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REGISTRATION

Germplasm

Registration of soybean germplasm DS1260-2, with improved tolerance to mature seed damage and Phomopsis seed decay

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Abstract

Damage to mature soybean [*Glycine max* (L.) Merr.] seed occurs when mature seeds are subjected to weathering, fungi, and insects under hot humid conditions. Such damage can be exacerbated by delays in harvest. Mature seed damage (MSD) causes lost revenue to both producers and processors, as well as lower quality of the seed, protein meal, and oil to consumers. The release of DS1260-2 (Reg. no. GP-531, PI 705148) by the USDA-ARS is part of our effort to increase soybean tolerance to mature seed damage using traditional plant breeding. Tolerance to MSD was derived from exotic accession Huang mao bai shui dou (PI 587982A) and incorporated through pedigree selection into an agronomically improved conventional late maturity group IV germplasm adapted for production in the midsouthern United States. DS1260-2 has significantly lower levels of seed damage than cultivars 'P46T59R', 'AG4632', and 'P48A60X', which manifests as lower incidence of *Diaporthe longicolla* (Hobbs) J.M. Santos (Syn. *Phomopsis longicolla* Hobbs), less seed coat wrinkling and visual mold, lower incidence of fungal metabolites (nivalenol, cercosporin, cytochalasins H and J, tryptophol, fusaric acid, and beauvericin), and higher seed germination. DS1260-2 yielded similar to P46T59R in trials over 4 years in Mississippi, but less than 'AG46X6', 'AG48X9', and 'S16-7922C' in regional

Abbreviations: DKT, damaged kernels total; FGIS, Federal Grain Inspection Service; HT, heat-damaged kernels; MDK, mold-damaged kernels; MG, maturity group; MSD, mature seed damage; PMS, purple mottled or stained; PSD, Phomopsis seed decay; PSS, purple seed stain; RCBD, randomized complete block design; SKD, stinkbug damaged; SUST, Soybean Uniform Test, Southern States.

testing. DS1260-2 is resistant to southern stem canker, frogeye leaf spot, and race 3 (HG type 0) of soybean cyst nematode. DS1260-2 is a valuable source for developing cultivars with improved tolerance to the MSD that is caused by mold and weathering.

1 | INTRODUCTION

Mature seed diseases, such as Phomopsis seed decay (PSD) caused by *Diaporthe longicolla* (Hobbs) J.M. Santos (Syn. *Phomopsis longicolla* Hobbs) and elevated temperatures near the times of senescence through harvest, can damage soybean [*Glycine max* (L.) Merr.] seed (Bellaloui et al., 2017; Chebrolu et al., 2016; Egli et al., 2005a, 2005b; Gillman et al., 2019; Keith & Delouche, 1999; Li, 2011; Li et al., 2015; Mengistu et al., 2010). Such damage can render seed unacceptable for marketing as grain and unfit for future plantings. Mature seed damage (MSD) is exacerbated by weather conditions that delay harvest and expand the exposure of seeds to diseases and insects, especially to the insect species brown stink bug (*Euschistus servus* Say), green stink bug (*Chinavia hilaris* Say), southern green stink bug (*Nezara viridula* Linnaeus), and redbanded stink bug (*Piezodorus guildinii* Westwood) (Greene and Davis, 2015). Delays in harvest allow seed to weather (Pinheiro et al., 2021), rot, and even precociously germinate. In severe cases, fungi such as *Fusarium* spp. produce toxins that can be harmful to humans and farm animals (Hagler et al., 1989; Jacobsen et al., 1995; Trempus et al., 1989). Damaged seed may be punctured by stink bug feeding and/or appear shriveled, wrinkled, elongated, cracked, moldy, and discolored with shades of tan, green, or purple (Bellaloui et al., 2017). Damaged seed may have no visible symptoms, yet still be unable to germinate due to impermeable seed coats (Kebede et al., 2014), or from non-obvious physiological damage (Egli et al., 2005a, 2005b). MSD caused economic losses to US farmers in 2001 (Muzzi, 2002) and 2009 (Koenning, 2010), and was extremely damaging in 2017 for midsouthern US growers in Mississippi, Arkansas, Louisiana, and Tennessee due to grain elevator dockages estimated at $32 million (Heatherly, 2018). As the world climate warms and as harvest-rain patterns may change, major losses in soybean production are projected (Yu et al., 2021).

Smith et al. (2008) identified multiple accessions obtained from the USDA soybean germplasm collection at Urbana, IL, with improved tolerance to both elevated temperatures (36°C and 40°C) and PSD. Selected accessions were used from their study to develop germplasm lines with improved levels of tolerance to MSD. One of these accessions, PI 603756, was used to develop DS49-142 (PI 703498, released by USDA in 2023; also called 10049-1-4-2-3-1 in GRIN) and another two accessions, PI 417050 and PI 587982A, were used to develop DS43-72 (also called 11043-225-72 in Li et al. [2023]). PI 587982A was used to develop DS65-1 (Li et al., 2023), DS25-1 (PI 684675, released by USDA in 2017; Chebrolu et al., 2016; Gillman et al., 2019; Krishnan et al., 2020; and Narayanan et al., 2020; and also called 25-1-1-4-1-1 in Bellaloui et al. [2017]), and DS31-243 (PI 700941, released by USDA in 2022; Li et al., 2023). Gillman et al. (2021) recently mapped a major quantitative trait locus (QTL) for resistance to heat-induced seed degradation of soybean in a recombinant inbred line (RIL) population derived indirectly from PI 587982A.

DS1260-2 (Reg. no. GP-531, PI 705148) was developed to maintain the high level of tolerance to MSD found in PI 587982A and its derivatives (DS65-1, DS43-72, DS31-243, and DS25-1), and has substantially higher seed yield compared to the original PI and prior releases. DS1260-2 was released by the USDA-ARS in 2024. It is a late maturity group (MG) IV with indeterminate stem termination, which traits are ideal for optimum seed production in the midsouthern United States. DS1260-2 is useful for developing cultivars with improved tolerance to MSD caused by mold and weathering.

2 | METHODS

2.1 | Parental selection and pedigree

DS1260-2 (also designated as 12060-260-2 and DS1260-260-2) was derived from a single F_5 plant from a cross between DS34-1 (34-3-1-2-4-1 in Bellaloui et al., 2017) × 'LD00-3309' (PI 639740; Diers et al., 2006). DS34-1 is tolerant to heat-induced seed degradation (Bellaloui et al., 2017) and LD00-3309 is a high yielding public cultivar with resistance to Race 3 (HG Type 0) soybean cyst nematode (*Heterodera glycines* Ichinohe) (Diers et al., 2006), but susceptibility to heat-induced seed degradation, reduced standard germination, lower accelerated aging germination, and increased levels of seed wrinkling, green seed damage, and hard seed, relative to DS34-1 (Bellaloui et al., 2017). DS34-1 is derived from the pedigree of DT97-4290 (PI 642055; Paris et al., 2006) × PI 587982A, and LD00-3309 was derived from the pedigree of 'Maverick' (PI 598124; Sleper et al., 1998) × 'Dwight' (PI 597386; Nickell et al., 1998). DT97-4290 is resistant to races 2, 4, and 10 of *Phytophthora sojae* M.J. Kaufmann and J.W. Gerdemann (Paris et al., 2006), whereas PI 587982A is resistant to race 17 (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1524141) and LD00-3309 is susceptible to races 4 and 7 (Diers et al., 2006).

Based solely on pedigree analysis, DS1260-2 has 25% exotic germplasm.

2.2 | Breeding line development

The cross of DS34-1 × LD00-3309 was made at Stoneville, MS, in 2012. In 2013, F_1 plants were harvested from the USDA Tropical Agriculture Research Station at Isabela, Puerto Rico. Derived from one F_1 plant, 301 F_2 plants were harvested in 2013 at Stoneville for the initiation of a recombinant inbred line (RIL) population. The F_3 and F_4 RIL generations were advanced by randomly selecting a single plant from each heterogeneous progeny row at Homestead, FL (27 Farms of Homestead, Inc.) during the winters of 2013–2014 and 2014–2015. Three F_5 plants were selected from RIL number 260 in 2016 at Stoneville based on pod load and lodging resistance. Progeny of plant number 2 were selected for advancement in 2017 at Stoneville based on a standard germination score of 90% germination and zero hard seed using 50 seeds assayed at the State of Mississippi Seed Testing Laboratory (Mississippi State, MS) using official protocols (Association of Official Seed Analysts, 2001). The germination protocol consists of placing seed on moistened germination paper, with two sheets below the seed and one sheet covering the seed. The sheets are rolled and placed in a plastic container with aeration holes in the top, and then the containers are placed in a 20–30°C walk-in germinator for 7 days. The lower temperature is maintained for 16 h a day and then alternates to the higher temperature for 8 hours a day. At the end of 7 days, the numbers of normal seedlings, dead seeds, and hard seeds are recorded. Based on pod load, standability, and uniformity, the $F_{5:7}$ progeny derived from plant number 2 were bulk harvested and named 12060-260-2. The seed was weighed for plot yield and assayed for standard germination and hard seed percentages using 200 seeds as per above. The seed weight (g per plot) from the plot of 12060-260-2 was large (data not shown) relative to the high yielding check LG01-5087-5 (PI 667734). Seed germination for the $F_{5:7}$ seed was only moderate (62% with zero hard seed) due to extended periods of warm wet weather during the fall of 2017 (Heatherly, 2018), but was still substantially higher than the germinations of checks representing the typical susceptible gene pool (LG01-5087-5 had 28% germination and zero hard seed and DT97-4290 had 8% germination and zero hard seed).

2.3 | Breeding line evaluation

2.3.1 | Stoneville irrigated yield trials

DS1260-2 was evaluated over 5 years in yield trials planted at Stoneville, MS, which included four early plantings (April 18, 2018, April 22, 2019, April 5, 2021, and April 27, 2022) and one late planting (June 1, 2020), with the late planting necessitated by unsuitable early-season planting conditions. "Early planting" refers to a production system developed for the midsouthern United States described by Heatherly (1999), Bellaloui et al. (2017), and Smith et al. (2019), where early-maturing cultivars (MGs III, IV, and V) are planted earlier (April and early May) than in the traditional production system that typically planted late-maturing cultivars (MGs VI and VII) later (late May and June). The purpose for changing to the early-production system was to take advantage of early-season rains and to avoid late-season droughts, which typically come each season in August and September. The early-production system increased seed yields, but also increased seed damage, as cultivars were now maturing during the hottest times of the year, when any late-season wet weather could promote mold damage to the seed. Hence, planting our experiments in April (early) increased the chances of mold damage at senescence and maturity. The adoption of the early-production system by a majority of growers necessitated the search for damage-tolerant germplasm.

A randomized complete block design (RCBD) with three replications was used each year. Seed were sown with a machine planter into rows at a depth of 2.5 cm, with a seeding rate of 25 seed m^{-1} of row. Plots (experimental units) consisted of four rows 5.79-m long with a row spacing of 0.91 m. Plots were end-trimmed to 4.88-m long after R5 (Beginning Seed) (Fehr & Caviness, 1977), but before R6 (Full Seed) (Fehr & Caviness, 1977). Furrow irrigation was used to apply water as needed throughout the growing season to alleviate moisture-deficit stress. The timely harvest of seed was completed for each plot shortly after full maturity (R8, Fehr & Caviness, 1977) by cutting and threshing the two middle rows with a small plot combine. A field design was employed that allowed each plot to be timely and directly harvested by combine (Almaco SPC40) to minimize any bias due to the unequal weathering of plots. The harvested seed was dried for 3 days at 32°C, weighed, and the weights then converted to yield based on moisture of 130 g kg^{-1}.

Dates of Beginning Bloom (R1) (Fehr & Caviness, 1977) and R8 were recorded for each plot. Plant height (distance in cm from the ground to the top of the stem) and lodging (1–5 scale, where 1 = all plants erect, 2 = either all plants leaning slightly or a few plants down, 3 = most plants leaning at a 45° angle, 4 = either all plants leaning considerably or 50% to 80% of the plants down, and 5 = all plants prostrate) were estimated for each plot at R8. Seed size (estimated as g 100 $seeds^{-1}$ per Gillen [2021], [2022]; and Gillen & Shelton [2020]) was estimated on 200 seeds (per Smith et al. [2008] to provide a better estimate than weighing just 100 seeds) from each plot prior to the germination assays at the Mississippi State Seed Testing Laboratory, as per above. Harvested seed were visually graded

for individual components of damage, manifest as seed wrinkling, mold, green seed damage, purple seed stain (PSS), and stink bug piercing, per the protocols of Bellaloui et al. (2017) and Smith et al. (2008). In brief, the grading was reported for each harvested seed lot for each separate component as the percentage of visibly damaged (wrinkled, moldy, green, purple, and insect-pierced) seed. Included in trials in multiple combinations were the commercial cultivar checks 'AG46X6', 'AG4632', 'AG49X6' (Bayer Crop Science), 'P46T59R', and 'P48A60X' (Corteva Agriscience). Two lines were included as MSD tolerant checks; late MG IV DS25-1 and early MG V DS49-142.

2.3.2 | Disease and seed damage nurseries

Two seed damage-testing nurseries were planted at Stoneville in RCBDs that featured replicated single-row plots, where harvest was delayed 2 weeks after R8. The first was an irrigated PSD nursery with supplemental overhead irrigation. The second was a dryland (rainfed) nursery.

Stoneville, MS, irrigated PSD nursery

The PSD nursery featured 3-m-long plots with 0.66 m between rows in three replications planted on April 29, 2019, May 4, 2020, April 30, 2021, and April 28, 2022. Plots were furrow irrigated as needed, and then additionally watered with an overhead watering system after plots were inoculated at the R5 growth stage with a spore suspension of *D. longicolla*, as per Smith et al. (2019). Unless it rained, the overhead irrigation system was used to apply water to the foliage twice daily on weekdays at approximately 7:00 a.m. and at dusk, and once a day at dusk on weekends as per Smith et al. (2019). Cultivars AG4632, P46T59R, P48A60X, 'AG51X8', 'AG5335', 'AG55X7' (Bayer Crop Science), and 'Manokin' (Kenworthy et al., 1996), and "tolerant" germplasms DS25-1 and DS49-142, were included as controls. Following the hand-harvest of plots, plot bundles were threshed individually in a machine bundle thresher. The seed was then stored at 21°C and 60% relative humidity until all plots were harvested and threshed.

Following the harvest of all plots, a 125-g unselected seed sample from each plot was graded for total seed damage (DKT, damaged kernels total) using Federal Grain Inspection Service (FGIS) standards (FGIS, 2020) by Midsouth Grain, Inc. For FGIS ratings, damage is recorded as a percentage of the weight of damaged grain.

A random 25-seed sample of non-mechanically damaged seed (i.e., non-split seed or cracked seed coats) was taken from each harvested plot and plated on acidified potato dextrose agar (APDA) as per Smith et al. (2019). After incubation, the number of seeds infected with *D. longicolla* was recorded and calculated as percent seed infection (Li et al., 2015, 2023).

Seed samples (~300–400 seeds each) from the 2020 and 2021 PSD nurseries were air dried at room temperature for 72 h or until the moisture levels were below 10%. The dried seeds were ground to a consistency of flour using a coffee grinder (Fresh Grind, Hamilton Beach) and then stored at 4°C until used. To extract potential toxins from the ground samples, 250 mL of 50% methanol in water were added to 473 mL-plastic extracting cups (Berry Global) containing 50 g of ground sample. The cups were covered with lids, and the lids were punctured with a needle-sized hole to avoid pressure buildup of released gases during the shaking process. The cups were placed on a fixed-speed benchtop reciprocal shaker (Eberbach) and shaken for 30 min on low speed (280 oscillations per min). The contents of the cups were allowed to settle for 1 h, and then the samples were initially filtered through Whatman grade 1 qualitative filter paper (Cytiva Life Sciences). The rough filtrate of each sample was pushed through a Whatman nylon membrane syringe filter with a 0.22-μm pore size using a 3-mL disposable polypropylene luer lock syringe (BD). Ten mL of sterile filtrate from each sample were collected in a 20-mL glass scintillation vial. The vials were dried to remove methanol volatiles in a ventilated forced air oven (VWR International) set to 35°C for at least 48 h. The vials were then stored at −20°C prior to final freeze drying. A VirTis general-purpose freeze dryer (Scientific Products) was set to −40°C shelf and −50°C condenser, at 500 μBarr of pressure. The frozen vials containing the concentrated filtrates were transferred to the freeze dryer, uncapped, and dried for a minimum of 5 days or until the samples were completely dry. The freeze-dried residues were immediately capped and stored at −20°C until used. The dried extracts were re-dissolved in 5 mL of acetonitrile/water/acetic acid (79/20/1, v/v/v) for 90 min on a rotary shaker and an aliquot of 500 μL was diluted 1:1 with acetonitrile/water/acetic acid (20/79/1, v/v/v). Aliquots (5 μL) of this diluted extract were injected into the LC-MS/MS system and analyzed as described in Sulyok et al. (2024). In brief, a 1290 Series high performance liquid chromatography System (Agilent) was coupled to a QTrap 5500 LC-MS/MS System (Applied Biosystems SCIEX) equipped with Turbo Ion Spray electrospray ionization source. Chromatographic separation was performed at 25°C running an acidified methanol/water gradient on a Gemini C_{18}-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C_{18} 4 × 3 mm i.d. security guard cartridge (Phenomenex). ESI-MS/MS data were acquired in the scheduled multiple reaction monitoring mode both in positive and negative polarity in two separate chromatographic runs.

Stoneville, MS, non-irrigated stress nursery

The second Stoneville seed damage nursery (non-irrigated, rain fed) featured two replications of 3-m-long plots with 0.91 m between rows planted April 22, 2019, and June 2,

2020. No artificial inoculation or overhead waterings were employed; seed infection and insect damage were due entirely to natural conditions. Rainfed nurseries are more likely to suffer green seed damage, have smaller seeds, and impermeable seed coats (Bellaloui et al., 2017). DS1260-2 was tested in comparison to cultivars P46T59R, AG4632, and P48A60X. DS25-1 and DS49-142 were grown as "tolerant" controls and grandparent DT97-4290 was grown as a "susceptible" control. As with the above PSD nursery, all plots were hand-harvested 2 weeks after R8 and threshed. Bulked harvested seeds were visually rated for seed wrinkling, visual mold, green seed damage, stink bug feeding, and PSS as per above and Bellaloui et al. (2017) and Smith et al. (2008). An unselected 200-seed sample was assayed for standard germination and hard seed as described above, at the Mississippi State Seed Testing Laboratory. Seed were also graded at Midsouth Grain, Inc. in 2020, where damage was reported as the percentage of the weight of damaged grain for DKT, mold-damaged kernels (MDK), and purple mottled or stained (PMS).

Jackson, TN, PSD and frogeye leaf spot assays

DS1260-2 and appropriate controls were assayed for multiple soybean diseases (PSD in 2019 and 2022, and frogeye leaf spot, *Cercospora sojina* Hara, in 2019–2022) at the Jackson, TN, USDA worksite. For PSD, single row plots (305-cm long by 76-cm wide) were planted in three replications in a RCBD. In 2019, DT97-4290, P46T59R, AG4632, P48A60X, 'P48A32X' (Corteva Agriscience), AG51X8, AG5335, 'CZ3841LL' (Credenz, BASF), DS49-142, and DS25-1 were used as controls. In 2020, only DT97-4290 and DS25-1 were used as controls. Plots were inoculated with *D. longicolla* at the R1 growth stage and delay-harvested 2 weeks after R8. A random sample of 100 seeds was surface-disinfected, plated on APDA, and incubated at 24°C for 5 days as per Mengistu et al. (2010) and Smith et al. (2019). Levels of infection for each genotype were estimated as the percentage of the number of seeds infected with *D. longicolla*. For frogeye leaf spot ratings, cultivar 'AG4703' (susceptible control, Bayer Crop Science) and DS1260-2 were planted in plots in a RCBD with three replications from 2019 to 2021, and similarly 'NK48R2X' (susceptible control, Syngenta) was compared with DS1260-2 in 2022. These field plots are known to have a consistent natural infection from year-to-year and therefore were not inoculated. Disease severity ratings were taken between the R5 and R6 growth stages based on a severity scale of 0% to 100% (Mengistu et al., 2018), where 0 is resistant, 1–5 moderately resistant and >5% is susceptible.

West Lafayette, IN, Phytophthora assay

DS1260-2 was tested at West Lafayette, IN, in 2021 and 2023, along with the 15-genotype differential set for Phytophthora root and stem rot, caused by *P. sojae*. The 15 lines are: 'Williams' (*rps1*; Bernard & Lindahl, 1972), 'Union' (*Rps1a*; Bernard & Cremeens, 1982), L77-1863 (*Rps1b*; Sugimoto et al., 2012), L75-3735 (*Rps1c*; Sugimoto et al., 2012), PI 103091 (*Rps1d*), 'Williams 82' (*Rps1k*, Bernard & Cremeens, 1988), L82-1449 (*Rps2*, Dorrance et al., 2004), PI 171442 (*Rps3a*), L91-8347 (*Rps3b*; Sugimoto et al., 2012), PRX145-48 (*Rps3c*; Yang et al., 2020), L85-2352 (*Rps4*; Sugimoto et al., 2012), L85-3059 (*Rps5*; Sugimoto et al., 2012), L89-1581 (*Rps6*; Sugimoto et al., 2012), L93-3258 (*Rps7*; Sugimoto et al., 2012), and PI 399073 (*Rps8*). *P. sojae* isolates belonging to races 1 (defeats *Rps7*), 3 (defeats *Rps1a* and *Rps7*), 4 (defeats *Rps1a*, *Rps1c*, and *Rps7*), 7 (defeats *Rps1a*, *Rps3a*, *Rps6*, and *Rps7*), 17 (defeats *Rps1b*, *Rps1d*, *Rps3a*, *Rps6*, and *Rps7*), and 25 (defeats *Rps1a*, *Rps1b*, *Rps1c*, *Rps1k*, and *Rps7*) were used individually to infect the soybean lines with the hypocotyl inoculation method (Dorrance et al., 2004). Briefly, *P. sojae* isolates were grown on half-strength lima bean agar (LBA) for 7 days on a bench top at room temperature. The LBA, covered by mycelium and oospores, was cut into strips using a sterile razor blade. The agar strips were forced through a syringe to produce macerated, colonized LBA as inoculum. Soybean lines were grown in the greenhouse to the V0 to V1 growth stages. A syringe needle was inserted entirely through the hypocotyl to apply inoculum on both sides of the hypocotyl. Ten to 20 plants of each soybean line were inoculated with each isolate. A transparent plastic cover was placed on top of the inoculated plants for 48 h to maintain high moisture. Disease was rated 7 days after inoculation as follows: resistant if more than 70% of inoculated plants were alive; susceptible if less than 30% of inoculated plants were alive; and intermediate if in-between.

2.3.3 | USDA Uniform Soybean Tests, Southern States

DS1260-2 was entered into the USDA Uniform Soybean Tests, Southern States (SUST) in 2019 in the Preliminary Group IV-S Test and then in 2020 and 2021 in the Uniform IV-S Test (Gillen, 2021, 2022; Gillen & Shelton, 2020). Diverse planting dates, soil types, and plot sizes were used across multiple locations and years as described by Gillen (2021, 2022) and Gillen and Shelton (2020). Agronomic traits measured were seed yield, R8, plant height, lodging, seed size, seed quality, seed composition (protein and oil), and responses to multiple diseases (Gillen, 2021, 2022; Gillen & Shelton, 2020). All seed protein and oil data are reported on a 13% moisture basis. As multiple lines were grown in the Uniform Tests, and entries varied from year to year; comparisons with DS1260-2 were restricted to cultivars AG46X6, 'AG48X9' (Bayer Crop Science), 'S16-7922C' (Chen et al., 2022), and 'Ellis' (Pantalone et al., 2017).

2.4 | Seed purification and increase

DS1260-2 was derived from a single F_5 plant in 2016, and subsequent rogued bulk-harvested rows were used as the seed source for an increase/purification block in 2019. The increase block was four rows wide and 100 m long, with 0.91 m between rows. All rows were rogued for off-type plants at R2 (full bloom; Fehr & Caviness, 1977) for flower color and at R8 for pod and pubescence colors, and maturity. Shortly after R8, the middle two rows were harvested with a plot combine that was thoroughly cleaned of seed and debris before use. The seed was mechanically cleaned and then hand-picked to remove any off-type seeds, focusing especially on hila color, seed coat color, seed size, and seed shape. The cleaned seed was then stored at 4° C and 50% relative humidity until distribution.

2.5 | Statistical analyses

Analysis of raw data from the Uniform Test trials was performed only on data from entries and controls that were present in all years. Analysis of variance was performed to obtain adjusted means using the Generalized Linear Mixed (GLIMMIX) procedure of SAS Version 9.4 (TS1M6) for Windows (SAS Institute). Analysis of residuals was performed using the 'plots = residual panel' option of GLIMMIX. All ANOVA analyses in this study were performed using this version of SAS. Yield, maturity, lodging, height, seed size and seed quality were analyzed with a model using genotype as a fixed effect and location, year, location × year, replication (location year), location × genotype, year × genotype, and location × year × genotype as random effects. Protein, oil, and meal protein data were analyzed using a model with genotype as a fixed effect and location, year, and location × year, location × genotype, and year × genotype as random effects. The least significant difference (LSD) was calculated at alpha = 0.05.

Analysis of irrigated yield and seed quality trials at Stoneville was performed only on data from entries and controls which were present in all 5 years for R1, R8, yield, lodging, height germination, hard seed, and seed weight. Analysis of variance and residuals was performed to obtain adjusted means using the GLIMIMIX procedure of SAS using a model with genotype as a fixed effect and year, replication(year), and year × genotype as random effects. The 'plots = residual panel' option was used. Fisher's protected LSD was calculated at alpha = 0.05. Two years of data for visual seed quality, including seed coat wrinkling, mold, green seed, purple seed stain, stink bug damage, and R8 (2020 and 2022) from these trials were separately analyzed to facilitate a comparison to more current commercial controls. The analysis to obtain adjusted means used the GLIMIMIX procedure of SAS. The 'plots = residual panel' and 'lines' options were used. The model with genotype and year as fixed effects and replication(year), and year × genotype as random effects was used. The LSD was calculated at alpha = 0.05.

Analyses of the Stoneville PSD nursery and the Tennessee PSD nursery were performed using only data from DS1260-2 and specific control lines. The Tennessee PSD nursery also included breeding lines. The analysis was done by year for the Stoneville PSD nursery, as it was obvious that PSD levels varied by year, and the controls varied over years. Years were also different, because the number of seeds rated per plot for PSD was 25 in 2019, 2020, and 2022, but 50 seeds were evaluated in 2021. Only data from 2019 was analyzed for the Tennessee PSD nursery because of low disease pressure in 2022. Analysis of variance was performed to obtain adjusted means of the percentage of seed infested with *D. longicolla* (PSD) using the GLIMMIX procedure in SAS with a binomial distribution, and the 'lines' and 'ilink' options for LSmeans statement. Analysis of residuals was performed using the 'plots = residual panel' option of GLIMMIX. The binomial distribution was used because each seed had only two possible ratings, infested or not infested. The lines option output the Conservative T Grouping using letters to indicate significant differences at alpha = 0.05, which facilitated visualization of the results. PSD, R8, DKT, and all toxin data were analyzed by year using a model with genotype as a fixed effect and replication as a random effect. Analyses were performed by year using the GLIMMIX procedure of SAS using the 'lines' option for model statement and the 'plots = residual panel' option. The residual analyses indicated that transformation of the DKT and toxin data may be useful. Therefore, DKT was transformed using the formula log(N+ 0.5). The toxin data were transformed using the formula log(N +1). Results were back transformed and presented using the original scale of the data.

Analyses of the Stoneville non-irrigated seed quality trials were performed on data from entries that were in common between 2019 and 2020. Analysis of variance was performed to obtain adjusted means using the GLIMIMIX procedure of SAS. Analyses of residuals were performed using the 'plots = residual panel' option. R8, seed coat wrinkling, mold, and germination were analyzed using a model with genotype and year as fixed effects and replication(year), and year × genotype as random effects. LSDs were calculated at alpha = 0.05. In 2020, the seed from the non-irrigated seed quality trials were sent to Midsouth Grain Inspection service for evaluation of DKT, MDK, and PMS. Analyses of variance were performed to obtain adjusted means using the GLIMIMIX procedure of SAS. Analyses of residuals were performed using the 'plots = residual panel' option. DKT, MDK, PMS and R8 were analyzed using a model with genotype as a fixed effect and replication as random effect. LSDs were calculated at alpha = 0.05.

TABLE 1 LSmeans of agronomic and seed characteristics of DS1260-2 and check lines from an analysis of lines in common to all trials ($n = 8$) in the Uniform Soybean Tests—Southern States, Uniform Maturity Group IV-S Tests in 2020–2021.

Genotype	Environments No.	Yield kg ha^{-1}	Relative maturity days[a]	Height cm	Lodging (1–5)[b]	Seed weight g 100^{-1} seeds	Seed protein g kg^{-1}	Seed oil g kg^{-1}	Meal protein %
AG48X9	28	4699	66	91	1.4	16.4	399	228	47.1
AG46X6	28	4413	66	88	1.6	17.5	402	224	47.1
S16-7922C	28	4407	69	83	2.3	15.0	406	221	47.5
Ellis	28	3989	69	66	1.0	13.2	403	215	46.8
DS1260-2	28	3733	63	86	1.3	11.9	407	209	47.0
LSD 0.05		337	2.5	6.1	0.3	2.1	6	3	0.6
Environment no.		28	24	26	26	24	23	23	23

[a]Days after August 1.

[b]1 = no lodging; 2 = either all plants leaning slightly or a few plants down; 3 = either all plants leaning moderately or 25% to 50% of the plants down; 4 = either all plants leaning considerably or 50% to 80% of the plants down; 5 = all lodged.

TABLE 2 LSmeans of agronomic and seed characteristics of DS1260-2 and common check lines from yield trials in Stoneville, MS, in 2019–2022.

Genotype[a]	R1 days[b]	R8 days	Yield kg ha^{-1}	Height cm	Lodging (1–5)[c]	Germ %	Hard seed %	Seed weight g 100^{-1} seeds
DS1260-2	89	173	4715	103	1.1	89	1.0	10.1
DS25-1	90	172	4087	88	1.8	89	0.0	9.9
P46T59R	72	171	4638	76	1.0	41	0.0	16.6
Mean	84	172	4480	89	1.3	73	0.3	12.2
LSD(0.05)[d]	6.5	4.8	583	24.0	0.5	32.0	0.7	1.0

[a]These trials had damage consistent with exposure to the herbicide Dicamba. All genotypes are susceptible to Dicamba.

[b]Days after 31 March (1 April = 1).

[c]1 = all plants erect; 2 = either all plants leaning slightly or a few plants down; 3 = most plants leaning at a 45° angle; 4 = either all plants leaning considerably or 50 to 80% of the plants down; 5 = all plants prostrate.

[d]LSD values for R8, yield, height, and lodging are presented for information only. Differences among the genotypes were not significant based on Fishers Protected LSD.

3 | CHARACTERISTICS

3.1 | Botanical description and seed composition

Plants of DS1260-2 have an indeterminate growth habit with purple flowers, gray pubescence, tan pod walls, imperfect black hila, and yellow seed coats. Across 24 environments and 2 years in the Uniform Tests, DS1260-2 was 3 days earlier than AG48X9 and AG46X6 and 6 days earlier than Ellis and S16-7922C (Table 1), whereas it was 1 and 2 days later than DS25-1 and P46T59R, respectively, across 4 years at Stoneville (Table 2). DS1260-2 is classified as late MG IV. DS1260-2 had similar protein (407 g kg^{-1}), but less oil (209 g kg^{-1}) than AG46X6 (402 and 224 g kg^{-1}, respectively), S16-7922C (406 and 221 g kg^{-1}, respectively), and Ellis (403 and 215 g kg^{-1}, respectively) (Table 1). Meal protein of DS1260-2 (47.0%) was similar to the cultivars (47.1, 47.5, and 46.8%, respectively) (Table 1). The seed size of DS1260-2 was similar (11.9 g 100^{-1} seed) to Ellis (13.2 g 100^{-1} seed), but smaller than S16-7922C (15.0 g 100^{-1} seed), AG48X9 (16.4 g 100^{-1} seeds) and AG46X6 (17.5 g 100^{-1} seed) (Table 1). The smaller seed size of DS1260-2 is derived from its grandparent PI 587982A, which has an even smaller seed size of 7.6 g 100^{-1} seeds (Smith et al., 2008). Two cycles of meiotic recombination and selection from PI 587982A to develop DS1260-2 produced an apparent increase in seed size of over 4 g 100^{-1} seeds (11.9 vs 7.6 g 100^{-1} seeds), while maintaining the same tolerance to MSD. Continued cycles of meiotic recombination and selection using appropriate materials could be expected to produce improved cultivars with larger seed. In terms of appropriate breeding material, Smith et al. (2008) found that PI 603756 had tolerance to heat-induced seed degradation with low levels of *D. longicolla*, while also having larger seed (17.8 g 100^{-1} seeds). Tolerant check DS49-142 was developed from PI 603756 and has low levels of damage and infection by *D. longicolla* (Table 3), and has a seed size of 13.3 g 100^{-1} seeds. DS49-142 would be a good parent to use for increasing seed size and even possibly for improving tolerance to MSD, as combining tolerances

TABLE 3 LSmeans for total damaged kernels percentage, Phomopsis percentage, and days from April 1 to growth stage R8 of DS1260-2 and check lines from a mature seed damage/Phomopsis seed decay (PSD) nursery in Stoneville, MS, in 2019–2022. Damaged kernels total (DKT) and PSD were back-transformed to the original scale.

Genotype	DKT	PSD	R8	Genotype	DKT	PSD	R8
	w/w %	%	days		w/w %	%	days
	2019				2020		
DT97-4290	1.6 abc	21.8 b	170 d	Manokin	0.8 c	25.3 ab	174 c
P46T59R	2.2 ab	37.9 a	174 c	DS1260-2	2.3 bc	16.0 bc	178 c
DS1260-2	0.1 d	7.9 cd	175 bc	DS25-1	1.6 bc	14.7 bc	183 b
DS25-1	0.5 cd	7.9 cd	175 bc	P46T59R	9.6 a	32.0 a	183 b
AG4632	2.9 a	48.0 a	176 abc	AG4632	11.8 a	22.7 ab	185 b
DS49-142	0.2 d	4.6 d	176 abc	DS49-142	3.0 bcd	9.3 c	186 ab
AG51X8	0.7 bcd	19.8 bc	177 ab	AG5335	9.0 a	24.0 ab	187 ab
P48A60X	2.0 ab	39.2 a	178 a	P48A60X	8.8 a	24.0 ab	191 a
AG5335	0.5 cd	20.5 b	178 a	Mean	4.3	20.1	183
Mean	0.9	19.1	175				
	2021				2022		
DS1260-2	0.2 d	44.7 d	172 c	DS1260-2	0.8 c	10.4 b	169 d
P46T59R	2.8 ab	85.4 a	172 c	P46T59R	6.6 a	25.0 a	171 cd
AG4632	0.4 cd	82.0 a	174 c	DS25-1	0.9 c	5.2 b	171 cd
DS25-1	0.6 cd	70.0 b	174 c	AG55X7	0.4 c	9.1 b	171 cd
AG55X7	1.4 bc	58.0 c	177 b	P48A60X	4.4 ab	9.1 b	172 c
DS49-142	0.9 c	28.0 e	177 b	DS49-142	0.8 c	2.6 b	175 b
P48A60X	3.9 a	82.0 a	177 b	AG5335	2.6 b	5.2 b	180 a
AG5335	5.1 a	46.0 cd	184 a	Mean	1.7	7.8	173
Mean	1.4	64.1	176				

Note: Means with a letter in common within a column are not significantly different based on a Conservative T grouping at alpha = 0.05.

from PIs 587982A and 603756 may increase total tolerance. In terms of the effects of seed size on seed damage, Smith et al. (2008) determined that seed size had only a small effect on levels of *D. longicolla*, standard germination, and seed vigor.

3.2 | Seed damage and disease performance

3.2.1 | Irrigated PSD nurseries

The levels of MSD, as defined by FGIS terminology (DKT) and standards, are estimated each year for producers at grain elevators at the time soybean seeds are sold. Damage above minimal levels (determined by each elevator, but generally about 2%) results in grain "dockage," the loss in revenue due to seed damage. The most damaging subcomponents of DKT for a given seed lot may vary from environment to environment, but at Stoneville, mold (MDK, mold-damaged kernels) and stink bugs (SKD, stinkbug damaged) typically cause the most seed damage. Other potentially important damage components of DKT in the midsouthern United States are heat (HT = heat-damaged kernels), "distinctly green kernels" (DGK), and seed that is "distinctly discolored" (DISC). Seed wrinkling and impermeable seed coats are not considered "damage" by FGIS standards. In addition, low levels of DGK, usually due to the rapid dry-down of seeds resulting from premature plant death from frost, drought, etc., are not considered "damage" under FGIS standards, unless the degree of green in the seed reaches a high intensity. Levels of "purple mottled or stained" (PMS) caused by the purple seed stain (PSS) fungus [*Cercospora kikuchii* (Matsumoto and Tomoyasu) Gardner] can be recorded on the grading ticket but are not included in DKT. Table 3 presents DKT levels for DS1260-2 in comparison to multiple controls (tolerant and susceptible) across 4 years from the Stoneville PSD nursery, where all plots were inoculated with *D. longicolla* and harvested 2 weeks after R8. As each year provided a unique testing environment, the 2019–2022 data are presented by year. For each of the 4 years, DS1260-2 had similar total seed damage (DKT) compared to damage-tolerant DS25-1, but significantly less damage than susceptible cultivars P46T59R and P48A60X (Table 3). For example, in 2022 DS1260-2 and DS25-1 had DKT scores of 0.8 and 0.9%, respectively, whereas P46T59R and P48A60X had DKT scores of 6.6%

TABLE 4 LSmeans for Phomopsis percentage and days from April 1 to growth stage R8 of DS1260-2 and check lines from a Phomopsis seed decay (PSD) nursery in Jackson, TN, in 2019. PSD percentages were back-transformed to the original scale.

Genotype	PSD %	R8 days
CZ3841LL	53 cd	176 d
DB06X006-93	24 fg	178 d
P48A60X	36 efg	185 c
AG4632	79 ab	186 bc
DT97-4290	64 bc	188 bc
AG51X8	42 def	189 bc
P48A32X	61 c	190 bc
DS1260-2	23 g	190 bc
P46T59R	77 ab	191 bc
AG5335	64 bc	192 ab
DA10X30-09F	81 a	192 ab
DA13099-008F	49 cde	192 ab
DS25-1	33 efg	192 ab
DS49-142	31 fg	197 a
Mean	52	190

Note: Means with a letter in common within a column are not significantly different based on a Conservative T grouping at alpha = 0.05.

and 4.4%, respectively; an eight-fold increase in damage in P46T59R compared to that of DS1260-2.

D. longicolla can be an important component of DKT and was estimated for 4 years at Stoneville. During that time, DS1260-2 had less than or nearly half the incidence of *D. longicolla* compared with P46T59R (Table 3). In independent PSD assays at Jackson, TN, DS1260-2 had less than a third of the incidence of *D. longicolla* compared to P46T59R (Table 4).

3.2.2 | Mycotoxin contamination

It is well known that fungi in soybean seed can produce mycotoxins (Hagler et al., 1989; Jacobsen et al., 1995; Trempus et al., 1989). The incidence of fungal metabolites was measured over 2 years (2020–2021) in seed harvested from the Stoneville PSD nursery. Due to variability between years, data are presented by year. Toxin levels are not part of the ratings for DKT and MDK, but they are an important component of seed quality, and their toxicity can present significant problems to end users of contaminated grain (CAST, 2003). In 2020, the levels of cercosporin, cytochalasin H, and cytochalasin J were less in DS1260-2 (3.1, 0.0, and 0.8 ng g^{-1}, respectively) compared to those in P46T59R (151, 22.6, and 37.4 ng g^{-1}, respectively) and to those in AG4632 (317, 9.4, and 27.5 ng g^{-1}, respectively) (Table 5). However, the levels in DS25-1 (10.2, 4.8, and 6.2 ng g^{-1}, respectively) were not different from those in DS1260-2. Cercosporin is a metabolite of numerous species of *Cercospora* that becomes toxic when activated by light (Daub & Ehrenshaft, 2000). Cytochalasins are toxic metabolites that have been isolated from species of *Diaporthe* (*Phomopsis*) (Xu et al., 2021).

The 2020 levels of tryptophol were different among genotypes, but the level in DS1260-2 was no different than in any other genotype. Tryptophol is an aromatic alcohol produced from tryptophan by *Rhizoctonia* spp. and other microbial species (Furukawa et al., 1996). In 2020, DS1260-2 and DS25-1 had significantly lower levels of beauvericin (0.5 and 0.4 ng g^{-1}, respectively) than both AG4632 and P48A60X (27.0 and 21.0 ng g^{-1}, respectively) (Table 5). Likewise in 2020, DS1260-2 had lower fusaric acid (3.0 ng g^{-1}) than P46T59R (1044 ng g^{-1}) and P48A60X (1276 ng g^{-1}). Fusaric acid and beauvericin are mycotoxins produced by *Fusarium* spp. that have a variety of biological activities, including antibiotic and insecticidal effects (Escrivá et al., 2015). Levels of nivalenol were only estimated in 2020, when DS1260-2 (0.0 ng g^{-1}) and DS25-1 (0.0 ng g^{-1}) had lower levels than P46T59R (4.31 ng g^{-1}) and AG4632 (3.44 ng g^{-1}) (Table 6). Likewise, diacetoxyscirpenol was only measured in 2020, when there were no differences among genotypes, except for the later maturing P48A60X that had 0.41 ng g^{-1} compared to none for DS1260-2 and DS25-1 (Table 6). T-2 toxin and monoacetoxyscirpenol were also only estimated in 2020, and neither showed any genotypic differences (Table 6). Trichothecene toxins isolated from *Fusarium* species include nivalenol, diacetoxyscirpenol, T-2 toxin and monoacetoxyscirpenol (McCormick et al., 2011). Their toxicity in humans is unknown, but in animals they are associated with "feed refusal," vomiting, gastrological irritation, immunological dysfunction, and hematotoxicity (Abbas et al., 1984).

In 2021, levels of cercosporin, cytochalasin H, cytochalasin J, and tryptophol were higher in P46T59R (12.6, 1.5, 4.7, and 21.5 ng g^{-1}, respectively) than in DS1260-2 (0.0, 0.0, 0.3, and 6.5 ng g^{-1}, respectively), and in DS25-1 for cercosporin, cytochalasin J and tryptophol (0.5, 0.8, and 6.5 ng g^{-1}, respectively) (Table 5). In both 2020 and 2021, levels of zearalenone were not different among genotypes. Likewise, levels of fusaric acid and beauvericin were not different across genotypes in 2021. The presence of the above fungal metabolites is consistent with the presence of species of *Diaporthe*, *Cercospora*, and *Fusarium* on moldy soybean seed assayed in this study.

3.2.3 | Irrigated Stoneville trials harvested on-time and without artificial inoculation

Four-year (2019–2022) seed germination percentages at Stoneville for DS1260-2 and DS25-1 were double (both 89%) that of P46T59R (41%) (Table 2). DS1260-2 had 1% seed

TABLE 5 LSmeans back-transformed to the original data scale for fungal metabolites in seed and days from April 1 to growth stage R8 of DS1260-2 and check lines from a mature seed damage/Phomopsis seed decay (PSD) nursery in Stoneville, MS, in 2020 and 2021.

Genotype	Cercos	CytoH	CytoJ	Trypto	Zear	FusAci	Beau	R8
	ng g^{-1}							
2020								
Manokin	0.0 d	0.0 c	2.8 cde	38.9 ab	0.0 a	0.0 c	0.1 b	174 c
DS1260-2	3.1 cd	0.0 c	0.8 de	47.7 ab	1.0 a	3.0 bc	0.5 b	178 c
DS25-1	10.2 cb	4.8 abc	6.2 bcd	62.4 a	1.4 a	23.5 abc	0.4 b	183 b
P46T59R	151.1 a	22.6 a	37.4 a	64.6 a	2.5 a	1043.7 a	6.1 ab	183 b
AG4632	316.5 a	9.4 ab	27.5 ab	51.9 ab	0.9 a	155.4 ab	27.0 a	185 b
DS49-142	21.4 b	0.0 c	0.0 e	33.4 b	0.0 a	72.3 abc	8.3 ab	186 ab
AG5335	262.6 a	1.1 bc	6.7 bc	36.5 b	0.0 a	76.6 abc	4.6 ab	187 ab
P48A60X	512.2 a	18.8 a	27.5 ab	39.4 ab	1.8 a	1276.2 a	21.0 a	191 a
2021								
DS1260-2	0.0 c	0.0 b	0.3 cd	6.5 bc	0.0 a	0.0 a	0.07 a	172 c
P46T59R	12.6 a	1.5 a	4.7 a	21.5 a	0.1 a	8.0 a	0.07 a	172 c
AG4632	0.6 c	1.5 a	1.1 bc	8.3 b	0.0 a	2.0 a	0.07 a	174 c
DS25-1	0.5 c	0.4 ab	0.8 bcd	6.5 bc	0.0 a	1.5 a	0.30 a	174 c
AG55X7	1.6 bc	1.1 ab	1.8 ab	8.3 b	0.0 a	0.0 a	0.00 a	177 b
DS49-142	1.3 bc	0.8 ab	0.0 d	4.1 c	0.0 a	1.2 a	0.10 a	177 b
P48A60X	6.0 ab	2.0 a	4.3 a	6.3 bc	0.0 a	4.4 a	0.09 a	177 b
AG5335	8.8 ab	1.8 a	4.7 a	14.7 a	0.0 a	4.8 a	0.50 a	184 a

Note: Means with a letter in common within a column are not significantly different based on a Conservative T grouping at alpha = 0.05.
Abbreviations: Cercos = cercosporin; CytoH = cytochalasin H; CytoJ = cytochalasin J; Trypto = Tryptophol; Zear = zearalenone; FusAci = fusaric acid, Beau = beauvericin.

TABLE 6 LSmeans back-transformed to the original scale for fungal metabolites in seed and days from April 1 of growth stage R8 of DS1260-2 and check lines from a mature seed damage/Phomopsis seed decay (PSD) nursery in Stoneville, MS, in 2020.

Genotype	T2	Niv	Mono	Diac	R8
	ng g^{-1}				
Manokin	0.00 a	1.15 ab	0.00 a	0.00 b	174 c
DS1260-2	0.00 a	0.00 b	0.00 a	0.00 b	178 c
DS25-1	2.67 a	0.00 b	0.00 a	0.00 b	183 b
P46T59R	0.00 a	4.31 a	0.00 a	0.09 b	183 b
AG4632	0.24 a	3.44 a	0.00 a	0.09 b	185 b
DS49-142	0.82 a	0.00 b	0.00 a	0.00 b	186 ab
AG5335	0.69 a	3.73 a	0.00 a	0.00 b	187 ab
P48A60X	0.72 a	1.03 ab	0.56 a	0.41 a	191 a

Note: Means with a letter in common within a column are not significantly different based on a Conservative T grouping at alpha = 0.05.
Abbreviations: T2 = T-2 toxin; Niv = nivalenol; Mono = monoacetoxyscirpenol; and Diac = diacetoxyscirpenol.

coat impermeability, whereas those for DS25-1 and P46T59R were 0% (Table 2). However, such a small difference is not meaningful. Visual ratings of seed coat wrinkling were lower for DS1260-2 (none) and DS25-1 (1.7%) than for P46T59R (15%). Likewise, visual mold was lower for both DS1260-2 and DS25-1 (both none) than for P46T59R (5.0%), AG46X6 (6.7%), and AG49X6 (10.0%) across 2 years (2020 and 2022) (Table 7). No differences among lines were noted for green seed damage, PSS, and stink bug feeding (Table 7).

3.2.4 | Non-irrigated Stoneville nursery harvested 2 weeks after R8 and without artificial inoculation

Seed damage was also estimated under rainfed conditions for 2 years (2019 and 2020) at Stoneville after a 2-week delay in harvest. DS1260-2 and DS25-1 had germination rates (81% and 85%, respectively) more than three times as high as those of P46T59R (20%) and AG 4632 (24%) (Table 8).

DS1260-2 had less seed coat wrinkling (5%) than P46T59R (18%) and less visual mold (none) than AG4632 (15%) (Table 8). In 2020, DS1260-2 had less (none) PSS compared with DS25-1 (10%), AG4632 (10%), and P46T59R (25%), but stink bug damage was equally present for all genotypes (data not shown). Total seed damage on a weight basis (DKT) in 2020 was less for DS1260-2 and DS25-1 (both 1.1%) than in DT97-4290 (2.8%) and P48A60X (2.7%). Mold damage on a weight basis (MDK) was less for DS1260-2 (0.7%) and DS25-1 (0.5%) than for DT97-4290 (1.8%) (Table 9). There were

TABLE 7 LSmeans of seed quality characteristics and days from April 1 to growth stage R8 of DS1260-2 and check lines from irrigated yield trials in Stoneville, MS, in 2020 and 2022.

Genotype	R8	Wri	Mold	GrnSd	PSS	StBug
	days	%	%	%	%	%
DS1260-2	178	0.0	0.0	0.0	0.0	15.0
DS25-1	178	1.7	0.0	0.0	3.3	11.7
P46T59R	179	15.0	5.0	0.0	13.3	18.3
AG46X6	183	8.3	6.7	6.7	3.3	20.0
AG49X6	185	6.7	10.0	3.3	5.0	23.3
Mean	180	6.3	4.3	2.0	5.0	17.7
LSD 0.05	3.6	9.9	4.1	7.2	14.3	14.2

Abbreviations: Wri = seed coat wrinkling; Mold = visual mold (fungal growth); GrnSd = green seed damage; PSS = purple seed stain; StBug = stink bug damage.

TABLE 8 LSmeans of seed quality characteristics and days from April 1 to growth stage R8 of DS1260-2 and check lines from non-irrigated trials in Stoneville, MS, in 2019 and 2020.

Genotype	Wri	Mold	Germ	R8
	——————%——————			days
P46T59R	18	10	20	174
DS1260-2	5	0	81	177
DS25-1	13	5	85	178
AG4632	13	15	24	178
Mean	12	8	52	177
LSD 0.05	9	13	46	14

Abbreviations: Wri = seed coat wrinkling; Mold = visual mold or fungal infestation; Germ = germination.

TABLE 9 LSmeans of seed damage characteristics and days from April 1 to growth stage R8 of DS1260-2 and check lines from a non-irrigated trial in Stoneville, MS, 2020.

Genotype	DKT	MDK	PMS	R8
	——————w/w %——————			days
DS1260-2	1.1	0.7	2.4	185
DS25-1	1.1	0.5	2.6	186
Manokin	1.4	0.6	0.8	187
DT97-4290	2.8	1.8	3.4	187
DS49-142	0.7	0.3	0.1	189
AG4632	1.7	0.6	3.4	190
P48A60X	2.7	1.3	1.9	192
AG5335	1.3	0.3	1.6	192
Mean	1.6	0.8	2.0	188
LSD 0.05	1.3	1.1	2.9	4

Abbreviations: DKT = damaged kernels total by weight; MDK = mold-damaged kernels by weight; PMS = purple mottled or stained by weight.

differences among genotypes for PMS: (DS49-142 had 0.1%, whereas DT97-4290 and AG4632 had 3.4%), but DS1260-2 was not different from the other genotypes for PMS (Table 9).

3.2.5 | Other disease ratings

DS1260-2 had lower levels (3%) of frogeye leaf spot than AG 4703 (27%) averaged over 3 years of testing (2019–2021) at Jackson, TN (data not shown). In 1 year (2022) of testing at Jackson, DS1260-2 had lower incidence (none) than NKS48R2X (21.7%) (data not shown). In tests at West Lafayette, DS1260-2 was susceptible to races 1, 3, 7, and 25 of Phytophthora root and stem rot, with mixed reactions to races 4 and 17 (data not shown). Based on data from the SUST (2019 and 2021), DS1260-2 is resistant to stem canker, caused by *Diaporthe aspalathi* Jansen, Castlebury, and Crous (syn. *Diaporthe phaseolorum* var. *meridionalis* Fernandez) (Gillen, 2022; Gillen & Shelton, 2020). Further, DS1260-2 is resistant to soybean cyst nematode race 3 (HG type 0) (Gillen, 2022; Gillen & Shelton, 2020), but susceptible to races 2 (HG type 1.2.5.7) and 5 (HG type 2.5.7) (Gillen, 2021; Gillen & Shelton, 2020). DS1260-2 is susceptible to peanut root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood), southern root-knot nematode (*M. incognita* (Kofoid and White) Chitwood), and Javanese root-knot nematode (*M. javanica* (Treub) Chitwood) (Gillen, 2021, 2022).

3.3 | Agronomic performance

3.3.1 | Mississippi yield trials

DS1260-2 (4715 kg ha^{-1}) had similar seed yield to P46T59R (4638 kg ha^{-1}) over 4 years (2019-2022) at Stoneville, even though P46T59R had larger seed (16.6 g 100^{-1} seed) and a longer reproductive period (R8–R1 = 99 days) than DS1260-2 (10.1 g 100^{-1} seeds, and 84 days, respectively) (Table 2). The two lines matured within 2 days of each other. DS25-1 had similar seed size (9.9 g 100^{-1} seeds) and reproductive period

(82 days) but was lower yielding (4087 kg ha^{-1}) than DS1260-2 (Table 2). DS1260-2 was taller (10 cm) than P46T59R (76 cm) but was similar (1.1) in lodging with P46T59R (1.0) (Table 2). Across 3 years at Stoneville (2020–2022), DS1260-2 (4766 kg ha^{-1}) was not significantly different in yield compared with AG49X6 (4577 kg ha^{-1}), AG46X6 (5243 kg ha^{-1}), and P46T59R (4829 kg ha^{-1}) (data not shown).

3.3.2 | Uniform test trials

Over 28 locations across 2 years (2020–2021) in the SUST, DS1260-2 (3733 kg ha^{-1}) yielded similar to Ellis (3989 kg ha^{-1}), but less than S16-7922C (4407 kg ha^{-1}), AG46X6 (4413 kg ha^{-1}), and AG48X9 (4699 kg ha^{-1}) (Table 1). DS1260-2 was not different in height (86 cm) or lodging (1.3) than AG48X9 (91 cm and 1.4, respectively). DS1260-2 was similar in height with AG46X6 (88 cm) and S16-7922C (83 cm), but had less lodging (1.6 and 2.3, respectively). DS1260-2 was taller and lodged more than determinant Ellis (66 cm and 1.0, respectively) (Table 1).

4 | CONCLUSIONS

DS1260-2 is an improved germplasm line derived from exotic accession PI 587982A, with significantly lower levels of seed damage from fungi and weathering. Its improved tolerance to seed damage is manifest as lower total seed damage (DKT), lower incidence of *D. longicolla*, lower seed coat wrinkling, lower visual mold, lower incidence of fungal metabolites (nivalenol, cercosporin, cytochalasin H, cytochalasin J, tryptophol, fusaric acid, and beauvericin), and higher seed germination. These lower levels of seed damage will result in lower levels of grain dockage at elevators and again when sold to domestic and international processors. Higher levels of seed germination would allow DS1260-2 to produce viable seed in hot humid environments suitable for replanting; such conditions will be more prevalent in the future due to climate change. DS1260-2 is highly useful for developing cultivars with improved tolerance to mature seed damage that is caused by mold and weathering.

5 | AVAILABILITY

Seed of DS1260-2 is available immediately through material transfer agreement from the senior author (USDA-ARS, Crop Genetics Research Unit, P.O. Box 345, Stoneville, MS, rusty.smith@usda.gov), or by requests to the USDA ARS Germplasm Resource Information Network (GRIN) at https://npgsweb.ars-grin.gov/gringlobal/search?q=PI+705148, or by searching GRIN using the advanced search option with PI 705148. Seed of DS1260-2 was deposited into the USDA-ARS National Laboratory for Genetic Resources Preservation at Ft. Collins, CO, and into the soybean working collection at Urbana, IL, where it will be available for research purposes. We ask for appropriate recognition if seed of this germplasm line is used in the development of new germplasm lines and/or in the development and commercialization of new cultivars.

AUTHOR CONTRIBUTIONS

James R. Smith: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; supervision; validation; writing—original draft; writing—review and editing. **Anne M. Gillen**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; writing—original draft; writing—review and editing. **Shuxian Li**: Data curation; investigation; methodology; supervision; validation; writing—review and editing. **Hamed K. Abbas**: Conceptualization; data curation; investigation; methodology; supervision; validation; writing—original draft; writing—review and editing. **Michael Sulyok**: Data curation; formal analysis; investigation; methodology; validation; writing—review and editing.**W. Thomas Shier**: Data curation; formal analysis; investigation; methodology; validation; writing—review and editing. **Alemu Mengistu**: Data curation; investigation; methodology; validation; writing—review and editing. **Guohong Cai**: Data curation; formal analysis; investigation; methodology; validation; writing—review and editing. **Jason Gillman**: Conceptualization; investigation; methodology; validation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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