbohydrate mobilization during cereal grain germination.

Kader and Jutzi [24] examined the impact of temperature, seed treatment, storage conditions, and osmotic stress on primed and untreated sorghum seeds. Their findings revealed that imbibition rates were consistently higher in untreated seeds than in primed seeds after 24 hours of soaking. Additionally, increases in priming temperature enhanced imbibition to a greater extent in untreated seeds. Recent studies validate that osmoprimer and haloprimer strategies can improve germination vigour and stress tolerance in sorghum under variable climatic conditions [25].

Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) synthesis occurs in de-embryonated sorghum seeds through interactions among metabolites and post-translational enzyme regulation via phosphorylation. During germination, two PEPC polypeptides (108 and 110 kDa), identified through SDS–PAGE, showed increasing abundance corresponding with rising PEPC activity. Sodium chloride (NaCl) severely inhibited both germination and PEPC accumulation and phosphorylation, though PEPC kinase (PEPCK) content remained unchanged. In vitro, NaCl did not directly affect PEPCK or PEPC phosphorylation. Conversely, L-malate inhibited PEPCK activity in vitro. NaCl also reduced L-malate consumption rates in imbibing seeds, suggesting that salt inhibition of PEPC phosphorylation results from concentration-dependent suppression of PEPCK activity in vivo. Further, germination and PEPC phosphorylation were inhibited in seeds germinated in the presence of L-malate, despite unaltered PEPCK levels. Contemporary research corroborates the involvement of PEPC regulation in early seed metabolic reactivation under stress conditions in C<sub>4</sub> cereals [26].

## Seedling Establishment

In field experiments, fertilization of the parental crop with up to 120 kg N + 80 kg  $P_2O_5$   $ha^{-1}$  resulted in seeds with higher protein content, lower electrolyte leakage even seven months post-harvest, and enhanced seedling root and shoot growth. Such seeds produced a field emergence rate of about 90%, whether derived from the 120 kg N + 80 kg  $P_2O_5$  regime or from 80 kg N + 40 kg  $P_2O_5$   $ha^{-1}$  [27].

A visual scoring method has been established to screen genotypes for seedling vigour; vigour correlates with rapid emergence and early drought tolerance. Field data indicate that sowing seeds with reduced viability significantly lowers seedling vigour and ultimately reduces yield [28].

Establishment of sorghum in semi-arid tropic (SAT) environments is often hampered by abiotic stresses such as dry sowing, variable planting depth, limited moisture, high soil temperatures, soil salinity, and surface crust formation. To mitigate these constraints, many researchers have developed screening procedures to identify genotypes resilient under such stress, and to uncover physiological, biochemical, and molecular bases of stress resistance.

Considerable genotypic variation exists in seed and seedling traits across sorghum germplasm under different stress conditions-offering opportunities to breed for improved stress tolerance. For instance, genotypes differ in their capacity to emerge when planted deep [29, 30], to penetrate soil crusts [31, 32], and to tolerate high soil temperatures [3, 32, 33]. Tolerance to drought and salinity [34] has also been reported. Adaptation to dry sowing [35], as well as combined stress tolerance (drought + salinity) [3, 34, 36] has been observed in certain genotypes. Glossy-seeded sorghum lines additionally show variability in resistance to both biotic and abiotic stressors [3, 37].

Given this diversity, improving breeder's lines for establishment-related traits should remain a key objective in sorghum breeding programs [3, 38]. Earlier reviews summarized the inherent seed and seedling traits underlying sorghum's adaptation to SAT conditions [8]. Subsequent work has continued to explore sorghum's responses to drought, temperature extremes, and salinity stress [38].

Recent studies reinforce and expand these findings. For example, a study on 22 fodder sorghum genotypes showed that polyethylene glycol (PEG)-induced drought stress significantly reduced germination percentage, seedling length, and seedling vigour index-with substantial genotypic variation in drought tolerance [39]. Another recent investigation demonstrated that osmotic stress during early seedling development can be effectively used to screen sorghum germplasm for stress tolerance [40].

Moreover, an integrative physiological and transcriptomic study comparing drought-tolerant and drought-sensitive sorghum genotypes revealed marked differences in stress-response pathways during seedling growth, including hormone signaling, carbohydrate metabolism, and antioxidant defense mechanisms (e.g., for osmotic adjustment and reactive oxygen species scavenging) [41]. In saline and saline-alkaline soils with physical soil crusts, seed priming with Abscisic Acid (ABA) was recently shown to significantly improve emergence and early growth of *Sorghum bicolor*, by reprogramming hormonal balance and enhancing metabolic resilience [42].

Thus, combining traditional agronomic practices (e.g., optimized fertilization, seed quality maintenance) with newer approaches (osmopriming, stress screening, molecular-based selection) offers promising pathways to develop sorghum lines with reliable seedling establishment under diverse and stressful agro-climatic conditions.

## Low Temperature Effects

Cold temperatures markedly influence germination and early seedling growth in *Sorghum bicolor*. In one study using randomized complete block design (RCBD) with standard germination tests, cold tests, accelerated ageing, and electrical-conductivity assays, sorghum lines KFS1, KFS2 and KFS4 differed significantly in seed vigour, field emergence percentage, and forage seedling fresh and dry matter yields under low-temperature regimes [43].

Another investigation evaluated 12 diverse sorghum lines and their hybrids (with two testers) for germination and emergence under low soil temperatures (20–30 °C in a greenhouse and 15 °C in a growth chamber). While under optimal temperature there were significant genotypic differences in total germination percentage and early shoot growth rate, these differences were absent under cold conditions. The authors concluded that selecting cold-tolerance traits in seed parents (rather than male parents) would be more effective [44].

Subsequently, the same authors developed rapid screening protocols for cold tolerance in sorghum seed germination and early growth [45]. In a related study, ten genotypes of different genetic backgrounds were subjected to seven germination temperatures (4–25 °C) combined with two treatments: control and osmopriming (250 g  $L^{-1}$  PEG at 15 °C for two days). Osmopriming substantially improved germination percentages at low temperatures (8–10 °C), effectively lowering the base temperature required for germination by almost one degree compared with untreated seeds. The observed genotypic differences in base temperature suggest potential for breeding cold-tolerant sweet and fibre sorghum varieties [46].

These earlier findings have been further reinforced by more recent research. A comprehensive evaluation of 257 grain-sorghum accessions under cold stress (10 °C for germination; 4 °C for seedling survival) identified a subset of lines with high cold tolerance, based on germination rate, seedling survival rate, and seed quality traits (fat and protein content) [47]. Similarly, a 2025 study screening 71 sorghum germplasm accessions from the Chishui River Basin demonstrated substantial variation for low-temperature germination, seedling emergence, and agronomic performance under field chilling conditions. These results confirm that existing germplasm contains valuable cold-tolerant genotypes.

At the genetic level, a genome-wide association study (GWAS) with a diversity panel of 194 biomass sorghum lines revealed a quantitative trait locus (QTL) on chromosome Sb06 that is significantly associated with improved seedling emergence under chilling conditions. Heritability estimates were high ( $h^2 = 0.87$  for field emergence,  $h^2 = 0.93$  for controlled environment seedling survival), supporting the feasibility of selecting cold-tolerant germplasm (recent GWAS study, 2025). Moreover, physiological and biochemical studies have shown that cold stress triggers oxidative stress in sorghum seedlings, but treatments such as low-dose ethanol application can mitigate damage-improving antioxidant enzyme activity, osmolyte accumulation, and overall seedling performance under low temperature.

## Soil Moisture

Soil moisture deficit has a substantial influence on seedling emergence and early growth in sorghum [38]. Under progressive moisture stress, the reduction in shoot elongation has been found to be more pronounced than that of root elongation, across all levels of soil moisture deficit.

This differential growth response indicates that sorghum seedlings tend to invest more in root proliferation under drought, which serves as a major drought-avoidance mechanism. Consequently, traits such as percentage germination, seedling shoot dry mass, specific root length, and leaf area have been proposed as reliable selection criteria for evaluating seedling-stage drought tolerance in sorghum [48].

## Salinity

Pre-exposure of sorghum seeds to sublethal salinity, such as 150 mM NaCl, has been shown to enhance their subsequent salt-adaptation capacity. Mixing seeds of different sorghum genotypes further improved this adaptive response. The findings suggest that root-released chemical signals may regulate the physiological mechanisms underlying salt adaptation in *Sorghum bicolor*, implying a possible relationship between allelopathic interactions and internal developmental regulation [49].

## Fertilizer

Germination percentage, seedling growth, and dry matter accumulation showed a progressive decline with increasing concentrations of industrial effluent. The highest germination, seedling vigour, and dry weight were recorded at a 10% effluent concentration. While undiluted effluent exhibited a strong inhibitory effect on seedling development, the 10% concentration demonstrated a growth-promoting influence that was significantly superior to the control. These findings suggest that effluent can be safely utilized for irrigation after appropriate dilution, thereby contributing beneficially to crop cultivation [50].

## Cyanogenic Materials

Germination and kilning substantially influence the cyanogenic potential, amylase activity, and alcohol production capacity of sorghum malts used in the preparation of the traditional beverage buruktu. In an evaluation of red and white grain sorghum varieties, grains were steeped for 18 hours, germinated for five days, and kilned at 50°C before assessing amylase activity, cyanogenic potential, and fermentation characteristics. Kilning at 50°C reduced enzymatic activity in both varieties. Dhurrin content increased during germination in both red and white malts but was consistently higher in the white variety, where evidence of dhurrin mobilisation was observed. In red malts, dhurrin increased during germination but declined progressively after kilning. Buruktu produced from red malts resulted in higher alcohol content than that produced from white malts.

The concentration of the cyanogenic glucoside dhurrin in *Sorghum bicolor* varies with plant age and environmental conditions. Dhurrin synthesis is regulated at the transcriptional level and is strongly influenced by nitrogen fertilisation in older plants. Cyanogenic potential is highest shortly after germination, a developmental stage at which nitrogen application has no measurable effect on dhurrin content. However, in mature plants, nitrogen supplementation significantly elevates dhurrin levels. Across all growth stages, dhurrin concentration correlates closely with the activity, protein abundance, and transcript levels of the key biosynthetic enzymes CYP79A1 and CYP71E1. The lower activity of CYP79A1 relative to CYP71E1 during development suggests that CYP79A1 catalyses the rate-limiting step in dhurrin synthesis. The site of dhurrin biosynthesis shifts from leaves to the stem as the plant matures. Overall, the findings indicate that dhurrin accumulation in sorghum is primarily governed by transcriptional regulation of CYP79A1 and CYP71E1 [51].

## Storage Atmosphere Effects

Studies on germination and seed viability in soybean and sorghum indicate that elevated CO<sub>2</sub> concentrations in the storage atmosphere do not adversely affect seed quality to the same extent as high temperature or increased seed moisture content during storage. Thus, temperature and moisture remain the dominant factors influencing seed deterioration compared to CO<sub>2</sub> levels.

## Waterlogging

Waterlogging conditions resulted in the lowest germination percentage, accompanied by an increase in potassium leakage from seeds. Conversely, the concentrations of total and reducing sugars in seed leachates decreased with longer imbibition periods. A significant negative correlation ( $P < 0.05$ ) was observed between germination percentage and electrical conductivity, indicating that electrical conductivity can serve as a reliable indicator of germination ability in sudangrass. The study demonstrated that germination is severely affected when seeds are exposed to waterlogging prior to imbibition, and that electrical conductivity of seed leachates is an effective tool for assessing germination potential [52].

Recent studies further support that early seed metabolic leakage and ion conductivity parameters are reliable predictors of seed vigour under hypoxic stress in cereals [53].

## Herbicide

A study evaluating the influence of endosulfan and methyl-parathion on hydrolytic enzyme activities during germination revealed that lower concentrations of endosulfan (0.05–0.1% v/v) stimulated alpha-amylase, protease, acid phosphatase, and alkaline phosphatase activities. In contrast, methyl-parathion treatments suppressed alpha-amylase activity but substantially increased protease activity at lower doses. Overall, methyl-parathion induced greater osmotic stress during sorghum seed germination compared with endosulfan.

Another study reported the responses of four glossy sorghum lines (IS-8311, IS-4473, IS-1096, and IS-4476) and a non-glossy line (SR-224; control) to 2,4-D during germination, seedling growth, and callus development. Although the herbicide generally inhibited seedling and callus growth, the glossy line IS-8311 exhibited superior tolerance, as confirmed through transmission electron microscopy, which showed a higher abundance of cellular organelles under herbicide stress. These findings indicate that IS-8311 possesses enhanced tolerance to 2,4-D at the early seedling stage [54]. Recent advancements have shown similar genotypic differences in herbicide tolerance among sorghum hybrids, linked to differential antioxidant enzyme responses [55, 56].

## Allelopathy

*Sorghum bicolor* exhibits strong allelopathic potential, reducing germination and yield of succeeding crops such as peanut (*Arachis hypogaea*) [57]. Sorghum sap demonstrated species-specific and concentration-dependent effects on the germination of wheat and certain weeds including *Avena fatua*, *Phalaris minor*, *Convolvulus arvensis*, and *Chenopodium album*. Lower sap concentrations (25%) promoted germination in *P. minor*, *C. album*, and wheat, whereas higher concentrations inhibited germination across all tested species [58].

Seedling length in sorghum increased significantly following application of *Acacia arabica* extracts. Moreover, leaf extracts from *A. arabica*, *Samanea saman*, and *Azadirachta indica* at 5% and 10% concentrations enhanced the vigour index. Conversely, extracts of *Eucalyptus tereticornis* and *Syzygium cumini* reduced vigour index and seedling dry matter [1].

Shoot and root growth of sorghum cultivars were reduced when subjected to increasing concentrations of *Fusarium moniliforme*, with early-stage growth reduction not compensated at later developmental stages [59].

Additionally, the phytotoxic compound clerod-14-ene-3 $\alpha$ ,4 $\beta$ ,13 $\zeta$ -triol, isolated from *Viguiera tucumanensis*, inhibited germination and root growth in *Sorghum halepense* and *Chenopodium album*, with mild inhibition also observed in *Ipomoea purpurea*. Some crop species showed similar inhibitory effects. Recent research highlights the role of sorgoleone and phenolic acids as major allelochemicals in sorghum-based weed suppression strategies [60].

## Fungicide

Seed treatment with captan and thiram at concentrations of 0.5, 1, and 2 g a.i./kg completely inhibited macroconidium germination of one *Colletotrichum* isolate, while reducing germination of another isolate to less than 0.2%. Thiram applied to honeydew-coated sorghum seeds completely inhibited macroconidium germination at a rate lower than the currently registered dosage (2.4 g a.i./kg) for sorghum seed treatment in Australia. These findings have important implications for the safe movement of sorghum seed from regions where *Colletotrichum africana* is endemic to pathogen-free areas.

Recent studies have confirmed that fungicidal seed treatments remain an effective strategy for controlling *Colletotrichum* spp. in sorghum, while also reducing post-harvest seed infection and enhancing seedling vigour [61]. Additionally, novel formulations combining thiram with biocontrol agents have shown improved efficacy against anthracnose in sorghum under field conditions [62].

## Soil Management for Seedling Establishment

The soil environment within the seedling zone plays a critical role in successful seedling establishment and early growth of sorghum. Key factors influencing seedling establishment include:

**Soil moisture:** Sorghum seeds should ideally be sown at a depth of approximately 5 cm within the moist soil layer to ensure optimal germination. If the seedling zone is dry, post-sowing irrigation is recommended to support uniform seedling emergence and establishment [38, 62].

**Seed-soil contact:** Poor seed-soil contact in lighter soils under receding moisture conditions can adversely affect seedling emergence. Post-sowing compaction using press-wheel attachments in seed drills improves seed-soil contact and enhances germination [48, 63].

**Soil crust formation:** Surface crusting, resulting from soil physical and chemical processes after wetting and rainfall, can significantly impede seedling emergence. The incidence of crusting can be minimized through the application of organic manures, mulching with crop residues, and ridge-and-furrow seedbed preparation. In severe cases, mechanical crust breakers can be employed to facilitate seedling emergence [31, 64].

**Soil temperature:** Extremely high or low soil temperatures at sowing can hinder seedling establishment. Maintaining higher soil organic matter and using organic mulches can moderate soil temperature fluctuations, thereby improving seedling survival and growth [3].

**Soil aeration:** Poorly drained or waterlogged soils reduce soil aeration, which adversely affects seedling establishment. Improving surface drainage, employing raised bed planting, or using the broad bed and furrow (BBF) system can enhance soil aeration and promote uniform seedling establishment [48, 62].

Recent studies have further highlighted the importance of integrated soil management practices, including conservation tillage, residue retention, and precise irrigation scheduling, in enhancing sorghum seedling establishment under varying agro-climatic conditions [2, 65].

## Conclusion

The research on seed and seedling traits in grain sorghum demonstrates substantial variability among genotypes, particularly in traits related to adaptation under multiple abiotic stresses prevalent in the semi-arid tropics. This inherent genetic diversity provides significant opportunities for the selection and improvement of desirable seed and seedling traits that enhance tolerance to individual or combined stress factors such as deep sowing, soil crust formation, elevated soil surface temperatures, drought, and salinity. Certain seedling characteristics, including high seedling vigour, mesocotyl elongation, and glossy-trichome traits, have been consistently associated with multi-stress resistance. These traits represent valuable targets for sorghum breeding programs aimed at developing stress-resilient cultivars suitable for challenging agro-climatic conditions [8, 38].

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