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REVIEW

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Synthesis, function, and genetic variation of sorgoleone, the major biological nitrification inhibitor in sorghum

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Abstract

Sorghum is the third most important food crop, grown on nearly 40 million ha globally, and is known for its resilience under unfavorable conditions. Sorghum is reported to have a strong biological nitrification inhibition (BNI) capacity in root systems, a plant function that suppresses soil nitrifier activity, which in turn prevents the nitrogen (N) loss by reducing nitrous oxide (N_2O), nitric oxide (NO) emission, and nitrate (NO_3^-) leaching into water bodies. Sorgoleone, a major hydrophobic phytochemical released from sorghum roots, provides a significant part of BNI function in sorghum. The function of sorgoleone in suppressing nitrifying bacteria in pure cultures has been established. In addition, sorgoleone suppresses transformation of ammonium (NH_3) to NO_3^- and N_2O emissions from soils. Therefore, introducing high-sorgoleone phenotype into elite sorghum hybrids can increase nitrogen use efficiency while decreasing the environmental footprint of sorghum production systems. In recent years, significant progress has been made in identifying the mechanisms of sorgoleone production and secretion. Moreover, studies using both wild accessions and elite breeding materials reported significant genetic variation for sorgoleone secretion, and sorgoleone secretion was found to be highly heritable, making it a

Abbreviations: AMO, ammonia monooxygenase; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; ARS, alkylresorcinol synthase; BNI, biological nitrification inhibition; CRE, *cis*-regulatory element; CRISPR, clustered regularly interspaced short palindromic repeats; CYP, cytochrome P450; DES, fatty acid desaturase; ER, endoplasmic reticulum; GHG, greenhouse gas; GWAS, genome-wide association study; HAO, hydroxylamine oxidoreductase; NASS, National Agricultural Statistics Service; NUE, nitrogen use efficiency; OMT, *O*-methyltransferase; PKS, polyketide synthase; QTL, quantitative trait loci; RFW, root fresh weight; RHL, root hair length; SNP, single-nucleotide polymorphism; UAV, unmanned aerial vehicle.

good target for breeding. This review distills the current understanding of sorgoleone release in relation to BNI function and opportunities to exploit this trait. Also, we provide our assessment for genetic interventions of Sorgoleone biosynthesis and secretion pathways to enhance BNI capacity in sorghum. High-BNI sorghum hybrids can be an important component of low-nitrifying, low-N_2O-emitting agricultural production systems that are eco-friendly, productive, and sustainable.

Plain Language Summary

Nitrogen is one of the most important nutrients for plants, and its availability directly affects the plant's productivity. In most ecosystems, mineral nitrogen available for plant roots is less than optimal. For this reason, many plant species evolved a mechanism to keep the ammoniacal nitrogen longer in the soil around their roots, termed biological nitrification inhibition. Enhancing such a trait in modern crop plants could increase nitrogen fertilizer efficiency and decrease nitrogen-containing pollutants such as greenhouse gases (nitrogen oxides) and water pollutant (nitrate) released from agricultural fields. In this review, we will focus on one such compound from sorghum, called sorgoleone. We summarize what is known about the synthetic pathway of sorgoleone, its function, and its potential as a reagent to increase nitrogen use efficiency and decrease N pollution. We will also discuss the future paths for enhancing sorgoleone secretion in commercially grown sorghum plants.

1 | INTRODUCTION

Nitrogen (N) is an essential nutrient required for all life on earth, including plants. In the modern agricultural production environment, soil nitrogen mineralized from organic material is usually not sufficient to support the yield levels expected in modern production systems. For this reason, the field applications of nitrogen fertilizer are linearly correlated with yield, and relatively cheap nitrogen fertilizer from the Haber–Bosch process was the biggest driver of yield increase in the 20th century (Smil, 2004).

However, after >100 years of chemical nitrogen fertilizer use, human society recognizes the need for nitrogen but must minimize the negative impacts of nitrogen use in agriculture. First, the production of N fertilizers consumes a substantial amount of energy. Approximately 2% of global energy is used for manufacturing N fertilizers, leading to considerable greenhouse gas (GHG) emissions at the stage of production (Rosa & Gabrielli, 2023). Also, the energy-intensive nature of nitrogen fertilizer production means that the cost of energy has a direct impact on nitrogen fertilizer prices, and global energy price spikes put N fertilizers out of the reach of smallholder farmers in developing countries. Second, N fertilizers have a serious impact on the environment. N fertilizer use in agriculture from the last seven decades led to a near doubling of reactive N in the environment compared to the pre-industrialization level (Erisman et al., 2011). N fertilizer, typically applied as ammonia/ammonium (NH_3/NH_4^+) is readily converted into nitrite (NO_2^-) and nitrate (NO_3^-) through a soil-biological process called nitrification, mediated by microorganisms (Van Huynh et al., 2023) (Figure 1).

Unlike the positively charged NH_4^+ molecule that can be absorbed by the soil's cation exchange capacity, nitrate anion leaches out of farmlands and into the surface and groundwater.

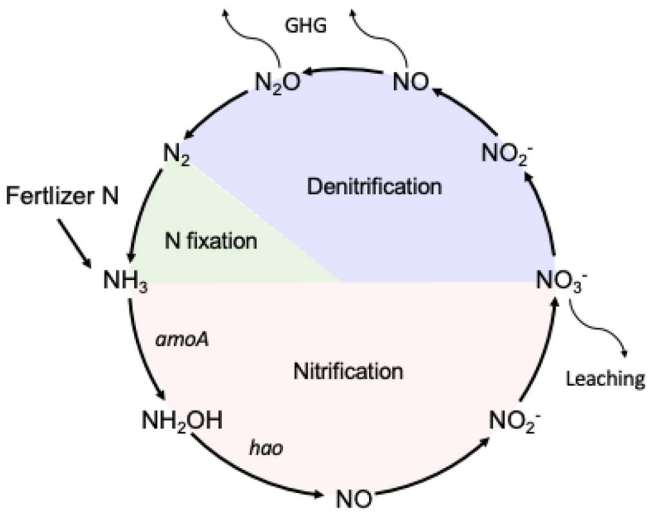

FIGURE 1 Nitrogen cycle. Reactions involved in N fixation, nitrification, and denitrification are marked with different background colors. Enzymes that are the main target of nitrification inhibitors are shown in italics. *amo*, ammonia monooxygenase; GHG, greenhouse gas; *hao*, hydroxylamine oxidoreductase.

This has global ecological implications, leading to problems such as eutrophication and the formation of dead zones and contamination of groundwater with nitrates. For example, the Gulf of Mexico is affected by a significant dead zone, fluctuating in size but covering >11,000 km^2 on average between 2019 and 2024 (NOAA, 2024). This dead zone is primarily caused by the excessive export of N from US croplands through rivers like the Mississippi. The fertilizers carried by the river into the Gulf of Mexico contribute to the growth of algal blooms, leading to hypoxia (low oxygen levels) and the subsequent formation of dead zones where marine life cannot survive. Moreover, nitrate that leaches into an anoxic environment (deeper soil layer or an aquatic system) can be reduced by various microorganisms to produce nitric oxide (NO) and nitrous oxide (N_2O) (denitrification). N_2O is a powerful GHG; it remains in the atmosphere for over 100 years as there are no significant natural sinks for N_2O, and it is 300 times more powerful compared to carbon dioxide (CO_2) (IPCC, 2014). In addition, N_2O is the main ozone-depleting chemical, surpassing the impact of chlorofluorocarbons in the 21st century after it was banned (USDA-NASS, 2024).

Nitrification is the first step required for the subsequent NO_3^-, NO, and N_2O generation; therefore, it is considered the starting point for N pollution in the environment. There are ways to prevent excessive nitrification from happening through agronomical practices such as precision N application, split N application, and the use of synthetic nitrification inhibitors. While these methods have proven effective in reducing N pollution, they come with additional costs for equipment and chemicals or additional labor (Norton & Ouyang, 2019).

Biological nitrification inhibition (BNI) is an alternative approach that relies on the plant's inherent ability to suppress nitrifying bacteria to limit nitrate production in the rhizosphere. Since mineral N is scarce in the vast majority of ecosystems, many plants have evolved sophisticated mechanisms to increase the retention of nitrogen in the root zone. One such mechanism is secreting specialized root metabolites that prevent nitrification, termed BNI (Subbarao et al., 2006, 2009). Initially predicted by the observation that the population of nitrifying bacteria is lower in the rhizosphere of some pasture grass species (Meiklejohn, 1968; Rice & Pancholy, 1973; Sylvesterbradley et al., 1988), later studies established that many plant species possess this ability, including *Brachiaria* (Gopalakrishnan et al., 2009; Subbarao et al., 2006, 2009), sorghum (Di et al., 2018; Nardi et al., 2013; Subbarao, Nakahara, et al., 2013; Tesfamariam et al., 2014), maize (Otaka et al., 2023, 2022; Petroli et al., 2023), rice (Lu et al., 2019; L. Sun et al., 2016; Tanaka et al., 2010), wheat (O'Sullivan et al., 2016; Subbarao et al., 2021), *Hibiscus splendens*, *Solanum echinatum* (Janke et al., 2018), and multiple species of weeds (O'Sullivan et al., 2017). Many of these phytochemicals are unrelated to each other yet target the same pathway, suggesting an evolutional benefit for nitrification inhibition. BNI, compared to synthetic nitrification inhibitors, offers a unique advantage in that no additional input or labor is required to suppress nitrification and subsequent N losses, hence making the adoption of BNI-crop-varieties or BNI-technology easier for farmers.

Core Ideas

- Sorgoleone, a specialized chemical secreted from sorghum root hair, is a potent nitrification inhibitor.
- High sorgoleone secretion is associated with decreased nitrification and reduced N_2O emission.
- Sorgoleone secretion is a quantitative and heritable trait, making it a good target for breeding.

BNI, however, has not been an active target of breeding efforts in any of the major crops thus far, because the yield benefit will be undetectable under an excessive supply of N. In addition, the inherent difficulty of phenotyping root traits has so far hindered high-throughput approaches that facilitate breeding efforts. However, recent advances in genomic marker availability and predictions, as well as advances in genetics tools, have made it possible to target BNI specifically in cultivar development. In this review, we will focus on sorgoleone, the main hydrophobic BNI component secreted from sorghum roots. Sorghum shows the strongest BNI activities among commonly cultivated crops (Subbarao et al., 2007), of which sorgoleone is the major compound (Di et al., 2018). Importantly, sorgoleone secretion shows a clear negative correlation with nitrification rate (Gao et al., 2022; Tesfamariam et al., 2014). Sorgoleone is one of the best-characterized BNI compounds in terms of biochemical pathways for production, and the genetic tools in sorghum have advanced dramatically in recent years. We will summarize the research on sorgoleone BNI function using genetics and breeding approaches and will lay out the roadmap forward for improving BNI function in sorghum through increased secretion of sorgoleone from its roots.

1.1 | Sorgoleone biosynthesis and secretion

1.1.1 | History of sorgoleone research

Sorgoleone, which is the main component of yellow/brown droplets secreted from the root hair of *Sorghum bicolor*, was first documented in 1986 (Netzly & Butler, 1986) in search of the active allelopathic compound specific to sorghum. Shortly after this first report, the same group identified the chemical structures of sorgoleone and its reduced form, dihydrosorgoleone, and also reported that dihydrosorgoleone stimulates germination of parasitic weed, that is, *Striga asiatica* (Chang et al., 1986). However, later studies found that sorgolactone,

which belongs to the family of plant hormone strigolactone, is active at a far lower concentration ($\sim 10^{-9}$ M, compared to 10^{-6} M for dihydrosorgoleone) (Wigchert & Zwanenburg, 1999). In addition, dihydrosorgoleone is quickly oxidized to sorgoleone, which does not have any stimulatory activity. Therefore, the function of dihydrosorgoleone as a Striga germination stimulant has not been investigated very actively since then.

Sorgoleone is also known to possess allelopathic properties. Several earlier studies reported that sorghum suppresses weed growth (Einhellig & Leather, 1988; Einhellig & Rasmussen, 1989; Putnam et al., 1983), and sorgoleone was identified as the major component in sorghum root exudate, which conferred a weed suppression effect (Einhellig & Souza, 1992; Kagan et al., 2003). The target site of sorgoleone for its allelopathic effect is not completely understood but likely involves multiple targets, such as photosystem II (Gonzalez et al., 1997), mitochondrial respiratory chain (Rasmussen et al., 1992), and root H^+-ATPase (Hejl & Koster, 2004). For further information on sorgoleone's allelopathic functions, the readers are referred to excellent reviews published previously (Hussain et al., 2021; Weston et al., 2013).

BNI activity in sorghum was reported by an earlier study, identifying sorghum as one of few commonly cultivated cereal crops that have detectable BNI activity within the elite germplasms (Subbarao et al., 2007). Later studies identified sorgoleone as the main component of the hydrophobic BNI activity secreted from sorghum roots (Subbarao, Nakahara, et al., 2013; Tesfamariam et al., 2014). Due largely to the interest in this compound as an allelopathic agent, the pathway for sorgoleone biosynthesis has been elucidated (see below), making sorgoleone one of few BNI compounds for which the genes involved in biosynthesis are known.

1.1.2 | Sorgoleone biosynthetic pathway

Sorgoleone (2-hydroxy-5-methoxy-3-[(*Z*,*Z*)-8′,11′,14′-pentadecatriene]-*p*-benzoquinone) is a benzoquinone alkylresorcinolic allelochemical synthesized exclusively in root hair cells of *Sorghum spp*. (Baerson et al., 2008; Weston et al., 2013). Sorgoleone belongs to the phenolic lipid family of compounds, which includes alkylphenols, alkylresorcinols, anacardic acids, and alkylcatechols. Alkylresorcinols represent the most prevalent phenolic lipid subfamily in nature and have been identified in numerous plants, fungal and bacterial taxa, but relatively few animal species (Kozubek & Tyman, 2005). These compounds are typically produced in plants as homologous mixtures possessing side chains of 13–27 carbons with varying degrees of saturation, in contrast to fungi and bacteria, which produce mixtures exclusively containing saturated side chains (Kozubek & Tyman, 1999). Sorgoleone has been shown to account for approximately 40%–90% of the root exudate material (w/w) in various *S. bicolor* accessions, with the remaining exudate consisting primarily of 4,6-dimethoxy-2-[(*Z*,*Z*)-8′,11′,14′-pentadecatriene] resorcinol (methoxy-dihydrosorgoleone) and sorgoleone congeners (Weston et al., 2013). Many alkylresorcinolic derivatives possess significant antimicrobial activity, which has led to the suggestion that these compounds primarily serve defensive roles in nature. Additionally, alkylresorcinols typically accumulate in layers surrounding plant structures, for example, within cuticle layers surrounding the seed coats of some cereal grains (Landberg et al., 2008; Ross et al., 2003), as well as in leaf cuticles (Ji & Jetter, 2008). Therefore, they may also play a critical role in the formation of defensive chemical barriers (Baerson et al., 2010).

The biosynthesis of phenolic lipids occurs through a convergence of fatty acid and polyketide synthase pathways, involving specialized type III polyketide synthase enzymes utilizing fatty acyl-CoA starter units, referred to as alkylresorcinol synthases (ARSs) (Austin & Noel, 2003; Miyanaga & Horinouchi, 2010). Initial labeling studies indicated that the aromatic moiety within sorgoleone's structure was likely derived from an ARS utilizing a $16{:}3D^{9,12,15}$ fatty acyl-CoA precursor (Fate & Lynn, 1996). This hypothesis was later confirmed with the functional characterization of two root hair-specific ARSs, SbARS1 and SbARS2 (Cook et al., 2010). To date, all of the enzymes required for the biosynthesis of dihydrosorgoleone from the precursor palmitoleoyl-CoA have been identified through the analysis of sequences expressed in root hair cells isolated from *S. bicolor* genotype BTx623 (Baerson et al., 2008; Yang, Owens, et al., 2004).

Sorgoleone biosynthesis initiates with the generation of the hexadecatrienyl moiety ($16{:}3D^{9,12,15}$) from the ubiquitous precursor palmitoleoyl moiety ($16{:}1D^{9}$). Two root hair-specific fatty acid desaturases, designated SbDES2 and SbDES3, were found to sequentially introduce double bonds at the Δ^{12} and Δ^{15} positions, respectively, yielding $16{:}3D^{9,12,15}$ (Z. Q. Pan et al., 2007) (Figure 2). Both SbDES2 and SbDES3 are highly specific to root hairs (Z. Q. Pan et al., 2007; Yang, Owens, et al., 2004). The work involved heterologous co-expression of the two *S. bicolor* desaturase sequences in *S. cerevisiae*, as SbDES2 was found to convert endogenous palmitoleic acid ($16{:}1D^{9}$) to hexadecadienoic acid ($16{:}2D^{9,12}$), thus providing a substrate for the generation of hexadecatrienoic acid ($16{:}3D^{9,12,15}$) by co-expressed recombinant SbDES3 (Z. Q. Pan et al., 2007). Interestingly, the Δ^{15} desaturation performed by SbDES3 produces an unusual terminal double bond, thus identifying a potentially commercially valuable property for this enzyme.

The hexadecatrienyl-CoA ($16{:}3D^{9,12,15}$) intermediate generated through SbDES2 and SbDES3 activities is subsequently used as a starter substrate by the root hair-specific ARSs SbARS1 and SbARS2 (Cook et al., 2010) (Figure 2). As mentioned, plant ARSs are members of the type III polyketide

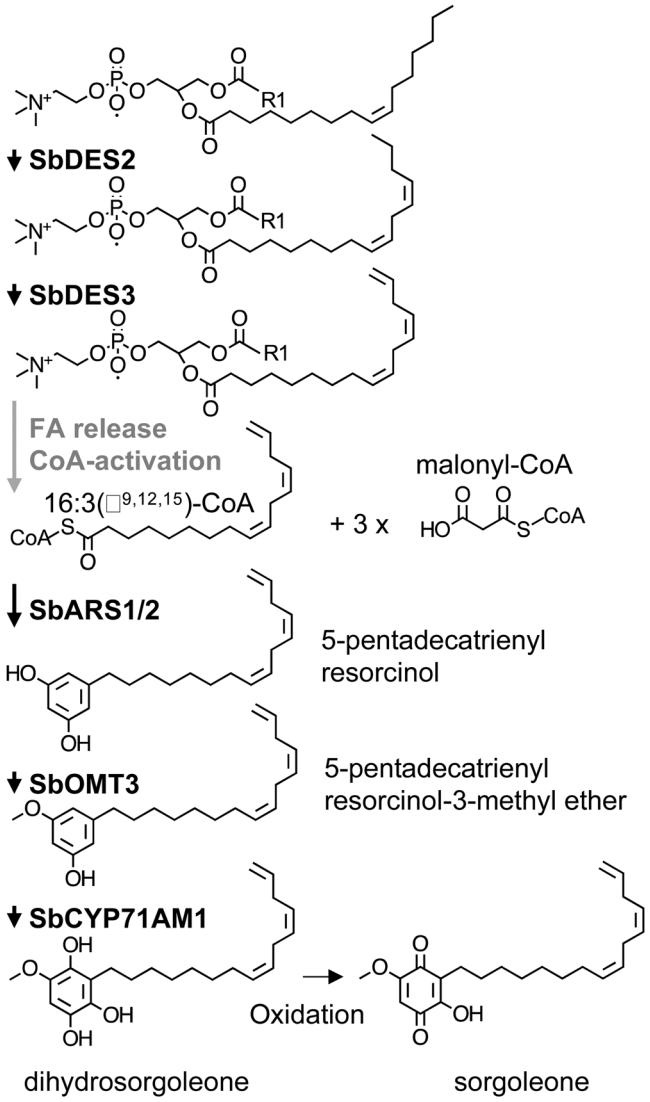

FIGURE 2 Proposed biosynthesis pathway of sorgoleone. Previously published enzymes are indicated in bold. The step for which the enzymes are unidentified is shown in gray. The figure has been modified from previously published work (Maharjan et al., 2023).

synthase (PKS) plant family of enzymes, which typically use malonyl-CoA extender units and perform iterative condensation reactions, followed by cyclization, for the generation of aromatic moieties. Enzymes from different sources may differ in the types of starter substrates utilized, the number of condensations performed, and the type of intramolecular cyclization mechanism used, all contributing to the great diversity of polyketide-derived compounds produced by plants. ARSs, which produce 5-*n*-alkylresorcinols utilize a stilbene synthase-type cyclization mechanism involving a C2→C7 aldol condensation, in contrast to chalcone synthase-type cyclization occurring via a C6→C1 Claisen condensation (Austin & Noel, 2003; Cook et al., 2010). With specific acyl-CoA starters, ARSs may also generate pyrone by-products via intramolecular C5 oxygen→C1 lactonization (Funa et al., 2007, 2006; Funabashi et al., 2008; Goyal et al., 2008). Recombinant enzyme studies demonstrated the ability of both SbARS1 and SbARS2 to generate 5-*n*-alkylresorcinols using a variety of short-, medium-, and long-chain acyl-CoA starter molecules when provided with malonyl-CoA as an extender. Importantly, both recombinant enzymes generated the 5-pentadecatrienyl resorcinol, sorgoleone pathway intermediate, when provided with the proposed physiological substrate hexadecatrienyl-CoA (16:3$\Delta^{9,12,15}$). Further evidence for their role in the sorgoleone biosynthetic pathway was obtained from RNA interference experiments directed against *SbARS1* and *SbARS2*, which resulted in multiple independent transformant events exhibiting dramatically reduced sorgoleone levels (Cook et al., 2010). At present, information on higher plant ARSs is quite limited, with several enzymes characterized from *Oryza sativa* exhibiting similar substrate profiles as SbARS1 and SbARS2 (Cook et al., 2010; Matsuzawa et al., 2010), and more recently, an ARS enzyme has been identified from *Secale cereale*, which accepts long-chain and very long-chain acyl-CoA starters and is proposed to play a role in cuticle formation (Y. L. Sun et al., 2020). Type III PKS enzymes producing 5-n-alkylresorcinols or alkylresorcilic acids have also been identified from soil-associated microbes, including *Azotobacter vinelandii*, *Streptomyces griseus*, and *Neurospora crassa* (Funa et al., 2006; Funabashi et al., 2008; Goyal et al., 2008), as well as from cyanobacteria (Costa et al., 2019). A 2′-oxoalkylresorcinol synthase has also been characterized from the moss *Physcomitrella patens*, proposed to contribute to the development of the gametophytic and sporophytic cuticle layers (Aslam et al., 2022).

Subsequent steps in sorgoleone biosynthesis involve a series of aromatic ring modification reactions, beginning with an *O*-methylation occurring at the 3′ position within the ring moiety (Baerson et al., 2008). A root hair-specific *O*-methyltransferase (OMT) identified within the *S. bicolor* Btx623 data set (designated SbOMT3) was shown to preferentially utilize alkylresorcinolic substrates among a panel of diverse phenolic substrates tested (Figure 2). Significantly, SbOMT3 was also shown to be capable of generating the 5-pentadecatrienyl resorcinol-3-methyl ether, sorgoleone pathway intermediate, when provided with the proposed physiological substrate 5-pentadecatrienyl resorcinol, strongly suggesting a role for this enzyme in the biosynthesis of sorgoleone (Baerson et al., 2008). To our knowledge, other AdoMet-dependent alkylresorcinol OMTs have not been described in plants to date; however alkylresorcinol-utilizing OMT enzymes have been identified in bacteria (Costa et al., 2019) as well as in *Dictyostelium* (Ghosh et al., 2008).

The final enzyme-mediated reactions involve the di-hydroxylation of 5-pentadecatrienyl resorcinol-3-methyl ether at the two ortho positions (C4 and C6) relative to the aliphatic side chain, resulting in the conversion of the intermediate into dihydrosorgoleone (a

hydroquinone: 5-methoxy-3-((8Z,11Z)-pentadeca-8,11,14-trien-1-yl)benzene-1,2,4-triol), the direct precursor to sorgoleone (Z. Q. Pan et al., 2018). This chemically unstable hydroquinone rapidly oxidizes upon rhizosecretion to the bioactive benzoquinone sorgoleone, which can persist in soil for extended periods (Weston et al., 2013). A root hair-specific bifunctional cytochrome P450 (CYP) monooxygenase has recently been identified, which can convert 5-pentadecatrienyl resorcinol-3-methyl ether to dihydrosorgoleone when heterologously expressed in *S. cerevisiae*, performing hydroxylation at both the 4′ and 6′ positions within the aromatic moiety. RNAi-mediated repression of the corresponding sequence in *S. bicolor* transformants resulted in decreased sorgoleone contents in multiple independent events, thus strongly suggesting a role for this P450 enzyme in sorgoleone biosynthesis (Z. Q. Pan et al., 2018). This P450 enzyme is positioned within a subfamily of the plant-specific CYP71 clan and has been designated CYP71AM1 (Figure 2). CYP71 members comprise the largest CYP clan, representing more than half of all higher plant CYPs (Nelson & Werck-Reichhart, 2011; Nelson et al., 2004). Biochemically characterized CYP71 members include CYP71BL1 and CYP71DD6, shown to be involved in the biosynthesis of eupatolide and inunolide, respectively (Frey et al., 2018). Importantly, the predicted reaction sequence for sorgoleone biosynthesis, wherein 3′*O*-methylation of the aromatic ring precedes 4′,6′-di-hydroxylation by CYP71AM1, is further supported by the detection of the proposed 5-pentadecatrienyl resorcinol-3-methyl intermediate in *S. bicolor* root hair tissues (Baerson et al., 2008).

Identification of sorgoleone biosynthesis enzymes enabled the assessment of cellular compartments that are involved in sorgoleone biosynthesis. The subcellular localization study of enzymes involved in the biosynthesis of sorgoleone showed that SbARS1 and SbOMT3 localize in the cytosol, whereas SbDES2, SbDES3, and SbCYP71AM1 localize in the ER (Maharjan et al., 2023). Furthermore, ARS1 interacted with DES2 and 3, supporting the formation of a multi-enzyme complex on the ER surface. This interaction likely explains why sorgoleone is disproportionally derived from $16{:}3^{\Delta 9,12,15}$, whereas recombinant ARS1 does not discriminate between fatty acid CoA with different chain lengths or unsaturated bonds (Cook et al., 2010).

1.1.3 | Cellular secretion of sorgoleone

The results above identified the endoplasmic reticulum (ER) as the site of sorgoleone biosynthesis. Transmission electron micrograph of sorghum root hair showed extensive layers of smooth ER adjacent to the plasma membrane, also suggesting their involvement in sorgoleone biosynthesis (Czarnota et al., 2003). From the ER, the compound will need to be transported to the plasma membrane and then secreted to the extracellular domain. The molecular mechanisms involved in this process are unknown. Earlier studies reported droplet-like inclusions inside the sorghum root hair cells and hypothesized that these vesicles contain dihydrosorgoleone/sorgoleone on the path to be secreted from the plasma membrane through the exocytotic pathway (Czarnota et al., 2003; Weston et al., 2013). However, temporal analysis of the intracellular vesicles and the expression of sorgoleone biosynthesis enzymes revealed that these vesicles likely contain sorgoleone precursors rather than sorgoleone themselves (Maharjan et al., 2023).

While the results above do not exclude exocytotic pathways where sorgoleone could be packaged directly from the ER membrane and brought to the plasma membrane, these data suggest that alternative pathways might be in play for sorgoleone secretion. One such alternative proposed pathway comes from molecular dynamics simulation of sorgoleone and selected precursors, which found that sorgoleone and its related molecules can readily transit from one membrane leaflet to the other and are highly lipophyllic (Raza et al., 2023). Thus, if the final biosynthesis enzymes are cytosolic and ER-localized, which has been found via confocal microscopy (Maharjan et al., 2023), there exists the possibility that sorgoleone biosynthesis can be completed in the outer leaflet of the ER prior to export.

Regardless of the mechanism by which sorgoleone or its precursors make their way to the cell surface, at some point sorgoleone needs to leave the membrane to create organic droplets that can be released into the soil. These droplets have been measured to be on the multi-micrometer scale (Dayan et al., 2009) and have mixed dihydrosorgoleone/sorgoleone compositions. Simulations have demonstrated that these droplets can be stable above a lipid bilayer if they are large enough and have shown that there is a minimal energy difference between sorgoleone in the membrane and sorgoleone in an organic phase (Raza et al., 2023). Thus, once a nascent droplet is established, the expectation is that the droplet could continue to grow via passive means without invoking an energy-intensive transport process. However, the initial extraction of sorgoleone or its precursors from a lipid bilayer is energetically unfavorable (Raza et al., 2023) and may require an active transport mechanism to nucleate an initial droplet. At this stage, a specific protein for sorgoleone droplet nucleation has not been determined. ATP-binding cassette transporters, many of which are involved in exporting lipidic compounds (Bessire et al., 2011; Pighin et al., 2004; Xu et al., 2010; Yadav et al., 2014), could be involved in this process. Alternatively, MATE efflux family proteins (Kuk et al., 2019) and Major Facilitator Submfamily proteins (Saier & Paulsen, 2001) involved in xenobiotic extrusion could also be involved. Once discovered, these genes can also be targets for genetic manipulation and haplotype discovery.

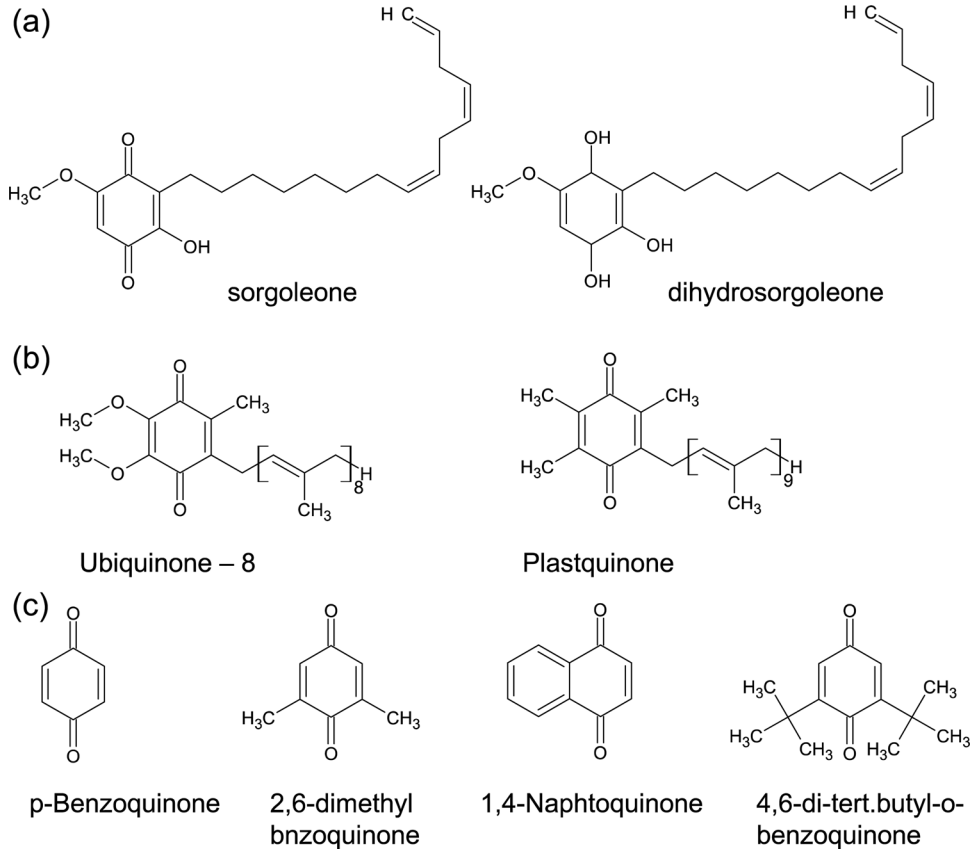

FIGURE 3 (a) Structures of sorgoleone and dihydrosorgoleone. (b) Benzoquinones involved in membrane electron transport. (c) Other benzoquinones with nitrification inhibition activities.

1.2 | The activity of sorgoleone as a BNI compound

1.2.1 | Structure of sorgoleone and potential mode of function

The structure of sorgoleone is similar to other biologically essential benzoquinones such as ubiquinones and plastoquinones (Figure 3). Ubiquinone-8 is required for the electron transfer to ammonia monooxygenase (AMO) during nitrification, as well as for mediating the membrane electron transfer required for energy production from nitrification (Hooper et al., 2004; Whittaker et al., 2000). Therefore, it is tempting to hypothesize that sorgoleone inhibits the binding of ubiquinone or its reduced form to proteins that utilize them as the electron donor or acceptor. Indeed, other benzoquinones and related compounds such as p-benzoquinone, 2,6-dirnethylbenzoquinone, 1,4 naphtoquinone, and 4,6-di-tert-butyl-o-benzoquinone show potent nitrification inhibition activities (Mishra & Flaig, 1979; Rodgers & Ashworth, 1982; Suarez-Ojeda et al., 2010).

Since AMO structure is still largely unknown due to the inherent difficulties in isolating this membrane protein, it is not clear whether sorgoleone indeed binds to AMO (Lancaster et al., 2018). Sorgoleone-induced inhibition, unlike more specific inhibitors of AMO such as allylthiourea (AT), is not recovered by adding hydroxylamine, suggesting that sorgoleone's inhibitory activity acts upon hydroxylamine oxidoreductase (HAO) rather than AMO (Subbarao, Nakahara et al., 2013). Since HAO structure has been resolved (Cedervall et al., 2013; Igarashi et al., 1997; Maalcke et al., 2014) and a relatively easy assay for HAO inhibition is now available (Nishigaya et al., 2016), it would be interesting to clarify whether sorgoleone directly binds HAO to inhibit the reaction.

Aside from AMO and HAO, completion of nitrification requires intricate transfer of electrons harvested from HAO, then AMO, to the electron transport chain via the membrane ubiquinone pool (Whittaker et al., 2000). One of the acceptors of electrons through ubiquinone/ubiquinol is the cytochrome *bc1* complex, a vital component of not only nitrification but also respiration found in mitochondria and the vast majority of aerobic bacteria. In this context, it is interesting to note that sorgoleone disrupts mitochondrial electron transport. From pharmacological and spectroscopic studies, it was suggested that sorgoleone inhibits electron flow between cytochrome b and c1 within complex III, the step also targeted by other respiratory inhibitors that are quinones (Rasmussen et al., 1992). Also considering the fact that photosystem II inhibition

by sorgoleone likely occurs at the plastquinone binding site (Gonzalez et al., 1997), sorgoleone might represent a general membrane electron transport inhibitor that interferes with the binding of endogenous benzoquinones. Further experiments, such as those using photoaffinity probe to label the protein directly interacting with the compound (Kroll et al., 2023), would be necessary to narrow down the functional target of sorgoleone.

1.2.2 | The impact of sorgoleone on soil nitrifier microbiome, nitrate formation, and N_2O emissions

In vitro studies involving soil microcosm laboratory incubation studies suggest substantial reductions in nitrification rates (about 50% reduction) in soils amended with sorgoleone (Tesfamariam et al., 2014) and in the rhizosphere soil of high-sorgoleone genotypes (Gao et al., 2022). Also, similar studies showed a reduction of N_2O emissions (>50%) in sorgoleone-amended soils (M. Zhang, Gao, et al., 2023). Further studies suggested reduced N_2O emissions were observed from root-zone soils collected from field-grown plants of sorghum; higher levels of suppression in N_2O emissions were observed in high-sorgoleone-producing sorghum genetic stocks compared to low-sorgoleone producing genetic stocks from glasshouse experiments involving pot-grown plants (Gao et al., 2022). This suggests that breeding for higher amounts of sorgoleone release from roots can lead to low-N_2O-emitting sorghum varieties in the future. Soil nitrifier populations of both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were suppressed in the root zone of sorghum (Subbarao, Nakahara et al., 2013); however, sorghum appears to inhibit AOA populations more strongly compared to AOB populations (Gao et al., 2022). This is in conformity to what has been reported in other crops such as wheat and *Brachiaria* pastures, where BNI function has strongly suppressed AOA populations compared to AOB populations in the root zone (Subbarao et al., 2021, 2009).

1.2.3 | Modification of sorgoleone secretion by the soil environment and management

It is well documented that BNI activities are often enhanced by NH_4^+ in different plant species, including sorghum (Pariasca Tanaka et al., 2010; Subbarao et al., 2009; Subbarao, Nakahara et al., 2013; Zakir et al., 2008; Zeng et al., 2016). Part of the stimulatory effect of NH_4^+ can be due to root zone acidification through NH_4^+ uptake and/or H^+-ATPase activation, but an experiment using a pH-stat system to stabilize the root zone pH showed that in sorghum, NH_4^+ stimulates secretion of BNI activity independent of the change in pH (Subbarao, Nakahara et al., 2013). However, while the secretion of hydrophilic BNI activity is clearly induced by NH_4^+, secretion of sorgoleone did not change with either the external NH_4^+ concentration (Subbarao, Nakahara et al., 2013) or pH (Di et al., 2018). Therefore, sorgoleone-mediated BNI is expected to be relatively stable across different environments and management systems (i.e., the type of N fertilizer applied). On the other hand, the contribution of sorgoleone to the total root BNI likely changes depending on the soil environment and management due to the altered contribution from other BNI compounds. Therefore, evaluating BNI activities in multiple production environments will be critically important.

1.3 | Value of sorgoleone in production environments

High surgeon genotypes could offer substantial production, environmental, and economic benefits by reducing fertilizer needs, as well as lowering N_2O emissions and nitrate leaching from croplands. The BNI trait in sorghum can improve nitrogen use efficiency (NUE), potentially allowing for reduced fertilizer application without compromising yield. Studies by He et al. (2018) showed that N fertilizer rates in wheat could be reduced by 25% without affecting grain yield when a nitrification inhibitor was used. This led to a 16.7% increase in NUE in wheat compared to full N application treatment. If sorgoleone in sorghum achieves similar results, it could significantly reduce fertilizer use and improve NUE.

The scale of this potential impact is significant when considering the extent of sorghum production in the U.S. and around the globe. According to the National Agricultural Statistics Service (NASS), 25,940 km^2 of sorghum were planted in the United States in 2024 (USDA-NASS, 2024), making it the largest producer of sorghum in the world. Given this large area under production, even modest improvements in NUE could translate into substantial reductions in fertilizer use. According to a 2019 agricultural chemical use survey by NASS, 89% of sorghum-planted acres in the United States received N fertilizer, amounting to 163,293 metric tons of N applied. A reduction in this input of N fertilizers could lead to meaningful economic savings for farmers, while also mitigating the environmental footprint of sorghum production.

In tandem with these agronomic benefits, the rise of ecosystem service markets in the United States in recent years offers a new avenue for providing incentive payments to agricultural producers who adopt practices that achieve GHG emission reductions, enhance water quality, promote biodiversity, and sequester soil carbon (Biggs et al., 2021; Lichtenfels et al., 2021; Reed, 2020). Evidence from other crops suggests that these benefits are achievable in BNI-based production

systems. For example, studies conducted with *Brachiaria humidicola*, a tropical forage grass known for its BNI properties, have shown significant reductions in N_2O emissions compared to fields planted with other crops (Byrnes et al., 2017; Lichtenfels et al., 2021; Subbarao, Rao et al., 2013). Since N_2O is produced during nitrification and denitrification processes, the natural suppression of nitrifying microbes by high sorgoleone levels in sorghum could result in similar reductions in emissions. This would not only provide environmental benefits but also position sorghum growers to take advantage of financial incentives offered through ecosystem service markets.

However, to fully realize the potential of BNI as a source of supplemental income for farmers, it will be necessary to conduct comprehensive studies that integrate sorghum management practices with GHG reduction strategies. These studies should aim to quantify the reductions in emissions and water quality improvements associated with sorgoleone-based BNI traits, ensuring that these outcomes can be credibly measured and translated into marketable ecosystem services. In addition, for BNI-based ecosystem services to gain widespread adoption among US sorghum growers, substantial outreach efforts will be required. Farmers will need to be informed about the economic opportunities available through ecosystem service markets through the planting of BNI crops on their farms.

1.4 | Improvement in sorgoleone secretion

The above studies collectively indicate that increasing sorgoleone secretion could help mitigate N_2O emission and NO_3^- leaching from sorghum fields. However, current elite hybrids have not been selected for this trait, and there is minimal knowledge regarding sorgoleone secretion in high-yield sorghum germplasm and its impact on N_2O emission and NO_3^- leaching. In this section, we discuss the two approaches (breeding and biotechnology) that can increase sorgoleone secretion, given the currently available knowledge and resources.

1.4.1 | Breeding

Multiple groups reported variations in sorgoleone secretion among different accessions, and recent studies further demonstrated that the trait is moderately to highly heritable, suggesting that this trait can be increased through breeding (Besançon et al., 2020; Czarnota et al., 2001; Maharjan et al., 2024; Sarr et al., 2020; Subbarao et al., 2015; Tesfamariam et al., 2014; Tibugari et al., 2019; Uddin et al., 2013). The current state of knowledge on the natural variation and heritability of sorgoleone, as well as the future paths for increasing sorgoleone secretion, is summarized below.

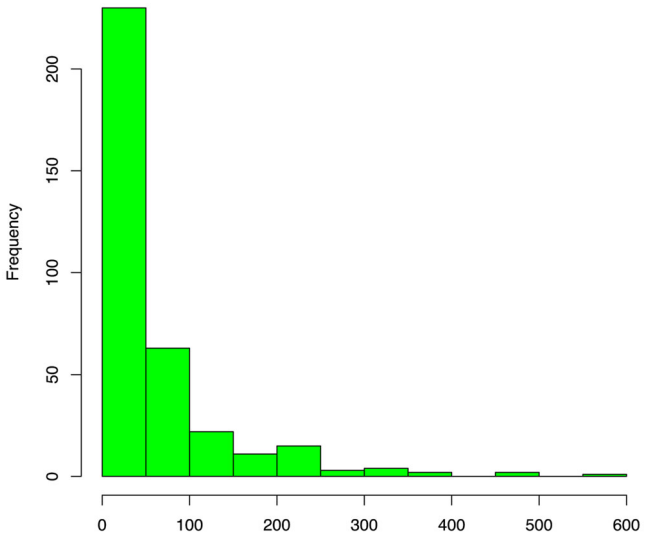

FIGURE 4 Frequency distribution for sorgoleone quantity across 348 genotypes from Southern African countries. Histogram was drawn using means as reported in Tibugari et al. (2019).

Genetic variation for sorgoleone production

Previous studies have shown that most sorghum accessions and some related species (e.g., johnsongrass and shattercane) secrete sorgoleone to varying degrees (Table 1) (Czarnota et al., 2001; Tibugari et al., 2019; Uddin et al., 2013). In the most comprehensive study for grain sorghum (*S. bicolor*) so far, 348 sorghum genotypes from Africa were phenotyped to reveal a wide range of sorgoleone production (0–584.69 μg/mg root fresh weight [RFW]) (Tibugari et al., 2019). Only seven genotypes had no measurable levels of sorgoleone. The frequency distribution of the same dataset shows continuous variation for the trait (Figure 4), with 84% of the genotypes (293) reporting sorgoleone concentration <100 μg/mg RFW. All of the 60 genotypes that reported ≥100 μg/mg RFW were landraces except two genotypes (IBS731 and IBS738). Given the wide variation found in landraces and wild accessions, studies using a mapping population or genome-wide association are likely to be effective in identifying genetic regions underlying the difference in sorgoleone secretion. Integration of such regions from wild accessions to elite lines provides a path to increasing sorgoleone secretion in elite germplasms.

Sorgoleone secretion in adapted/commercial varieties

A comprehensive screening of elite hybrids and inbred parents for sorgoleone secretion was not completed until recently. Using a structured set of 158 sorghum hybrids produced from 42 parental inbred lines, Marharjan et al. (2024) documented substantial variation (~8- and ~20-fold difference among the parental lines and hybrids, respectively) for sorgoleone secretion from 7-day-old seedlings. Sorgoleone secretion had high broad-sense heritability (H^2 = 0.87) and moderate to

TABLE 1 Reported concentrations of sorgoleone from sorghum roots at different growth stages.

Species	Number of genotypes	Specific sorgoleone (μg/mg root DW)		Specific sorgoleone (μg/mg root FW)		Reference
		5–7 days	25 days	5–7 days	10–14 days	
S. bicolor	2	1.6–2.4	1.5 – 5.0	N/A	N/A	Tesfamariam et al. (2014)
S. bicolor	36	N/A	N/A	0.06–0.75	N/A	Besançon et al. (2020)
S. halepense	1	N/A	N/A	N/A	0.64	Besançon et al. (2020)
S. bicolor ssp. *drumondii*	8	N/A	N/A	N/A	0.27–1.46	Besançon et al. (2020)
S. bicolor	17	N/A	N/A	0.33–6.98	N/A	Uddin et al. (2009)
S. bicolor ssp. *drumondii*	1	N/A	N/A	0.5	N/A	Czarnota et al. (2003)
S. halepense	1	N/A	N/A	N/A	14.75	Czarnota et al. (2003)
S. bicolor	5	N/A	N/A	1.33–1.85	N/A	Czarnota et al. (2003)
S. bicolor	348	N/A	N/A	0.0–584.69	N/A	Tibugari et al. (2019)
S. bicolor	3	5.6–19.1	N/A	N/A	N/A	Sarr et al. (2020)
S. bicolor	200	1.14–24.5	N/A	N/A	N/A	Maharjan et al. (2024)

high narrow-sense heritability ($h^2 = 0.76$) equally divided among seed ($h_f^2 = 0.39$) and pollinator ($h_m^2 = 0.35$) parents. These results indicate that selection for increased sorgoleone exudation in elite germplasm should be successful, presuming that sufficient numbers of progeny can be phenotyped. Consequently, the development of commercially viable high-sorgoleone hybrids might not require extensive efforts to introgress the trait from wild relatives.

Challenges in phenotyping

One significant challenge in breeding for sorgoleone secretion is the difficulty in phenotyping. Currently, the standard method involves growing the sorghum lines in semi-hydroponic culture, followed by extraction with organic solvents and quantification with chromatography. This process provides a throughput of roughly ~50 lines per week/person, which is sufficient for obtaining data from the target populations, such as genome-wide association study (GWAS) panels, nested association mapping, or recombinant inbred lines populations. The limitation of this lab-based assay is that it can only be performed for relatively young plants, and phenotyping at later stages becomes technically challenging due to the requirement for a hydroponic or semi-hydroponic method to harvest intact roots. Nevertheless, there is some documentation of high-secreting cultivars found in seedling screens showing high sorgoleone secretion and BNI activities in field conditions (Gao et al., 2022; Subbarao, Nakahara et al., 2013). Therefore, the lab assays provide a good entry point to assess the potential for different lines (Sarr et al., 2020).

However, the method described above is not practical for screening thousands of lines that might be found in a breeding program or a large germplasm collection. Some phenotypes that can be either remotely sensed or processed at a higher throughput would be useful as a proxy for sorgoleone secretion. For example, BNI is expected to promote N retention and N use efficiency, which will positively affect the plant's N content and performance at low-N conditions (Sarr et al., 2021). Multispectral indices acquired by unmanned aerial vehicle can be used to estimate aboveground N content (Dal Lago et al., 2024; Patel et al., 2024). Alternatively, leaf nitrate reductase activity and nitrate concentration might be useful criteria, since they are negatively correlated with BNI activity (Karwat et al., 2019). However, these suggested alternatives have only been tested with limited genetic stocks, and it is not clear whether a correlation between nitrate reductase activity and BNI is significant in a larger population. To evaluate the usefulness of these proxies, it would be valuable to test the set of lines and cultivars for which laboratory-based sorgoleone secretion amounts are available, such as those established in recent studies (Maharjan et al., 2024).

Genomic selection

Given the challenges of phenotyping the trait, selection for increased sorgoleone exudation will require alternative methods to direct selection. While the biosynthetic pathway of sorgoleone has been documented (Baerson et al., 2008; Cook et al., 2010; Z. Q. Pan et al., 2018, 2007), no markers have been used to facilitate selection. Further, given the quantitative variation observed in several studies (Maharjan et al., 2024; Sarr et al., 2020; Tesfamariam et al., 2014; Tibugari et al., 2019; Uddin et al., 2013), it is unlikely that individual molecular markers per se will be effective at further increases, especially given the length of time and costs associated with phenotyping.

An alternative approach to the improvement of sorgoleone exudation is the use of genomic selection. Originally applied in animal breeding programs, genomic selection evolved as a mechanism to predict the breeding values of genotypes for quantitative traits that are expensive or time-consuming to phenotype (Crossa et al., 2017; Georges et al., 2019).

Genomic prediction is based on the use of 1000 of genetic markers (typically single-nucleotide polymorphism [SNP] markers) distributed throughout the genome and developing a predictive breeding value for an individual based on a previously developed training model that integrated both genotypic and phenotypic data. The ability to generate genomic-estimated breeding values of new and existing breeding lines reduces the need to phenotype every new genotype in a selection nursery.

To breed for sorgoleone exudation using genomic prediction requires the development of genomic prediction models. Training models are developed by phenotyping a set of lines and hybrid for sorgoleone exudation and merging that phenotypic data with genotypic SNP data to build a prediction model using one of the myriads of statistical approaches to build the models. One method, genomic best linear unbiased prediction, is a process by which the genetic relationship between individuals can be used to estimate the performance of individuals prior to collecting data (Hayes et al., 2009; VanRaden, 2008).

Genomic prediction models have been developed in sorghum for several different traits, including grain yield, days to anthesis, plant height, and grain composition. Initial studies on genomic prediction models in sorghum indicated that genomic selection could be effective (de Oliveira et al., 2018; Hunt et al., 2018; Sapkota et al., 2020), but these studies focused on breeding value of lines. Given that sorghum is grown as a hybrid crop, more recent reports have focused on predicting general combining ability and specific combining ability values of both inbred lines and hybrids (Crozier et al., 2024; Fonseca et al., 2021; Winans et al., 2023). Initial efforts to build genomic prediction models for sorgoleone exudation are now underway.

1.4.2 | Biotechnology approaches

Breeding efforts described above will likely provide the fastest path to increased BNI in sorghum, due to well-established pipelines from the germline development to commercial licensing. However, the breeding approach is limited to the genetic diversity of the sorghum and its wild relatives. Biotechnology offers an alternative approach in targeted genome editing, which allows the creation of polymorphisms that do not exist in natural sources. The regulatory landscape around genome editing is rapidly evolving, but in many countries, plants carrying single nucleotide deletion or insertion will not be treated as traditional transgenic plants, removing a considerable burden for deregulation. Therefore, genome editing approaches provide a plausible pathway for increasing BNI. In this section, we summarize the potential target genes and the techniques that can be used to increase sorgoleone secretion.

Targeting sorgoleone biosynthesis pathway genes

Since biosynthetic pathway genes for sorgoleone are already known (Table 2), these genes are obvious targets for manipulation through conventional transgenic approaches or genome editing. Therefore, natural or induced variations in these genes and their cis-elements might result in a difference in sorgoleone secretion. Also, once discovered, transporters involved in sorgoleone secretion will be a target for increased BNI. In addition, most of the sorgoleone biosynthesis enzymes are root hair-specific (Baerson et al., 2008; Cook et al., 2010; Z. Q. Pan et al., 2018, 2007) and developmentally induced (Maharjan et al., 2023), suggesting that they might be co-regulated by a few common regulatory elements. Such regulatory elements will be interesting targets to improve sorgoleone synthesis and secretion. Taking advantage of cell-type specificity of this pathway, root-hair RNA-seq from either cultivars with varied sorgoleone secretion or loss-of-function mutants (Cook et al., 2010; Z. Q. Pan et al., 2018), followed by a subsequent gene network analysis, might reveal promising targets. In addition, if a negative regulator for biosynthesis and secretion can be found, a simple loss-of-function might be sufficient for creating a high BNI line.

In many scenarios, gain-of-function (i.e., increase in the activity of enzymes and transporters) will be of interest. Techniques such as root hair-specific expression of additional copies of these genes leading to the gain-of-function can be used to increase the biosynthesis and/or secretion and might lead to the increase in sorgoleone secretion. Alternatively, RNA-guided, clustered regularly interspaced short palindromic repeats/deactivated CRISPR-associated protein 9 (CRISPR/dCas9)-delivered transcriptional activators could be used to increase the expression of the target genes (Casas-Mollano et al., 2023; Gentzel et al., 2020; Z. X. Li et al., 2017). Although plants resulting from these approaches will be considered traditional transgenic plants and hence will be subject to substantial hurdles for commercialization, these approaches at least validate gene function in the laboratory. Another path for gain-of-function is through *cis*-regulatory element (CRE) editing. Techniques that allow precise modification of the target sequence (H. Y. Li et al., 2020; Lin et al., 2020) and insertion of additional CRE through homologous recombination (Shi et al., 2017) would help achieve these goals. Small transcriptional enhancers can also be inserted in the promoter to increase gene expression, as has been demonstrated in rice and maize (Claeys et al., 2024; Yao et al., 2024). Depending on the local governmental regulatory framework for genome-edited plants, some of these edits could be considered non-transgenic as long as the editing enzymes have been segregated out, providing a faster path for commercialization. Previously, the main bottleneck in these approaches was the low efficiency of sorghum transformation and regeneration, but recent advances in using morphogenic regulators, such as baby boom, Wuschel2, and Growth Regulator Factor 4-GRF

TABLE 2 Potential targets for genome editing for increasing sorgoleone.

Gene name	Gene ID (v3.3.1)	Chromosome	Coordinate (v3.3.1)
Biosynthesis genes			
SbDES2	Sobic.004G260600	4	60,583,495–60,585,071
SBDES3	Sobic.005G002700	5	207,067–209,446
SbARS1_1	Sobic.005G164200	5	63,964,679–36,995,963
SbARS1_2	Sobic.005G164300	5	63,975,290–63,976,997
SbARS2	Sobic.008G036800	8	3,476,907–3,478,489
SbOMT3	Sobic.008G007900	6	1,183,919–1,185,746
SbCyp71AM1	Sobic.004G139300	4	39,976,163–39,978,076
Root hair genes			
BHLH transcription factor (TaRSL4 homolog)	Sobic.002G359200	2	72,143,256–72,144,550
BHLH transcription factor (TaRSL4 homolog)	Sobic.008G147800	8	58,125,269–58,125,397
BHLH transcription factor (TaRSL4 homolog)	Sobic.005G195400	5	67,966,643–67,967,122
BHLH transcription factor (TaRSL4 homolog)	Sobic.001G166700	1	13,856,223–13,856,595
ZmTIP1 homolog	Sobic.010G205800	10	54,888,680–54,896,125
ZmTIP1 homolog	Sobic.004G068100	4	5,538,725–5,545,216

Interacting Factor 1, have led to improved transformation efficiencies in sorghum (J. P. Li et al., 2024; Nelson-Vasilchik et al., 2022; N. Wang et al., 2023). Moreover, the CRISPR-Combo technique can combine the activation of endogenous morphogenic regulators with CRISRP/Cas9 modifications for more efficient gene editing (Pan & Qi, 2023; Pan et al., 2022).

Targetting genetic variation for root hair traits

It is well documented that sorgoleone is produced exclusively in the living root hair cells (Czarnota et al., 2003). Fittingly, manipulation of root hair growth directly impacted sorgoleone production (Yang, Owens, et al., 2004). Therefore, it is conceivable to achieve a higher secretion of sorgoleone through an increase in root hair abundance and/or morphology. Root hair abundance and morphology will also influence the acquisition of diffusion-limited nutrients (e.g., phosphorous, ammonium, and potassium) since they represent the major site of uptake (Rongsawat et al., 2021; Saengwilai et al., 2021).

Genetic factors determining root hair length (RHL) and density have been studied extensively in model plant Arabidopsis (Balcerowicz et al., 2015; Cui et al., 2018; Y. Zhang, Yang, et al., 2023). The mechanisms involved in root hair initiation versus elongation have some common genes (Jones et al., 2002), yet the network of genes involved is largely independent of each other (Y. Zhang et al., 2023). Fittingly, results from quantitative trait loci (QTL) and GWAS analyses from rice and maize suggest a complex and likely independent control for RHL and root hair density (Hanlon et al., 2023; Zhu et al., 2005; Xuhui et al., 2023).

Although many genes involved in root hair traits were identified in Arabidopsis, reports of genes responsible for root hair traits in crop plants are limited. Okano et al. (2020) reported a QTL on chromosome 2A of Spanish spelt that co-localized with the wheat homolog of Root Hair Defective6-Like 4 (*TaRSL4*-2AS), a gene whose overexpression resulted in increased RHL (Han et al., 2016). Another study identified ZmTIP1, an S-acyltransferase that facilitates the localization of calcium-dependent protein kinase to the membrane, which determines the RHL in maize (Zhang et al., 2020). Sorghum genes homologous to those identified in these other crops are potential targets for an indirect sorgoleone secretion modification due to the reported similarities in the root system architecture (RSA) genetic control across the two crops (Zhang et al., 2020). Corresponding genes are summarized in Table 2. Among these, those that are expressed in root hairs would be good candidates for genome editing.

2 | CONCLUSION

Current understanding of sorgoleone biosynthesis and secretion offers immediate targets for manipulating sorgoleone secretion and BNI activity. In addition, recent remarkable progress in genetic tools such as well-annotated genomes from multiple breeding and association panel lines (Boatwright et al., 2022; Deschamps et al., 2018; McCormick et al., 2018; Tao et al., 2021; B. Wang et al., 2024) and sequenced ethyl methanesulfonate mutant collection (Jiao et al., 2016) provide opportunities for further gene discovery. Recent results on the variation and heritability of sorgoleone secretion also indicate that the trait can further be enhanced through breeding, especially with high-density genomic markers and genomic modeling capabilities.

New sorghum lines, either produced through biotechnology or breeding, will serve as precious model cases. Introducing this trait into elite hybrids will allow for assessing the impact

of BNI at scale under commercially relevant production practices.

AUTHOR CONTRIBUTIONS

Sakiko Okumoto: Writing—original draft. **Bal Maharjan**: Writing—original draft. **Nithya Rajan**: Writing—original draft. **Jing Xi**: Writing—original draft. **Scott R. Baerson**: Writing—original draft. **William L. Rooney**: Writing—original draft. **Michael J. Thomson**: Writing—original draft. **Damaris A. Odeny**: Writing—original draft. **Tadashi Yoshihashi**: Writing—original draft. **Josh V. Vermaas**: Writing—original draft. **Guntur V. Subbarao**: Writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Aslam, M., Aparato, V. P. M., & Suh, D. Y. (2022). (2′-Oxo)alkylresorcinols restore dehydration tolerance in a knockout line of PpORS, a bryophyte-specific type III polyketide synthase in *Physcomitrium* (*Physcomitrella*) *patens*. *Planta*, *255*(6), Article 129. https://doi.org/10.1007/s00425-022-03909-z

Austin, M. B., & Noel, J. P. (2003). The chalcone synthase superfamily of type III polyketide synthases. *Natural Product Reports*, *20*(1), 79–110. https://doi.org/10.1039/b100917f

Baerson, S. R., Dayan, F. E., Rimando, A. M., Nanayakkara, N. P. D., Liu, C. J., Schroerder, J., Fishbein, M., Pan, Z., Kagan, I. A., Pratt, L. H., Le Cordonnier-Pratt, M. M., & Duke, S. O. (2008). A functional genomics investigation of allelochemical biosynthesis in root hairs. *Journal of Biological Chemistry*, *283*(6), 3231–3247. https://doi.org/10.1074/jbc.M706587200

Baerson, S. R., Schröder, J., Cook, D., Rimando, A. M., Pan, Z. Q., Dayan, F. E., Noonan, B. P., & Duke, S. O. (2010). Alkylresorcinol biosynthesis in plants new insights from an ancient enzyme family? *Plant Signaling & Behavior*, *5*(10), 1286–1289. https://doi.org/10.4161/psb.5.10.13062

Balcerowicz, D., Schoenaers, S., & Vissenberg, K. (2015). Cell fate determination and the switch from diffuse growth to planar polarity in Arabidopsis root epidermal cells. *Frontiers in Plant Science*, *6*, 1163. https://doi.org/10.3389/fpls.2015.01163

Besançon, T. E., Dayan, F. E., Gannon, T. W., & Everman, W. J. (2020). Conservation and divergence in sorgoleone production of sorghum species. *Journal of Environmental Quality*, *49*(2), 368–377. https://doi.org/10.1002/jeq2.20038

Bessire, M., Borel, S., Fabre, G., Carraca, L., Efremova, N., Yephremov, A., Cao, Y., Jetter, R., Jacquat, A. C., Metraux, J. P., & Nawrath, C. (2011). A member of the PLEIOTROPIC DRUG RESISTANCE family of ATP binding cassette transporters is required for the formation of a functional cuticle in *Arabidopsis*. *Plant Cell*, *23*(5), 1958–1970. https://doi.org/10.1105/tpc.111.083121

Biggs, N. B., Hafner, J., Mashiri, F. E., Huntsinger, L., & Lambin, E. F. (2021). Payments for ecosystem services within the hybrid governance model: Evaluating policy alignment and complementarity on California rangelands. *Ecology & Society*, *26*(1), 19. https://doi.org/10.5751/Es-12254-260119

Boatwright, J. L., Sapkota, S., Jin, H., Schnable, J. C., Brenton, Z., Boyles, R., & Kresovich, S. (2022). Sorghum association panel whole-genome sequencing establishes cornerstone resource for dissecting genomic diversity. *Plant Journal*, *111*(3), 888–904. https://doi.org/10.1111/tpj.15853

Byrnes, R. C., Nuñez, J., Arenas, L., Rao, I., Trujillo, C., Alvarez, C., Arango, J., Rasche, F., & Chirinda, N. (2017). Biological nitrification inhibition by grasses mitigates soil nitrous oxide emissions from bovine urine patches. *Soil Biology & Biochemistry*, *107*, 156–163. https://doi.org/10.1016/j.soilbio.2016.12.029

Casas-Mollano, J. A., Zinselmeier, M. H., Sychla, A., & Smanski, M. J. (2023). Efficient gene activation in plants by the MoonTag programmable transcriptional activator. *Nucleic Acids Research*, *51*(13), 7083–7093. https://doi.org/10.1093/nar/gkad458

Cedervall, P., Hooper, A. B., & Wilmot, C. M. (2013). Structural studies of hydroxylamine oxidoreductase reveal a unique heme cofactor and a previously unidentified interaction partner. *Biochemistry*, *52*(36), 6211–6218. https://doi.org/10.1021/bi400960w

Chang, M., Netzly, D. H., Butler, L. G., & Lynn, D. G. (1986). Chemical-regulation of distance—Characterization of the 1st natural host germination stimulant for striga-asiatica. *Journal of the American Chemical Society*, *108*(24), 7858–7860. https://doi.org/10.1021/ja00284a074

Claeys, H., Neyrinck, E., Dumoulin, L., Pharazyn, A., Verstichele, A., Pauwels, L., Nuccio, M. L., & Van Ex, F. (2024). Coordinated gene upregulation in maize through CRISPR/Cas-mediated enhancer insertion. *Plant Biotechnology Journal*, *22*(1), 16–18. https://doi.org/10.1111/pbi.14191

Cook, D., Rimando, A. M., Clemente, T. E., Schroder, J., Dayan, F. E., Nanayakkara, N. P. D., Pan, Z. Q., Noonan, B. P., Fishbein, M., Abe, I., Duke, S. O., & Baerson, S. R. (2010). Alkylresorcinol synthases expressed in *Sorghum bicolor* root hairs play an essential role in the biosynthesis of the allelopathic benzoquinone sorgoleone. *Plant Cell*, *22*(3), 867–887. https://doi.org/10.1105/tpc.109.072397

Costa, M., Sampaio-Dias, I. E., Castelo-Branco, R., Scharfenstein, H., de Castro, R. R., Silva, A., Schneider, M. P. C., Araújo, M. J., Martins, R., Domingues, V. F., Nogueira, F., Camoes, V., Vasconcelos, V. M., & Leao, P. N. (2019). Structure of hierridin C, Synthesis of Hierridins B and C, and evidence for prevalent Alkylresorcinol biosynthesis

in Picocyanobacteria. *Journal of Natural Products*, *82*(2), 393–402. https://doi.org/10.1021/acs.jnatprod.8b01038

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., de los Campos, G., Burgueño, J., González-Camacho, J. M., Pérez-Elizalde, S., Beyene, Y., Dreisigacker, S., Singh, R., Zhang, X., Gowda, M., Roorkiwal, M., Rutkoski, J., & Varshney, R. K. (2017). Genomic selection in plant breeding: Methods, models, and perspectives. *Trends in Plant Science*, *22*(11), 961–975. https://doi.org/10.1016/j.tplants.2017.08.011

Crozier, D., Winans, N. D., Hoffmann, L., Patil, N. Y., Klein, P. E., Klein, R. R., & Rooney, W. L. (2024). Evaluating and predicting the performance of sorghum lines in an elite by exotic backcross-nested association mapping population. *Plants*, *13*(6), 879. https://www.mdpi.com/2223-7747/13/6/879. https://doi.org/10.3390/plants13060879

Cui, S., Suzaki, T., Tominaga-Wada, R., & Yoshida, S. (2018). Regulation and functional diversification of root hairs. *Seminars in Cell & Developmental Biology*, *83*, 115–122. https://doi.org/10.1016/j.semcdb.2017.10.003

Czarnota, M. A., Paul, R. N., Dayan, F. E., Nimbal, C. I., & Weston, L. A. (2001). Mode of action, localization of production, chemical nature, and activity of sorgoleone: A potent PSII inhibitor in spp. root exudates. *Weed Technology*, *15*(4), 813–825. https://doi.org/10.1614/0890-037X(2001)015%5b0813:MOALOP%5d2.0.CO;2

Czarnota, M. A., Paul, R. N., Weston, L. A., & Duke, S. O. (2003). Anatomy of sorgoleone-secreting root hairs of Sorghum species. *International Journal of Plant Sciences*, *164*(6), 861–866. https://doi.org/10.1086/378661

Dal Lago, P., Vavlas, N., Kooistra, L., & De Deyn, G. B. (2024). Estimation of nitrogen uptake, biomass, and nitrogen concentration, in cover crop monocultures and mixtures from optical UAV images. *Smart Agricultural Technology*, *9*, 100608. https://doi.org/10.1016/j.atech.2024.100608

Dayan, F. E., Howell, J., & Weidenhamer, J. D. (2009). Dynamic root exudation of sorgoleone and its in planta mechanism of action. *Journal of Experimental Botany*, *60*(7), 2107–2117. https://doi.org/10.1093/jxb/erp082

de Oliveira, A. A., Pastina, M. M., de Souza, V. F., da Costa Parrella, R. A., Noda, R. W., Simeone, M. L. F., Schaffert, R. E., de Magalhães, J. V., Damasceno, C. M. B., & Margarido, G. R. A. (2018). Genomic prediction applied to high-biomass sorghum for bioenergy production. *Molecular Breeding*, *38*(4), Article 49. https://doi.org/10.1007/s11032-018-0802-5

Deschamps, S., Zhang, Y., Llaca, V., Ye, L., Sanyal, A., King, M., May, G., & Lin, H. (2018). A chromosome-scale assembly of the sorghum genome using nanopore sequencing and optical mapping. *Nature Communications*, *9*(1), 4844. https://doi.org/10.1038/s41467-018-07271-1

Di, T., Afzal, M. R., Yoshihashi, T., Deshpande, S., Zhu, Y., & Subbarao, G. V. (2018). Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots. *Plant and Soil*, *423*(1), 99–110. https://doi.org/10.1007/s11104-017-3505-5

Einhellig, F. A., & Leather, G. R. (1988). Potentials for exploiting allelopathy to enhance crop production. *Journal of Chemical Ecology*, *14*(10), 1829–1844. https://doi.org/10.1007/BF01013480

Einhellig, F. A., & Rasmussen, J. A. (1989). Prior cropping with grain sorghum inhibits weeds. *Journal of Chemical Ecology*, *15*(3), 951–960. https://doi.org/10.1007/BF01015190

Einhellig, F. A., & Souza, I. F. (1992). Phytotoxicity of sorgoleone found in grain Sorghum root exudates. *Journal of Chemical Ecology*, *18*(1), 1–11. https://doi.org/10.1007/BF00997160

Erisman, J. W., Galloway, J., Seitzinger, S., Bleeker, A., & Butterbach-Bahl, K. (2011). Reactive nitrogen in the environment and its effect on climate change. *Current Opinion in Environmental Sustainability*, *3*(5), 281–290. https://doi.org/10.1016/j.cosust.2011.08.012

Fate, G. D., & Lynn, D. G. (1996). Xenognosin methylation is critical in defining the chemical potential gradient that regulates the spatial distribution in *Striga* pathogenesis. *Journal of the American Chemical Society*, *118*(46), 11369–11376. https://doi.org/10.1021/ja961395i

Fonseca, J. M. O., Klein, P. E., Crossa, J., Pacheco, A., Perez-Rodriguez, P., Ramasamy, P., Klein, R., & Rooney, W. L. (2021). Assessing combining abilities, genomic data, and genotype × environment interactions to predict hybrid grain sorghum performance. *The Plant Genome*, *14*(3), e20127. https://doi.org/10.1002/tpg2.20127

Frey, M., Schmauder, K., Pateraki, I., & Spring, O. (2018). Biosynthesis of Eupatolide-A Metabolic Route for Sesquiterpene Lactone Formation Involving the P450 Enzyme CYP71DD6. *Acs Chemical Biology*, *13*(6), 1536–1543. https://doi.org/10.1021/acschembio.8b00126

Funa, N., Awakawa, T., & Horinouchi, S. (2007). Pentaketide resorcylic acid synthesis by type III polyketide synthase from *Neurospora crassa*. *Journal of Biological Chemistry*, *282*(19), 14476–14481. https://doi.org/10.1074/jbc.M701239200

Funa, N., Ozawa, H., Hirata, A., & Horinouchi, S. (2006). Phenolic lipid synthesis by type III polyketide synthases is essential for cyst formation in *Azotobacter vinelandii*. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(16), 6356–6361. https://doi.org/10.1073/pnas.0511227103

Funabashi, M., Funa, N., & Horinouchi, S. (2008). Phenolic lipids synthesized by type III polyketide synthase confer penicillin resistance on *Streptomyces griseus*. *Journal of Biological Chemistry*, *283*(36), 25104. https://doi.org/10.1016/S0021-9258(19)49255-7

Gao, X., Uno, K., Sarr, P. S., Yoshihashi, T., Zhu, Y., & Subbarao, G. V. (2022). High-sorgoleone producing sorghum genetic stocks suppress soil nitrification and N_2O emissions better than low-sorgoleone producing genetic stocks. *Plant and Soil*, *477*(1), 793–805. https://doi.org/10.1007/s11104-022-05474-6

Gentzel, I. N., Park, C. H., Bellizzi, M., Xiao, G. Q., Gadhave, K. R., Murphree, C., Yang, Q., LaMantia, J., Redinbaugh, M. G., Balint-Kurti, P., Sit, T. L., & Wang, G. L. (2020). A CRISPR/dCas9 toolkit for functional analysis of maize genes. *Plant Methods*, *16*(1), Article 133. https://doi.org/10.1186/s13007-020-00675-5

Georges, M., Charlier, C., & Hayes, B. (2019). Harnessing genomic information for livestock improvement. *Nature Reviews Genetics*, *20*(3), 135–156. https://doi.org/10.1038/s41576-018-0082-2

Ghosh, R., Chhabra, A., Phatale, P. A., Samrat, S. K., Sharma, J., Gosain, A., Mohanty, D., Saran, S., & Gokhale, R. S. (2008). Dissecting the functional role of polyketide synthases in *Dictyostelium discoideum*—Biosynthesis of the differentiation regulating factor $_4$-methyl-$_5$-pentylbenzene-$_{1,3}$-diol. *Journal of Biological Chemistry*, *283*(17), 11348–11354. https://doi.org/10.1074/jbc.M709588200

Gonzalez, V. M., Kazimir, J., Nimbal, C., Weston, L. A., & Cheniae, G. M. (1997). Inhibition of a photosystem II electron transfer reaction by the natural product sorgoleone. *Journal of Agricultural and Food Chemistry*, *45*(4), 1415–1421. https://doi.org/10.1021/jf960733w

Gopalakrishnan, S., Watanabe, T., Pearse, S. J., Ito, O., Hossain, Z. A. K. M., & Subbarao, G. V. (2009). Biological nitrification inhibition by *Brachiaria humidicola* roots varies with soil type and inhibits

nitrifying bacteria, but not other major soil microorganisms. *Soil Science and Plant Nutrition*, *55*(5), 725–733. https://doi.org/10.1111/j.1747-0765.2009.00398.x

Goyal, A., Saxena, P., Rahman, A., Singh, P. K., Kasbekar, D. P., Gokhale, R. S., & Sankaranarayanan, R. (2008). Structural insights into biosynthesis of resorcinolic lipids by a type III polyketide synthase in *Neurospora crassa*. *Journal of Structural Biology*, *162*(3), 411–421. https://doi.org/10.1016/j.jsb.2008.02.009

Han, Y., Xin, M., Huang, K., Xu, Y., Liu, Z., Hu, Z., Yao, Y., Peng, H., Ni, Z., & Sun, Q. (2016). Altered expression of TaRSL4 gene by genome interplay shapes root hair length in allopolyploid wheat. *New Phytologist*, *209*(2), 721–732. https://doi.org/10.1111/nph.13615

Hanlon, M. T., Vejchasarn, P., Fonta, J. E., Schneider, H. M., McCouch, S. R., & Brown, K. M. (2023). Genome wide association analysis of root hair traits in rice reveals novel genomic regions controlling epidermal cell differentiation. *BMC Plant Biology*, *23*(1), Article 6. https://doi.org/10.1186/s12870-022-04026-5

Hayes, B. J., Visscher, P. M., & Goddard, M. E. (2009). Increased accuracy of artificial selection by using the realized relationship matrix. *Genetics Research*, *91*(1), 47–60. https://doi.org/10.1017/S0016672308009981

He, T. H., Liu, D. Y., Yuan, J. J., Luo, J. F., Lindsey, S., Bolan, N., & Ding, W. X. (2018). Effects of application of inhibitors and biochar to fertilizer on gaseous nitrogen emissions from an intensively managed wheat field. *Science of the Total Environment*, *628–629*, 121–130. https://doi.org/10.1016/j.scitotenv.2018.02.048

Hejl, A. M., & Koster, K. L. (2004). The allelochemical sorgoleone inhibits root H^+-ATPase and water uptake. *Journal of Chemical Ecology*, *30*(11), 2181–2191. https://doi.org/10.1023/B:JOEC.0000048782.87862.7f

Hooper, A. B., Arciero, D., Bergmann, D., & Hendrich, M. P. (2004). The oxidation of ammonia as an energy source in bacteria. In D. Zannoni (Ed.), *Respiration in archaea and bacteria: Diversity of prokaryotic respiratory systems* (pp. 121–147). Springer. https://doi.org/10.1007/978-1-4020-3163-2_6

Hunt, C. H., van Eeuwijk, F. A., Mace, E. S., Hayes, B. J., & Jordan, D. R. (2018). Development of genomic prediction in sorghum. *Crop Science*, *58*(2), 690–700. https://doi.org/10.2135/cropsci2017.08.0469

Hussain, M. I., Danish, S., Sanchez-Moreiras, A. M., Vicente, O., Jabran, K., Chaudhry, U. K., Branca, F., & Reigosa, M. J. (2021). Unraveling sorghum allelopathy in agriculture: Concepts and implications. *Plants*, *10*(9), 1795. https://doi.org/10.3390/plants10091795

Igarashi, N., Moriyama, H., Fujiwara, T., Fukumori, Y., & Tanaka, N. (1997). The 2.8 angstrom structure of hydroxylamine oxidoreductase from a nitrifying chemoautotrophic bacterium, *Nitrosomonas europaea*. *Nature Structural Biology*, *4*(4), 276–284. https://doi.org/10.1038/nsb0497-276

IPCC. (2014). Carbon and other biogeochemical cycles. In I. P. O. C. Change (Ed.), *Climate change 2013 – The physical science basis: Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change* (pp. 465–570). Cambridge University Press. https://doi.org/10.1017/CBO9781107415324.015

Janke, C. K., Wendling, L. A., & Fujinuma, R. (2018). Biological nitrification inhibition by root exudates of native species, Hibiscus splendens and Solanum echinatum. *Peerj*, *6*, e4960. https://doi.org/10.7717/peerj.4960

Ji, X. F., & Jetter, R. (2008). Very long chain alkylresorcinols accumulate in the intracuticular wax of rye (*Secale cereale* L.) leaves near the tissue surface. *Phytochemistry*, *69*(5), 1197–1207. https://doi.org/10.1016/j.phytochem.2007.12.008

Jiao, Y., Burke, J., Chopra, R., Burow, G., Chen, J., Wang, B., Hayes, C., Emendack, Y., Ware, D., & Xin, Z. (2016). A sorghum mutant resource as an efficient platform for gene discovery in grasses. *Plant Cell*, *28*(7), 1551–1562. https://doi.org/10.1105/tpc.16.00373

Jones, M. A., Shen, J. J., Fu, Y., Li, H., Yang, Z., & Grierson, C. S. (2002). The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *Plant Cell*, *14*(4), 763–776. https://doi.org/10.1105/tpc.010359

Kagan, I. A., Rimando, A. M., & Dayan, F. E. (2003). Chromatographic separation and in vitro activity of sorgoleone congeners from the roots of *Sorghum bicolor*. *Journal of Agricultural and Food Chemistry*, *51*(26), 7589–7595. https://doi.org/10.1021/jf034789j

Karwat, H., Sparke, M. A., Rasche, F., Arango, J., Nuñez, J., Rao, I., Moreta, D., & Cadisch, G. (2019). Nitrate reductase activity in leaves as a plant physiological indicator of biological nitrification inhibition by. *Plant Physiology and Biochemistry*, *137*, 113–120. https://doi.org/10.1016/j.plaphy.2019.02.002

Kozubek, A., & Tyman, J. H. P. (1999). Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chemical Reviews*, *99*(1), 1–25. https://doi.org/10.1021/cr970464o

Kozubek, A., & Tyman, J. H. P. (2005). Bioactive phenolic lipids. In A. ur-Rahman (Ed.), *Studies in natural products chemistry* (Vol. 30, pp. 111–190). Elsevier. https://doi.org/10.1016/S1572-5995(05)80032-8

Kroll, J. O., Fossen, E. M. V., Anderson, L. N., McNaughton, A. D., Herrera, D., Oda, Y., Wilson, A. J., Nelson, W. C., Kumar, N., Frank, A. R., Elmore, J. R., Handakumbura, P., Lin, V. S., & Egbert, R. G. (2023). Profiling sorghum-microbe interactions with a specialized photoaffinity probe identifies key sorgoleone binders in *Acinetobacter pittii*. *bioRxiv*. https://doi.org/10.1101/2023.05.31.542302

Kuk, A. C. Y., Hao, A., Guan, Z., & Lee, S.-Y. (2019). Visualizing conformation transitions of the Lipid II flippase MurJ. *Nature Communications*, *10*(1), Article 1736. https://doi.org/10.1038/s41467-019-09658-0

Lancaster, K. M., Caranto, J. D., Majer, S. H., & Smith, M. A. (2018). Alternative bioenergy: Updates to and challenges in nitrification metalloenzymology. *Joule*, *2*(3), 421–441. https://doi.org/10.1016/j.joule.2018.01.018

Landberg, R., Kamal-Eldin, A., Salmenkallio-Marttila, M., Rouau, X., & Åman, P. (2008). Localization of alkylresorcinols in wheat, rye and barley kernels. *Journal of Cereal Science*, *48*(2), 401–406. https://doi.org/10.1016/j.jcs.2007.09.013

Li, H. Y., Li, J. Y., Chen, J. L., Yan, L., & Xia, L. Q. (2020). Precise modifications of both exogenous and endogenous genes in rice by prime editing. *Molecular Plant*, *13*(5), 671–674. https://doi.org/10.1016/j.molp.2020.03.011

Li, J. P., Pan, W. B., Zhang, S., Ma, G. J., Li, A. X., Zhang, H. W., & Liu, L. J. (2024). A rapid and highly efficient sorghum transformation strategy using GRF4-GIF1/ternary vector system. *Plant Journal*, *117*(5), 1604–1613. https://doi.org/10.1111/tpj.16575

Li, Z. X., Zhang, D. D., Xiong, X. Y., Yan, B. Y., Xie, W., Sheen, J., & Li, J. F. (2017). A potent Cas9-derived gene activator for plant and mammalian cells. *Nature Plants*, *3*(12), 930–936. https://doi.org/10.1038/s41477-017-0046-0

Lichtenfels, M., Burtis, P., Hovani, A., Kuppalli, R., Lichtenfeld, M., & Miyata, Y. (2021). Improving markets for ecosystem services. In B. S. Gentry, Q. Newcomer, S. C. Anisfeld, & M. F. Fotos III (Eds.), *Emerging markets for ecosystem services* (pp. 337–364). CRC Press.

Lin, Q. P., Zong, Y., Xue, C. X., Wang, S. X., Jin, S., Zhu, Z. X., Wang, Y. P., Anzalone, A. V., Raguram, A., Doman, J. L., Liu, D. V. R., & Gao, C. X. (2020). Prime genome editing in rice and wheat. *Nature Biotechnology*, *38*(5), 582–585. https://doi.org/10.1038/s41587-020-0455-x

Lu, Y. F., Zhang, X. N., Jiang, J. F., Kronzucker, H. J., Shen, W. S., & Shi, W. M. (2019). Effects of the biological nitrification inhibitor 1,9-decanediol on nitrification and ammonia oxidizers in three agricultural soils. *Soil Biology & Biochemistry*, *129*, 48–59. https://doi.org/10.1016/j.soilbio.2018.11.008

Maalcke, W. J., Dietl, A., Marritt, S. J., Butt, J. N., Jetten, M. S. M., Keltjens, J. T., Barends, T. R. M., & Kartal, B. (2014). Structural basis of biological NO generation by octaheme oxidoreductases. *Journal of Biological Chemistry*, *289*(3), 1228–1242. https://doi.org/10.1074/jbc.M113.525147

Maharjan, B., Leon, F., Rooney, W. L., & Okumoto, S. (2024). Combining abilities and quantitative inheritance of sorgoleone exudation in *Sorghum bicolor*. *Crop Science*, *64*(6), 3219–3230. https://doi.org/10.1002/csc2.21366

Maharjan, B., Vitha, S., & Okumoto, S. (2023). Developmental regulation and physical interaction among enzymes involved in sorgoleone biosynthesis. *Plant Journal*, *115*(3), 820–832. https://doi.org/10.1111/tpj.16263

Matsuzawa, M., Katsuyama, Y., Funa, N., & Horinouchi, S. (2010). Alkylresorcylic acid synthesis by type III polyketide synthases from rice. *Phytochemistry*, *71*(10), 1059–1067. https://doi.org/10.1016/j.phytochem.2010.02.012

McCormick, R. F., Truong, S. K., Sreedasyam, A., Jenkins, J., Shu, S., Sims, D., Kennedy, M., Amirebrahimi, M., Weers, B. D., McKinley, B., Mattison, A., Morishige, D. T., Grimwood, J., Schmutz, J., & Mullet, J. E. (2018). The *Sorghum bicolor* reference genome: Improved assembly, gene annotations, a transcriptome atlas, and signatures of genome organization. *Plant Journal*, *93*(2), 338–354. https://doi.org/10.1111/tpj.13781

Meiklejohn, J. (1968). Numbers of nitrifying bacteria in some rhodesian soils under natural grass and improved pastures. *Journal of Applied Ecology*, *5*(2), 291–300. https://doi.org/10.2307/2401563

Mishra, M. M., & Flaig, W. (1979). Inhibition of mineralization of urea nitrogen in soil. *Plant and Soil*, *51*(3), 301–309. https://doi.org/10.1007/Bf02197777.[CrossRef]

Miyanaga, A., & Horinouchi, S. (2010). Phenolic lipids synthesized by type III polyketide synthases. In K. N. Timmis (Ed.), *Handbook of hydrocarbon and lipid microbiology* (pp. 445–450). Springer. https://doi.org/10.1007/978-3-540-77587-4

Nardi, P., Akutsu, M., Pariasca-Tanaka, J., & Wissuwa, M. (2013). Effect of methyl 3-4-hydroxyphenyl propionate, a root exudate, on N dynamic, potential nitrification activity and abundance of ammonia-oxidizing bacteria and archaea. *Plant and Soil*, *367*(1-2), 627–637. https://doi.org/10.1007/s11104-012-1494-y

Nelson, D., & Werck-Reichhart, D. (2011). A P450-centric view of plant evolution. *Plant Journal*, *66*(1), 194–211. https://doi.org/10.1111/j.1365-313X.2011.04529.x

Nelson, D. R., Schuler, M. A., Paquette, S. M., Werck-Reichhart, D., & Bak, S. (2004). Comparative genomics of rice and Arabidopsis. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. *Plant Physiology*, *135*(2), 756–772. https://doi.org/10.1104/pp.104.039826

Nelson-Vasilchik, K., Hague, J. P., Tilelli, M., & Kausch, A. P. (2022). Rapid transformation and plant regeneration of sorghum (*Sorghum bicolor* L.) mediated by altruistic *Baby boom* and *Wuschel2*. *In Vitro Cellular & Developmental Biology-Plant*, *58*(3), 331–342. https://doi.org/10.1007/s11627-021-10243-8

Netzly, D. H., & Butler, L. G. (1986). Roots of sorghum exude hydrophobic droplets containing biologically-active components. *Crop Science*, *26*(4), 775–778. https://doi.org/10.2135/cropsci1986.0011183X002600040031x

Nishigaya, Y., Fujimoto, Z., & Yamazaki, T. (2016). Optimized inhibition assays reveal different inhibitory responses of hydroxylamine oxidoreductases from beta- and gamma-proteobacterial ammonium-oxidizing bacteria. *Biochemical and Biophysical Research Communications*, *476*(3), 127–133. https://doi.org/10.1016/j.bbrc.2016.05.041

NOAA. (2024). *Gulf of mexico 'dead zone' larger than average, scientists find*. National Oceanic and Atmospheric Administration. https://www.noaa.gov/news-release/gulf-of-mexico-dead-zone-larger-than-average-scientists-find

Norton, J., & Ouyang, Y. (2019). Controls and adaptive management of nitrification in agricultural soils. *Frontiers in Microbiology*, *10*, 1931. https://doi.org/10.3389/fmicb.2019.01931

Okano, N., Goto, R., Kato, T., Saisho, D., Kato, K., Miura, H., Tani, M., & Onishi, K. (2020). Spanish spelt is unique germplasm for improvement of root hair length in hexaploid wheat. *Plant and Soil*, *452*(1/2), 171–184. https://www.jstor.org/stable/48732970

O'Sullivan, C. A., Fillery, I. R. P., Roper, M. M., & Richards, R. A. (2016). Identification of several wheat landraces with biological nitrification inhibition capacity. *Plant and Soil*, *404*(1-2), 61–74. https://doi.org/10.1007/s11104-016-2822-4

O'Sullivan, C. A., Whisson, K., Treble, K., Roper, M. M., Micin, S. F., & Ward, P. R. (2017). Biological nitrification inhibition by weeds: wild radish, brome grass, wild oats and annual ryegrass decrease nitrification rates in their rhizospheres. *Crop & Pasture Science*, *68*(8), 798–804. https://doi.org/10.1071/Cp17243

Otaka, J., Subbarao, G. V., Jiang, M. L., Ono, H., & Yoshihashi, T. (2023). Isolation and characterization of the hydrophilic BNI compound, 6-methoxy-2(3)-benzoxazolone (MBOA), from maize roots. *Plant and Soil*, *489*(1–2), 341–359. https://doi.org/10.1007/s11104-023-06021-7

Otaka, J., Subbarao, G. V., Ono, H., & Yoshihashi, T. (2022). Biological nitrification inhibition in maize-isolation and identification of hydrophobic inhibitors from root exudates. *Biology and Fertility of Soils*, *58*(3), 251–264. https://doi.org/10.1007/s00374-021-01577-x

Pan, C. T., Li, G., Malzahn, A. A., Cheng, Y. H., Leyson, B., Sretenovic, S., Gurel, F., Coleman, G. D., & Qi, Y. P. (2022). Boosting plant genome editing with a versatile CRISPR-Combo system. *Nature Plants*, *8*(5), 513–525. https://doi.org/10.1038/s41477-022-01151-9

Pan, C. T., & Qi, Y. P. (2023). CRISPR-Combo-mediated orthogonal genome editing and transcriptional activation for plant breeding. *Nature Protocols*, *18*(6), 1760–1794. https://doi.org/10.1038/s41596-023-00823-w

Pan, Z. Q., Baerson, S. R., Wang, M., Bajsa-Hirschel, J., Rimando, A. M., Wang, X. Q., Nanayakkara, N. P. D., Noonan, B. P., Fromm, M. E., Dayan, F. E., Khan, I. A., & Duke, S. O. (2018). A cytochrome P450 CYP71 enzyme expressed in *Sorghum bicolor* root hair cells participates in the biosynthesis of the benzoquinone allelochemical sorgoleone. *New Phytologist*, *218*(2), 616–629. https://doi.org/10.1111/nph.15037

Pan, Z. Q., Rimando, A. M., Baerson, S. R., Fishbein, M., & Duke, S. O. (2007). Functional characterization of desaturases involved in

the formation of the terminal double bond of an unusual 16:3Δ fatty acid isolated from root hairs. *Journal of Biological Chemistry*, *282*(7), 4326–4335. https://doi.org/10.1074/jbc.M606343200

Pariasca Tanaka, J., Nardi, P., & Wissuwa, M. (2010). Nitrification inhibition activity, a novel trait in root exudates of rice. *AoB Plants*, *2010*, plq014. https://doi.org/10.1093/aobpla/plq014

Patel, M. K., Ryu, D., Western, A. W., Fitzgerald, G. J., Perry, E. M., Suter, H., & Young, I. M. (2024). A new multispectral index for canopy nitrogen concentration applicable across growth stages in ryegrass and barley. *Precision Agriculture*, *25*(1), 486–519. https://doi.org/10.1007/s11119-023-10081-1

Petroli, C. D., Subbarao, G. V., Burgueño, J. A., Yoshihashi, T., Li, H. H., Franco Duran, J., & Pixley, K. V. (2023). Genetic variation among elite inbred lines suggests potential to breed for BNI-capacity in maize. *Scientific Reports*, *13*(1), 13422. https://doi.org/10.1038/s41598-023-39720-3

Pighin, J. A., Zheng, H., Balakshin, L. J., Goodman, I. P., Western, T. L., Jetter, R., Kunst, L., & Samuels, A. L. (2004). Plant cuticular lipid export requires an ABC transporter. *Science*, *306*(5696), 702–704. https://doi.org/10.1126/science.1102331

Putnam, A. R., Defrank, J., & Barnes, J. P. (1983). Exploitation of allelopathy for weed control in annual and perennial cropping systems. *Journal of Chemical Ecology*, *9*(8), 1001–1010. https://doi.org/10.1007/BF00982207

Rasmussen, J. A., Hejl, A. M., Einhellig, F. A., & Thomas, J. A. (1992). Sorgoleone from root exudate inhibits mitochondrial functions. *Journal of Chemical Ecology*, *18*(2), 197–207. https://doi.org/10.1007/BF00993753

Raza, S., Sievertsen, T. H., Okumoto, S., & Vermaas, J. V. (2023). Passive permeability controls synthesis for the allelochemical sorgoleone in sorghum root exudate. *Phytochemistry*, 113891. https://doi.org/10.1016/j.phytochem.2023.113891

Reed, D. (2020). Ecosystem services markets conceived and designed for US agriculture. In J. A. Delgado, C. J. Gantzer, & G. F. Sassenrath (Eds.), *Soil and water conservation: A Celebration of 75 years* (pp. 70–74). Soil and Water Conservation Society.

Rice, E. L., & Pancholy, S. K. (1973). Inhibition of nitrification by climax ecosystems. II. Additional Evidence and Possible Role of Tannins. *American Journal of Botany*, *60*(7), 691–702. https://doi.org/10.2307/2441448

Rodgers, G. A., & Ashworth, J. (1982). Bacteriostatic action of nitrification inhibitors. *Canadian Journal of Microbiology*, *28*(10), 1093–1100. https://doi.org/10.1139/m82-163

Rongsawat, T., Peltier, J. B., Boyer, J. C., Very, A. A., & Sentenac, H. (2021). Looking for root hairs to overcome poor soils. *Trends in Plant Science*, *26*(1), 83–94. https://doi.org/10.1016/j.tplants.2020.09.001

Rosa, L., & Gabrielli, P. (2023). Energy and food security implications of transitioning synthetic nitrogen fertilizers to net-zero emissions. *Environmental Research Letters*, *18*(1), 014008. https://doi.org/10.1088/1748-9326/aca815

Ross, A. B., Shepherd, M. J., Schüpphaus, M., Sinclair, V., Alfaro, B., Kamal-Eldin, A., & Åman, P. (2003). Alkylresorcinols in cereals and cereal products. *Journal of Agricultural and Food Chemistry*, *51*(14), 4111–4118. https://doi.org/10.1021/jf0340456

Saengwilai, P., Strock, C., Rangarajan, H., Chimungu, J., Salungyu, J., & Lynch, J. P. (2021). Root hair phenotypes influence nitrogen acquisition in maize. *Annals of Botany*, *128*(7), 849–858. https://doi.org/10.1093/aob/mcab104

Saier, J. M. H., & Paulsen, I. T. (2001). Phylogeny of multidrug transporters. *Seminars in Cell & Developmental Biology*, *12*(3), 205–213. https://doi.org/10.1006/scdb.2000.0246

Sapkota, S., Boatwright, J. L., Jordan, K., Boyles, R., & Kresovich, S. (2020). Multi-trait regressor stacking increased genomic prediction accuracy of sorghum grain composition. *bioRxiv*. https://doi.org/10.1101/2020.04.03.023531

Sarr, P. S., Ando, Y., Nakamura, S., Deshpande, S., & Subbarao, G. V. (2020). Sorgoleone release from sorghum roots shapes the composition of nitrifying populations, total bacteria, and archaea and determines the level of nitrification. *Biology and Fertility of Soils*, *56*(2), 167–167. https://doi.org/10.1007/s00374-019-01418-y

Sarr, P. S., Nakamura, S., Ando, Y., Iwasaki, S., & Subbarao, G. V. (2021). Sorgoleone production enhances mycorrhizal association and reduces soil nitrification in sorghum. *Rhizosphere*, *17*, 100283. https://doi.org/10.1016/j.rhisph.2020.100283

Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., Hakimi, S. M., Mo, H., & Habben, J. E. (2017). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, *15*(2), 207–216. https://doi.org/10.1111/pbi.12603

Smil, V. (2004). *Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food Production*. The MIT press. https://doi.org/10.7551/mitpress/2767.001.0001

Suarez-Ojeda, M. E., Guisasola, A., & Carrera, J. (2010). Inhibitory impact of quinone-like compounds over partial nitrification. *Chemosphere*, *80*(4), 474–480. https://doi.org/10.1016/j.chemosphere.2010.03.041

Subbarao, G. V., Ishikawa, T., Ito, O., Nakahara, K., Wang, H. Y., & Berry, W. L. (2006). A bioluminescence assay to detect nitrification inhibitors released from plant roots: A case study with *Brachiaria humidicola*. *Plant and Soil*, *288*(1), 101–112. https://doi.org/10.1007/s11104-006-9094-3

Subbarao, G. V., Kishii, M., Bozal-Leorri, A., Ortiz-Monasterio, I., Gao, X., Ibba, M. I., Karwat, H., Gonzalez-Moro, M. B., Gonzalez-Murua, C., Yoshihashi, T., Tobita, S., Kommerell, V., Braun, H. J., & Iwanaga, M. (2021). Enlisting wild grass genes to combat nitrification in wheat farming: A nature-based solution. *PNAS*, *118*(35), e2106595118. https://doi.org/10.1073/pnas.2106595118

Subbarao, G. V., Nakahara, K., Ando, Y., Sahrawat, K. L., Deshpande, S. P., Srinivasa, R. P., Upadhyaya, H. D., & Hash, C. T. (2015). Biological nitrification inhibition (BNI) activity in sorghum: Potential role for enhancing nitrogen-use efficiency (NUE). In *Millets: Promotion for food, feed, fodder, nutritional and environment security, Proceedings of Global Consultation on Millets Promotion for Health & Nutritional Security. society for millets research* (pp. 91–96). ICAR Indian Institute of Millets Research.

Subbarao, G. V., Nakahara, K., Hurtado, M. P., Ono, H., Moreta, D. E., Salcedo, A. F., Yoshihashi, A. T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I. M., Lascano, C. E., Berry, W. L., & Ito, O. (2009). Evidence for biological nitrification inhibition in *Brachiaria pastures*. *PNAS*, *106*(41), 17302–17307. https://doi.org/10.1073/pnas.0903694106

Subbarao, G. V., Nakahara, K., Ishikawa, T., Ono, H., Yoshida, M., Yoshihashi, T., Zhu, Y. Y., Zakir, H. A. K. M., Deshpande, S. P., Hash, C. T., & Sahrawat, K. L. (2013). Biological nitrification inhibition (BNI) activity in sorghum and its characterization. *Plant and Soil*, *366*(1–2), 243–259. https://doi.org/10.1007/s11104-012-1419-9

Subbarao, G. V., Rao, I. M., Nakahara, K., Sahrawat, K. L., Ando, Y., & Kawashima, T. (2013). Potential for biological nitrification inhibition to reduce nitrification and N_2O emissions in pasture crop-livestock systems. *Animal*, *7*, 322–332. https://doi.org/10.1017/S1751731113000761

Subbarao, G. V., Rondon, M., Ito, O., Ishikawa, T., Rao, I. M., Nakahara, K., Lascano, C., & Berry, W. L. (2007). Biological nitrification inhibition (BNI)—Is it a widespread phenomenon? *Plant and Soil*, *294*(1), 5–18. https://doi.org/10.1007/s11104-006-9159-3

Sun, L., Lu, Y. F., Yu, F. W., Kronzucker, H. J., & Shi, W. M. (2016). Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. *New Phytologist*, *212*(3), 646–656. https://doi.org/10.1111/nph.14057

Sun, Y. L., Yao, R. N., Ji, X. F., Wu, H. Q., Luna, A., Wang, Z. H., & Jetter, R. (2020). Characterization of an alkylresorcinol synthase that forms phenolics accumulating in the cuticular wax on various organs of rye (*Secale cereale*). *Plant Journal*, *102*(6), 1294–1312. https://doi.org/10.1111/tpj.14704

Sylvesterbradley, R., Mosquera, D., & Mendez, J. E. (1988). Inhibition of nitrate accumulation in tropical grassland soils—Effect of nitrogen-fertilization and soil disturbance. *Journal of Soil Science*, *39*(3), 407–416. https://doi.org/10.1111/j.1365-2389.1988.tb01226.x

Tanaka, J. P., Nardi, P., & Wissuwa, M. (2010). Nitrification inhibition activity, a novel trait in root exudates of rice. *Aob Plants*, plq014 https://doi.org/10.1093/aobpla/plq014

Tao, Y., Luo, H., Xu, J., Cruickshank, A., Zhao, X., Teng, F., Hathorn, A., Wu, X., Liu, Y., Shatte, T., Jordan, D., Jing, H., & Mace, E. (2021). Extensive variation within the pan-genome of cultivated and wild sorghum. *Nature Plants*, *7*(6), 766–773. https://doi.org/10.1038/s41477-021-00925-x

Tesfamariam, T., Yoshinaga, H., Deshpande, S., Srinivasa Rao, P., Sahrawat, K., Ando, Y., Nakahara, K., Hash, C., & Subbarao, G. (2014). Biological nitrification inhibition in sorghum: The role of sorgoleone production. *Plant and Soil*, *379*, 325–335. https://doi.org/10.1007/s11104-014-2075-z

Tibugari, H., Chiduza, C., Mashingaidze, A. B., & Mabasa, S. (2019). Quantification of sorgoleone in sorghum accessions from eight southern African countries. *South African Journal of Plant and Soil*, *36*(1), 41–50. https://doi.org/10.1080/02571862.2018.1469794

Uddin, R., Kim, Y.-K., Park, S. U., & Pyon, J. Y. (2009). Herbicidal sctivity of sorgoleone from grain sorghum root exudates and its contents among sorghum cultivars. *Weed & Turfgrass Science*, *29*(3), 229–236.

Uddin, M. R., Thwe, A. A., Kim, Y. B., Park, W. T., Chae, S. C., & Park, S. U. (2013). Effects of jasmonates on sorgoleone accumulation and expression of genes for sorgoleone biosynthesis in sorghum roots. *Journal of Chemical Ecology*, *39*(6), 712–722. https://doi.org/10.1007/s10886-013-0299-7

USDA-NASS. (2024). *Sorghum Acres*. https://www.nass.usda.gov/Charts_and_Maps/graphics/sorgac.pdf

Van Huynh, V., Ngo, M. T. T., Itayama, T., Nguyen, M. B., Vo, T. D. H., Vo, T. K. Q., Le, V. G., You, S. J., Nguyen, P. T., & Bui, X. T. (2023). Dynamic of microbial community in simultaneous nitrification and denitrification process: A review. *Bioresource Technology Reports*, *22*, 101415. https://doi.org/10.1016/j.biteb.2023.101415

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, *91*(11), 4414–4423. https://doi.org/10.3168/jds.2007-0980

Wang, B., Chougule, K., Jiao, Y., Olson, A., Kumar, V., Gladman, N., Huang, J., Llaca, V., Fengler, K., Wei, X., Wang, L., Wang, X., Regulski, M., Drenkow, J., Gingeras, T., Hayes, C., Armstrong, J. S., Huang, Y., Xin, Z., & Ware, D. (2024). High-quality chromosome scale genome assemblies of two important Sorghum inbred lines, Tx2783 and RTx436. *NAR Genom Bioinform*, *6*(3), lqae097. https://doi.org/10.1093/nargab/lqae097

Wang, N., Ryan, L., Sardesai, N., Wu, E. M. Y., Lenderts, B., Lowe, K., Che, P., Anand, A., Worden, A., van Dyk, D., Barone, P., Svitashev, S., Jones, T., & Gordon-Kamm, W. (2023). Leaf transformation for efficient random integration and targeted genome modification in maize and sorghum. *Nature Plants*, *9*(2), 255–270. https://doi.org/10.1038/s41477-022-01338-0

Weston, L. A., Alsaadawi, I. S., & Baerson, S. R. (2013). Sorghum allelopathy-from ecosystem to molecule. *Journal of Chemical Ecology*, *39*(2), 142–153. https://doi.org/10.1007/s10886-013-0245-8

Whittaker, M., Bergmann, D., Arciero, D., & Hooper, A. B. (2000). Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium *Nitrosomonas europaea*. *Biochimica Et Biophysica Acta-Bioenergetics*, *1459*(2–3), 346–355. https://doi.org/10.1016/S0005-2728(00)00171-7

Wigchert, S. C. M., & Zwanenburg, B. (1999). A critical account on the inception of Striga seed germination. *Journal of Agricultural and Food Chemistry*, *47*(4), 1320–1325. https://doi.org/10.1021/jf980926e

Winans, N. D., Klein, R. R., Fonseca, J. M. O., Klein, P. E., & Rooney, W. L. (2023). Evaluating introgression sorghum germplasm selected at the population level while exploring genomic resources as a screening method. *Plants*, *12*(3), 444. https://www.mdpi.com/2223-7747/12/3/444

Xu, J., Yang, C. Y., Yuan, Z., Zhang, D. S., Gondwe, M. Y., Ding, Z. W., Liang, W. Q., Zhang, D. B., & Wilson, Z. A. (2010). The ABORTED MICROSPORES Regulatory Network Is Required for Postmeiotic Male Reproductive Development in Arabidopsis thaliana. *Plant Cell*, *22*(1), 91–107. https://doi.org/10.1105/tpc.109.071803

Xuhui, L., Siqi, L., Weiwei, C., Hang, Z., Huanzhang, L., Danwen, F., Lina, F., Junteng, F., Yuanqiang, H., Xiangbo, Z., Hailong, C., & Yongwen, Q. (2023). Genome-wide association study of root hair length in maize. *Tropical Plant Biology*, *16*(1), 67–74. https://doi.org/10.1007/s12042-023-09331-3

Yadav, V., Molina, I., Ranathunge, K., Castillo, I. Q., Rothstein, S. J., & Reed, J. W. (2014). ABCG Transporters Are Required for Suberin and Pollen Wall Extracellular Barriers in Arabidopsis. *The Plant Cell*, *26*(9), 3569–3588. https://doi.org/10.1105/tpc.114.129049

Yang, X., Owens, T. G., Scheffler, B. E., & Weston, L. A. (2004). Manipulation of root hair development and sorgoleone production in sorghum seedlings. *Journal of Chemical Ecology*, *30*(1), 199–213. https://doi.org/10.1023/b:joec.0000013191.35181.03

Yao, Q., Shen, R. D., Shao, Y., Tian, Y. F., Han, P. J., Zhang, X. N., Zhu, J. K., & Lu, Y. M. (2024). Efficient and multiplex gene upregulation in plants through CRISPR-Cas-mediated knockin of enhancers. *Molecular Plant*, *17*(9), 1472–1483. https://doi.org/10.1016/j.molp.2024.07.009

Zakir, H., Subbarao, G. V., Pearse, S. J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H., & Yoshida, M. (2008). Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). *New Phytologist*, *180*(2), 442–451. https://doi.org/10.1111/j.1469-8137.2008.02576.x

Zeng, H., Di, T., Zhu, Y., & Subbarao, G. V. (2016). Transcriptional response of plasma membrane H^+-ATPase genes to ammonium nutrition and its functional link to the release of biological nitrification inhibitors from sorghum roots. *Plant and Soil*, *398*(1), 301–312. https://doi.org/10.1007/s11104-015-2675-2

Zhang, M., Gao, X., Chen, G., Afzal, M. R., Wei, T., Zeng, H., Subbarao, G. V., Wei, Z., & Zhu, Y. (2023). Intercropping with BNI-sorghum benefits neighbouring maize productivity and mitigates soil nitrification and N_2O emission. *Agriculture, Ecosystems & Environment*, *352*, 108510. https://doi.org/10.1016/j.agee.2023.108510

Zhang, X., Mi, Y., Mao, H., Liu, S., Chen, L., & Qin, F. (2020). Genetic variation in ZmTIP1 contributes to root hair elongation and drought tolerance in maize. *Plant Biotechnology Journal*, *18*(5), 1271–1283. https://doi.org/10.1111/pbi.13290

Zhang, Y., Yang, Z., Zhang, Z., Li, Y., Guo, J., Liu, L., Wang, C., Fan, H., Wang, B., & Han, G. (2023). Root hair development and adaptation to abiotic stress. *Journal of Agricultural and Food Chemistry*, *71*(25), 9573–9598. https://doi.org/10.1021/acs.jafc.2c07741

Zhu, J. M., Kaeppler, S. M., & Lynch, J. P. (2005). Mapping of QTL controlling root hair length in maize (Zea mays L.) under phosphorus deficiency. *Plant and Soil*, *270*(1-2), 299–310. https://doi.org/10.1007/s11104-004-1697-y

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