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RESEARCH ARTICLE

Enhancement of sorghum grain yield and nutrition: A role for arbuscular mycorrhizal fungi regardless of soil phosphorus availability

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Societal Impact Statement

Sorghum is an important cereal crop that provides calories and nutrients for much of the world's population, and it is often grown with low fertiliser input. Optimising the yield, nutritive content and bioavailability of sorghum grain with minimal input is of importance for human nutrition, and arbuscular mycorrhizal (AM) fungi have previously shown potential to assist in this. Across sorghum genetic diversity, AM fungi improved the yield, nutrition and zinc and iron bioavailability of grain in a low phosphorus soil. Thus, food production systems that effectively manage AM fungi may improve consumer outcomes.

Summary

- Sorghum is a C_4 cereal crop that is an important source of calories and nutrition across the world, predominantly cultivated and consumed in low- and middle-income countries. Sorghum can be highly colonised by arbuscular mycorrhizal (AM) fungi, and the plant-fungal association can lead to improvements in biomass and nutrient uptake. High-throughput phenotyping allows us to non-destructively interrogate the 'hidden' effects of AM fungi on sorghum growth and phenology.
- Eight genetically diverse sorghum genotypes were grown in a soil amended with 2 or 20 mg P kg^{-1} and inoculated with an AM fungal culture of *Rhizophagus irregularis*. High-throughput phenotyping uncovered the 'hidden' effects of AM fungi on growth and phenology, while grain biomass, nutrition, Zn and Fe bioavailability and root AM colonisation was determined after destructive harvest.
- Sorghum plants colonised by AM fungi generally performed better than non-AM control plants, with greater yield, harvest indices, and grain P, Zn and Fe contents. During the early growth stages, AM colonisation led to temporary growth depressions. There were also AM fungal and P fertilisation effects on sorghum time-of-flowering. The sorghum genotype with the highest AM colonisation could barely produce grain when non-inoculated.

- The two genotypes that failed to mature had very low AM colonisation. Generally, the genetically diverse sorghum genotypes were highly responsive to AM colonisation and produced more grain of greater nutritive quality when colonised, without adverse consequences for micronutrient bioavailability.

KEYWORDS
arbuscular mycorrhizal fungi, bioavailability, hidden hunger, micronutrients, phosphorus, phytate, *Sorghum bicolor*, zinc

1 | INTRODUCTION

Sorghum (*Sorghum bicolor*) is an important C_4 crop grown for food, feed and fibre. Grain varieties for food provide calories and essential nutrients for humans, and are particularly important as a subsistence crop in parts of Africa and Asia (Shakoor et al., 2014). The threat to food security in low- and middle-income countries is two-pronged: an intake both of sufficient calories and of sufficient amounts of essential micronutrients such as zinc (Zn), iron (Fe) and vitamin A are necessary to prevent disease and other health problems (Cakmak et al., 2017). A lack of micronutrients in the human diet (known as 'hidden hunger') can lead to a range of immune problems and diseases, due to the importance of Zn and Fe as co-factors in >300 enzymes. Thus, grain yield and increased nutrient concentrations, particularly of Zn and Fe, are important qualities for people who rely on sorghum as a staple food in their diet (Cakmak & Kutman, 2017).

While cereals provide calories and micronutrients for human consumption, the absorption of some mineral nutrients in the gut are hindered by the anti-nutritional compound phytic acid/phytate. Phytic acid is a major storage compound for phosphorus (P) in seeds/grain, and increased phytate content in the grain has a direct negative relationship with the bioavailability of Zn and Fe, as a result of their chelation by phytate. Many regions in the world with soils deficient in Zn (e.g., Australia) have overcome the limitation to crop growth by applying fertiliser (Alloway, 2009; Alloway et al., 2008), but this is not the case everywhere. Low soil Zn availability is a major constraint on cereal cropping systems in parts of Africa, and the resulting bioavailability of Zn in maize grain was reported to be dramatically low (Manzeke et al., 2012). Thus, the development of sorghum varieties with increased bioavailability of micronutrients would help to combat the hidden hunger that is experienced by ~30% of the world's population in low- and middle-income countries. Some work has been done to optimise post-harvest processes for improvement of bioavailability in sorghum (Afify et al., 2011; Mahgoub & Elhag, 1998). However, these interventions alone will not be sufficient to alleviate the problem, and research directed to pre-harvest processes (variety selection, soil nutrient management) will be beneficial.

The application of inorganic fertilisers, particularly of P and nitrogen (N), drastically increases the yield of cereal crops on low nutrient soils. However, subsistence farming practices often rely on a low-input system due to the high cost and inaccessibility of inorganic fertilisers (Nziguheba et al., 2016). In these situations, effective agronomic management is important, particularly management of the soil and choice of crop variety that best suits the environmental constraints (e.g., low nutrient, drought and salinity) (Chikowo et al., 2014; Whitbread et al., 2010). As part of soil management, the effective management of soil microbiota including arbuscular mycorrhizal (AM) fungi is an option to increase the availability and utilisation of soil-derived nutrients (Cardoso & Kuyper, 2006; Lekberg et al., 2008; Plenchette et al., 2005).

Arbuscular mycorrhizal fungi colonise the roots of the majority of terrestrial plants, including many important cereal crops. The host plant benefits from the association with AM fungi by improved uptake of inorganic nutrients including P and Zn. Sorghum varieties grown for biomass can be highly colonised by different species of AM fungi, and typically display positive biomass responses when colonised (Watts-Williams, Emmett, et al., 2019). AM fungi can improve sorghum P, K, Zn and Fe nutrition (Caris et al., 1998; Ortas et al., 1996; Raju et al., 1990), and the tolerance of sorghum to drought and salinity stress (Cho et al., 2006). This is also the case in many other cereal crops (maize, rice and wheat) which display positive growth responses to AM colonisation in both field and glasshouse experiments, except when intervention practices such as tillage or rotation with a non-host crop (e.g., canola) are taken into account (Zhang et al., 2019).

Recent studies in durum wheat demonstrated that AM fungi have a complex role in the bioavailability of micronutrients in cereal crops, as colonisation of roots by AM fungi affects both P and micronutrient uptake into the grain (Tran et al., 2019, 2021). This has also been shown in other crops (Ma et al., 2019; Ryan et al., 2008; Subramanian et al., 2013). Expanding this research to sorghum will determine whether AM colonisation can improve nutrition not only for sorghum plants *per se*, but also for the human consumer of the grain product (bioavailability).

To explore the effects of AM colonisation on the growth and nutrition of sorghum, we used a subset of a panel of diverse grain *S. bicolor* genotypes (Tao et al., 2020) and investigated the effects of *Rhizophagus irregularis* inoculation and soil P fertilisation on plant growth, phenology, yield, and nutrition, with the focus particularly on effects of AM fungi on sorghum grown at low P availability. The combination of high-throughput phenotyping (which allows for repeated analysis over time) and destructive endpoint harvesting allowed us to examine the effects of AM fungal inoculation over the life of the plant, and address the following questions:

i. Do genetically diverse genotypes of sorghum perform better in low soil P conditions when colonised by AM fungi?
ii. Does AM colonisation of sorghum affect sorghum growth and phenology and/or grain yield and nutrition?
iii. Is the bioavailability of sorghum grain Zn or Fe affected negatively by soil P fertilisation, or AM fungi?

2 | MATERIALS AND METHODS

The sorghum genotypes were grown in a sand/soil substrate consisting of twice-autoclaved fine sand and sieved soil from the Gawler River region of South Australia (Clay & Mineral Sales Pty Ltd, Adelaide). The soil alone had plant-available (Colwell) P concentration of 10.6 mg kg^{-1}, pH of 8.3 and a clay loam texture. The sand and soil were mixed thoroughly in a 1:4 ratio (on a mass basis) which resulted in plant-available (Colwell) P concentration of 4.12 mg P kg^{-1}. The mixed substrate was then amended with *R. irregularis* AM fungal pot culture inoculum (comprising soil, spores, external hyphae and colonised root pieces) to 10% of the substrate mass, or with a mock inoculum. There were two P addition treatments; half of the pots received 2 mg P pot^{-1} (Low P) and the other half received 20 mg P pot^{-1} (High P) delivered as KH_2PO_4. Following mixing of P additions the substrates had plant-available P concentrations of 6.55 and 28 mg P kg^{-1}. Plant-available soil P values fell below (for Low P) and above (for High P) the estimated critical Colwell P values for sorghum (15–22 mg P kg^{-1}) (Peverill et al., 1999). Pots were 1-L olive pots (Garden City Plastics) that held 1.4 kg of sand/soil/inoculum mix each.

The sorghum genotypes (Table S1) consisted of the original reference genome BTx623 (Paterson et al., 2009) and seven genotypes selected from a previously characterised diversity panel (Tao et al., 2020) predominantly consisting of genotypes from the Sorghum Conversion Program. The seven genotypes were chosen to represent the major racial groups identified (caudatum, guinea, kafir, Asian durra and East African durra). These racial groups contain genotypes adapted to particular agroecological niches. Seeds were surface-sterilised by shaking in a 10% (*v*/*v*) sodium hypochlorite solution for 15 min before being rinsed well in reverse osmosis (RO) water. Seeds were then planted into trays of fine sand and watered and allowed to germinate for 5 days before seedlings were moved into the prepared sand/soil/inocula substrates (0 DAP; 24 December 2019). Plants were grown on a bench in a controlled environment room without supplemental lighting for 12 days (mean temperature during the day/night was 25.7°C/21.7°C) prior to being moved into the Smarthouse at the Plant Accelerator facility, where mean temperature during the day/night was 28.5°C/23.6°C and mean light intensity at midday during the growing period was 806 μmol m^{-2} s^{-1}. The capture of high-throughput phenotyping data commenced from 13 DAP, and from then plants were also watered automatically to 10% (*w*/*w*) gravimetric soil water content on a daily basis. All plants were given 10 ml of a modified Long-Ashton solution (P omitted) once per week for the duration of growing. Plants were given N as ammonium nitrate at five events over the duration of growing, to total 80 mg N by harvest.

The experiment was conducted in the NE Smarthouse of the Plant Accelerator on the Waite campus of the University of Adelaide. It comprised five replicates of eight Genotypes and four treatment groups, for a total of 160 plants. It occupied 10 Lanes × 16 Positions in the Smarthouse, each replicate occupying a block of 2 Lanes × 16 Positions. Randomisation was based on a split-plot design with eight main-units per block, each main-unit comprising four carts arranged in a grid of 2 Lanes × 2 Positions. The eight Genotypes were allocated to the 40 main units using a near-A optimal, resolved, row-column design, such that each Genotype occurred exactly once in each Block and no more than once at any of the eight main-unit positions. The four combinations of Mycorrhiza and Phosphorus were randomized to the four carts within each main unit. The initial row-column design was generated using od (Butler et al., 2020) and the full design was randomized using dae (Brien, 2020b), both being packages for the R statistical computing environment (R Core Team, 2020).

Day of transplanting is denoted 0 DAP. Imaging and automated watering were carried out daily from 13 DAP to 84 DAP (72 days). After the imaging event at 15 DAP, one plant was found to be dying and replaced. Due to hardware errors, images were found to be missing for all plants on 4 days (25, 26, 29 and 63 DAP), and for single plants on a further 5 days (14, 22, 27, 79 and 84 DAP).

From the collected red-green-blue (RGB) images (see Movies S1–S4, for examples), the Projected Shoot Area (PSA), a proxy for shoot surface area, of the plant was obtained. It is calculated as the sum of the areas, measured in kilopixels, from three camera views, comprising two side views and a view from above (see Figure S7 in Al-Tamimi et al., 2016 for diagram of HTP set-up). With this HTP system, plant type and size, values of PSA on day of harvest are generally linearly correlated with shoot biomass and as such, the growth analysis can be conducted with PSA and transformed data is not required (Neilson et al., 2015).

From 84 DAP the plants were moved back into the controlled environment room for further growth (mean temperature during the day/night was 26.1°C/20.3°C), until the grain had fully developed. At 111 DAP the plants were destructively harvested.

2.1 | Harvest and sample analyses

Shoots were cut at the soil level, weighed for total aboveground biomass, then placed in a drying oven at 60°C for at least 48 h; once dried, the grain was separated, and dry mass of grain and the remaining shoot tissue were measured. A small, steel corer (15-mm diameter) was used to take a subsample of soil near the stem containing roots. The subsample of fresh roots (100–200 mg) was washed free of soil then placed in a 50% ethanol solution.

After 24 h the fresh root subsamples were rinsed well with RO water and moved into a 10% KOH solution and left at room temperature to clear for 7 days. The cleared roots were then stained in a 5% ink in vinegar solution and heated to 60°C for 15 min, following Vierheilig et al. (1998). The stained roots were then rinsed well and allowed to de-stain in a 5% vinegar in water solution for 24 h before being moved to a 50% glycerol solution for assessment and storage.

Arbuscular mycorrhizal colonisation of the sorghum roots was quantified following McGonigle et al. (1990), whereby the proportion of arbuscules, vesicles and internal hyphae in roots were each independently assessed using the gridline intersect method.

For the plants that produced grain, the dried grain samples were ground to a fine flour using a Retsch MM400 mixer mill grinder. A subsample of ground sorghum grain was weighed into a 50-ml Falcon tube and the sample digested in a 4:1 nitric acid/hydrogen peroxide (*v*/*v*) mix in a heat block for 180 min before being diluted with RO water. The diluted acid digested grain samples were then analysed for elemental concentrations of P, Mg, S, K, Zn, Fe, Mn and Cu by ICP-OES. Another subsample of ground grain was used to measure the proportion of phytic acid present in the grain, following the manufacturer's protocol (Megazyme) and quantified on a spectrophotometer at OD_{655}.

2.2 | Statistical analyses of HTP and harvest data

The imaging data was prepared employing the R package growthPheno (Brien, 2020c) for the computation and using the SET method described by Brien et al. (2020). The PSA average growth rate (AGR) was calculated from the PSA values by differencing consecutive PSA and ln (PSA) values, respectively, and dividing by the time differences. These traits were found to exhibit (a) the usual large day-to-day fluctuations and (b) evidence of medium-term responses to applications of nitrogen fertiliser. Following exploratory routine probeSmoothing from growthPheno, it was decided to produce the smoothed PSA (sPSA) by applying weak logarithmic smoothing (smoothing df set to 15) to the PSA in order to remove the day-to-day fluctuations while retaining the medium-term responses. Using the sPSA, the smoothed growth rate sPSA AGR was computed analogously to PSA AGR.

It was decided to investigate growth with respect to the 10 time-points 16, 24, 34, 40, 45, 50, 55, 65, 75 and 83 DAP.

2.3 | Three-factor analysis

To produce phenotypic predictions, traits defined for both the +AMF and −AMF groups (including all imaging and biomass/yield traits) were analysed by using the R packages ASReml-R (Butler et al., 2020) and asremlPlus (Brien, 2020a) to fit a linear mixed model, starting with the following maximal linear mixed model:

$$\mathbf{y} = \mathbf{X}_t\boldsymbol{\tau} + \mathbf{X}_s\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where $\mathbf{y}$ is the response vector of values for the trait being analysed, $\boldsymbol{\tau}$ is the vector of fixed treatment effects, $\boldsymbol{\beta}$ is the vector of fixed spatial effects, $\mathbf{u}$ is the vector of random spatial effect; and $\mathbf{e}$ is the vector of residual effects; the matrices $\mathbf{X}_t$, $\mathbf{X}_s$ and $\mathbf{Z}$ are the design matrices for the corresponding effects.

The vector of fixed treatment effects, $\boldsymbol{\tau}$, is partitioned as $\left[\mu\, \boldsymbol{\tau}_G^\top\ \boldsymbol{\tau}_M^\top\ \boldsymbol{\tau}_P^\top\ \boldsymbol{\tau}_{G:M}^\top\ \boldsymbol{\tau}_{G:P}^\top\ \boldsymbol{\tau}_{M:P}^\top\ \boldsymbol{\tau}_{G:M:P}^\top\right]$, where μ is the overall mean and the sub-vectors of $\boldsymbol{\tau}$ correspond to the main effects of the treatment factors, namely Genotype (G), Mycorrhiza (M) and Phosphorus (P), the two-way treatment interactions (G:M, G:P and M:P), and the three-way treatment interaction (G:M:P).

The vector of fixed spatial effects, $\boldsymbol{\beta}$, is partitioned as $\left[\boldsymbol{\beta}_B^\top\ \beta_{xC}\right]$, where $\boldsymbol{\beta}_B^\top$ contains the effects of Blocks and β_{xC} is the coefficient for the linear east–west trend across columns of main units in the same Position pairs. The random-effects vector $\mathbf{u}$ is partitioned as $\left[\mathbf{u}_{spl(xC)}^\top\ \mathbf{u}_{B:m}^\top\right]$, where $\mathbf{u}_{spl(xC)}$ captures nonlinearity in the east–west trend across columns using splines and $\mathbf{u}_{B:m}$ represents random spatial variation between main units within blocks. The residual effects $\mathbf{e}$ were assumed to be normally distributed with variance σ^2. In the case of a harvest trait, the variance σ^2 was allowed to differ between Phosphorus levels; in most cases, the difference was found to be statistically significant at the $\alpha = .2$ level. Wald F-statistics were used to choose the model for fixed treatment effects. Predictions, or adjusted means, were calculated for each trait using the chosen model; least significant pairwise differences at the $\alpha = .05$ significance level [LSD (5%)] were produced for determining the significance of differences between pairs of predictions.

2.4 | Two-factor analysis

A reduced mixed model was used for traits restricted to either the +AMF of the −AMF group. It excluded Mycorrhiza (M) as an experimental factor, resulting in the simplified treatment-effects vector $\boldsymbol{\tau} = \left[\mu\, \boldsymbol{\tau}_G^\top\ \boldsymbol{\tau}_P^\top\ \boldsymbol{\tau}_{G:P}^\top\right]$. Significance testing began with the two-way interaction (G:P); if this was not significant, the main effects were tested. In all other respects, the two-factor analysis was identical to the three-factor analysis outlined above.

2.5 | Flowering time

By interrogating the images of each plant taken daily, we determined the day of flowering (as first appearance of anthers and stigmas) for 106 of the 119 plants that produced grain by harvest. There were 13 plants that did not flower before the final day of imaging (84 DAP) but produced mature grain before harvest (111 DAP). We estimated that those plants must have begun flowering at least 14 days prior to being harvested in order to have mature grain, leaving a 13-day window (between 85 and 97 DAP) within which flowering must have occurred. If there was evidence of imminent flowering on 84 DAP (e.g., stem elongation and booting) we imputed the flowering DAP as 87 DAP. If there was no evidence of imminent flowering, we imputed flowering DAP as the mid-point of the 13-day window, as 91 DAP.

2.6 | Calculations of mycorrhizal responsiveness and mycorrhizal dependence

In this study, mycorrhizal dependence (MD_{grain}) is defined as the percent plant biomass lost by the non-AM plant by not being colonised by AM fungi; for example, a value of 100% denotes the non-AM plant

failed to produce grain. This definition and equation for MD follows Smith and Smith (2011):

$$Mycorrhizal\ dependence_{grain}\ (\%) = \frac{AMmean - NM}{AMmean} \times 100 \quad (1)$$

AM: AM-inoculated plant; NM: non AM-inoculated plant. Equation (1) is originally from Plenchette et al. (1983).

3 | RESULTS

3.1 | Aboveground biomass growth, accumulation and allocation

Sorghum plants fertilised with 20 mg P pot^{-1} (High P) accumulated more aboveground biomass than those fertilised with just 2 mg P pot^{-1} (Low P), although there were genotypic differences in the magnitude of the response to P fertilisation (Table S2 and Figure 1a). The largest per cent increase in biomass due to P application was observed in SC648-14E (137%) and the smallest increase observed in PI609055 (106%).

Although the overall aboveground biomass was not affected by AM fungal inoculation, the production of grain was significantly increased by AM fungal inoculation, primarily in the Low P treatment (Figure 1b). This resulted in harvest index (ratio grain to total aboveground biomass) patterns that were also significantly greater in the AM inoculated sorghum plants (Figure 1c). While the positive response to AM inoculation was observed in all six genotypes that produced grain, the effect was particularly striking in SC876-14E, which was essentially unable to produce grain when not colonised by AM fungi, even at High P.

By the final day of imaging (84 DAP) there were no differences in aboveground biomass between the AM and non-AM plants of any genotype, but there was marked variation between different sorghum genotypes (Figure 2 and Movie S1). For some genotypes (SC876-14E,

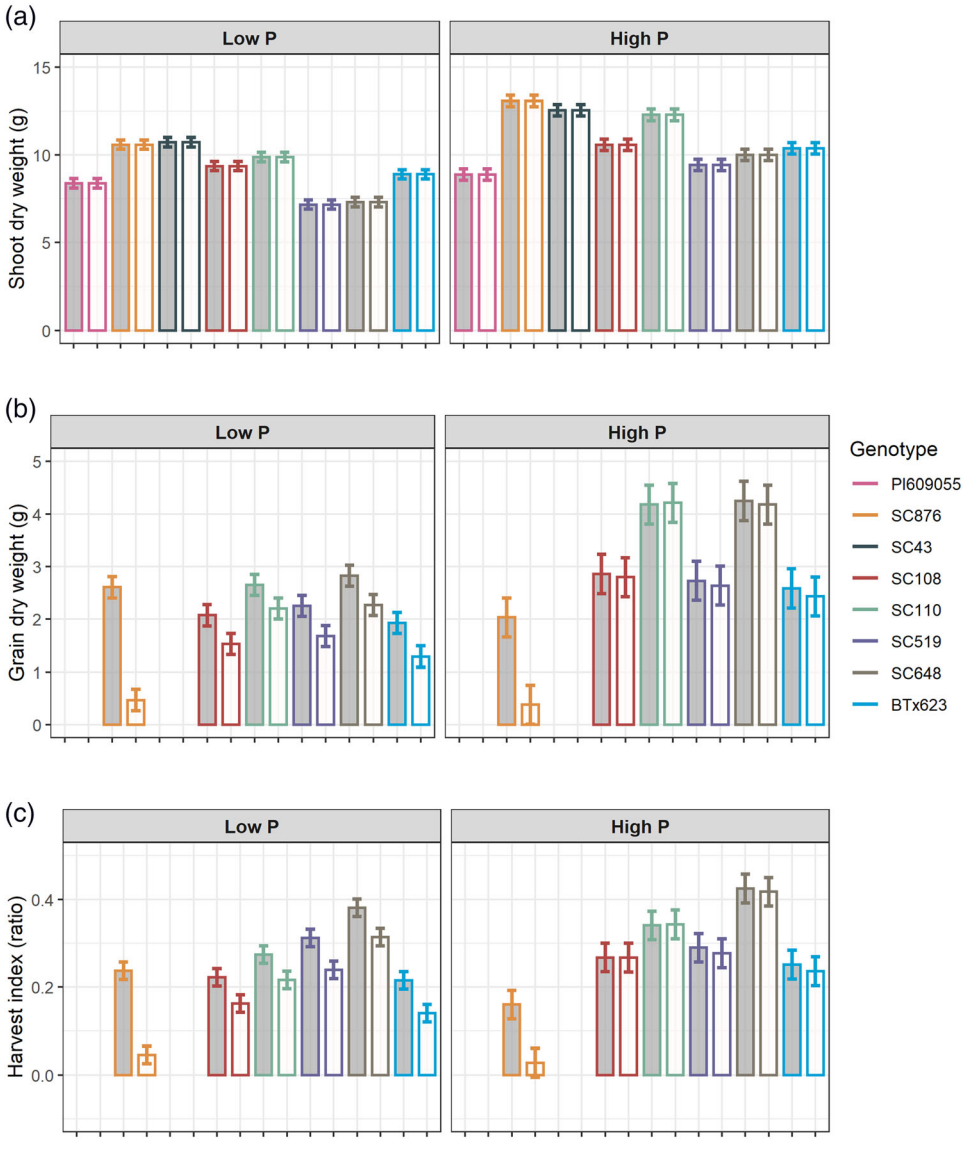

FIGURE 1 Shoot (all aboveground biomass) dry weights (a) at harvest of eight *Sorghum bicolor* genotypes, grain dry weights (b) and harvest index (c) at harvest of six *S. bicolor* genotypes (two did not produce grain) inoculated with the AMF *Rhizophagus irregularis* (grey bars) or mock-inoculated (white bars), grown at low or high plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi

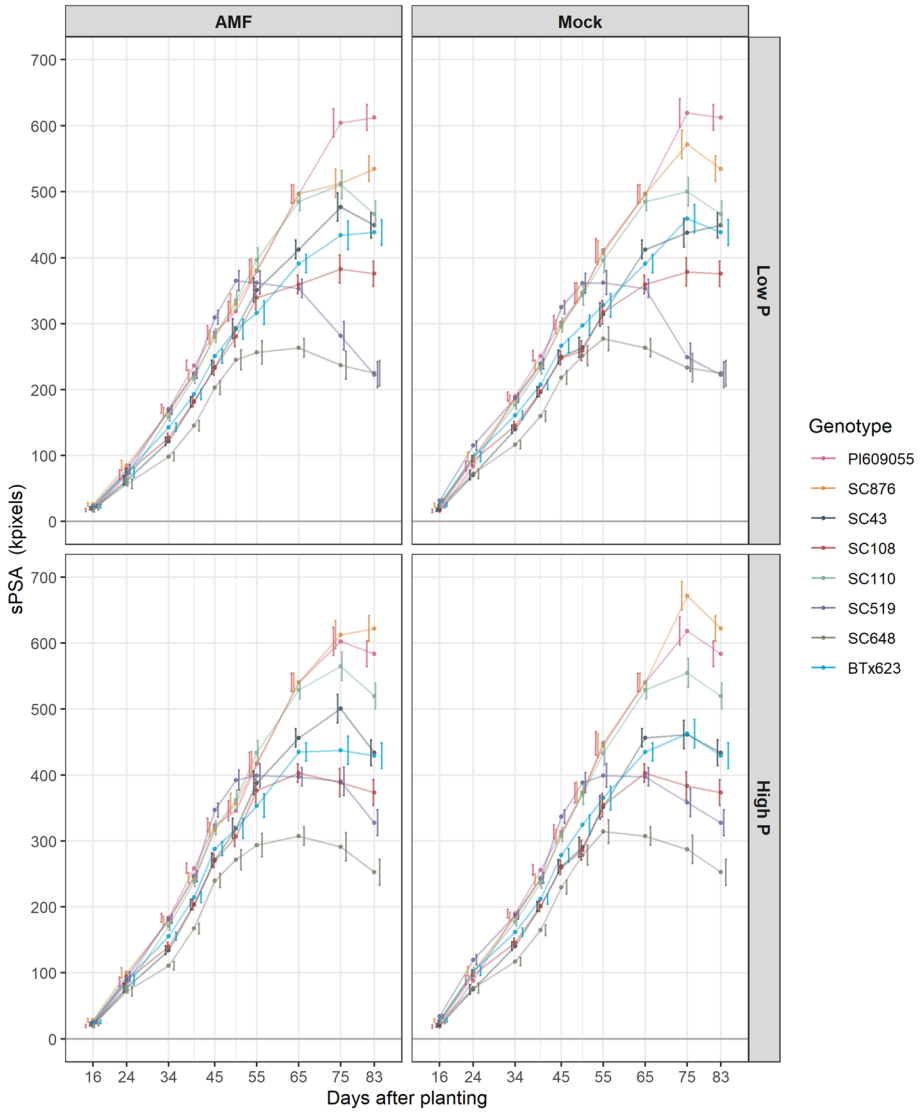

FIGURE 2 Predicted PSA at defined DAP of eight *Sorghum bicolor* genotypes inoculated with the AMF *Rhizophagus irregularis* (AMF) or mock-inoculated (Mock), grown at Low or High plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi; DAP, days after planting; PSA, projected shoot area

SC110-14E), the greater addition of P to the soil also positively affected growth.

However, the HTP revealed that there were some differences between AM fungal treatments over the course of the plants' growth that were not evident in the final harvest data (Figure 3). As early as 16 DAP, the mock-inoculated plants had significantly higher PSA than the AM plants in two genotypes (SC110-14E and SC519-14E); by 24 DAP two more genotypes also displayed this phenotype (SC108-14E and BTx623), and by 34 DAP the remaining four genotypes also exhibited this trend. For most genotypes, this trend was only observed in the Low P treatments, however. This was also reflected in the AGR (Figure S1) where at 20 DAP, the AGR was higher in the mock-inoculated plants than the AM plants for almost every genotype and P treatment (Figure S2).

For the genotypes that flowered, day of flowering was primarily determined by sorghum genotype, with SC519-14E flowering the earliest, and SC876-14E the latest (Figure S3); there was an approximately 25-day window between the earliest and the latest flowering genotypes. The flowering time of some genotypes was also affected by soil P fertilisation, and AM colonisation. In SC519-14E and SC648-14E, the two earliest flowering genotypes, the plants grown in High P treatment flowered later than those grown under Low P (Movie S2), while for SC108-14E, the opposite was true. In SC876-14E and SC648-14E, the AM plants flowered earlier than the non-AM plants (Movie S3), regardless of soil P availability. For the remaining two genotypes, SC110-14E and BTx623, flowering time was not significantly affected by soil P availability or AM colonisation (Movie S4).

The time point at which plants reach their maximum absolute growth rate (AGR_{max}) can provide an indication of their developmental rate. The day that plants reached their AGR_{max} value differed between genotypes, but not in response to soil P or AM inoculation (Figure S4a). This illustrates the strong influence of genotype on phenology of the diverse sorghum genotypes. With respect to AGR_{max}, SC876-14E peaked the latest, on 60 DAP, while SC648-14E peaked the earliest, at 42 DAP. However, the actual value of AGR (kpixels day^{-1}) on the day of AGR_{max} was affected by soil P fertilisation, and was greater in the High P treatment (Figure S4b). So, plant growth

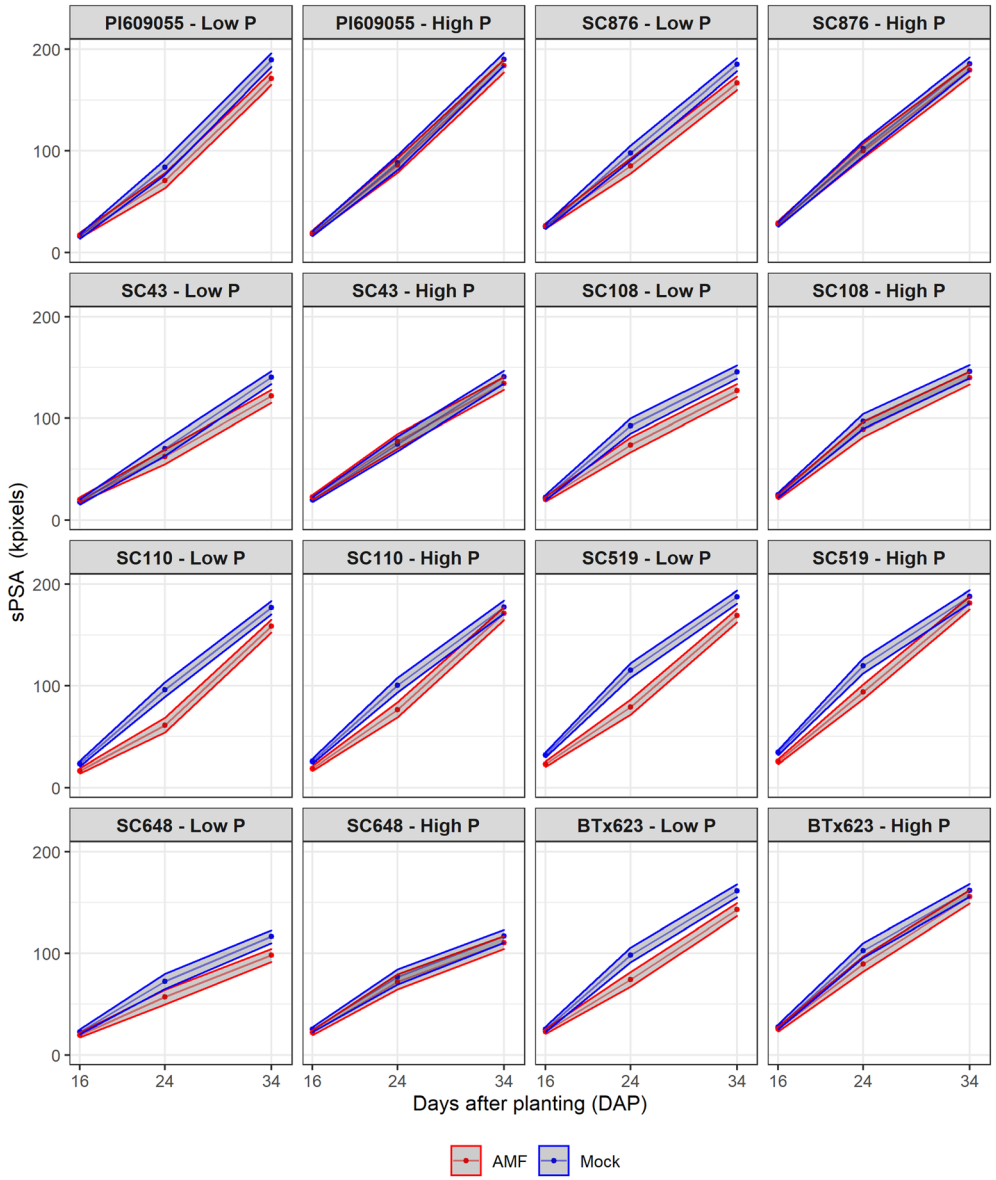

FIGURE 3 Predicted PSA at the three earliest analysed DAP of eight *Sorghum bicolor* genotypes inoculated with the AMF *Rhizophagus irregularis* (red) or mock-inoculated (blue), grown at low or high plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi; DAP, days after planting; PSA, projected shoot area

peaked at the same time depending on genotype, but the High P plants had a significantly greater peak than the Low P plants.

3.2 | Mycorrhizal colonisation

Sorghum roots were colonised by AM fungi in the *R. irregularis* treatments, and colonisation was highly variable across the different sorghum genotypes, ranging from 29.4% in PI609055 to 96.6% in SC519-14E (Figure 4a–c). All structural forms of AM colonisation observed (arbuscular, vesicular and hyphal) were reduced by increased soil P availability, but the increased P availability was particularly antagonistic to vesicular colonisation.

All six genotypes that produced grain displayed mycorrhizal dependence (as estimated by Equation (1)) to some degree when grown at Low P (Figure S5); but at High P, only SC876-14E and BTx623 were still dependent on the AM association to produce grain. SC876-14E had significantly higher mycorrhizal dependence at both soil P availabilities than the other five sorghum genotypes; interestingly, SC876-14E also had by far the greatest arbuscular colonisation (72%).

3.3 | Grain nutrition and micronutrient bioavailability

In the six sorghum genotypes that produced grain, we measured elemental concentrations of P, Zn and Fe on the dried grain samples, as well as phytic acid concentration. Grain Zn concentration was variable between genotypes, and in the non-AM plants was generally inversely related to the grain mass produced (i.e., more grain biomass, lower Zn concentrations; Figure S6a). In the AM plants, grain Zn concentration was higher than in the non-AM plants (except BTx623). Given that grain mass was also greater in the AM plants, the total Zn

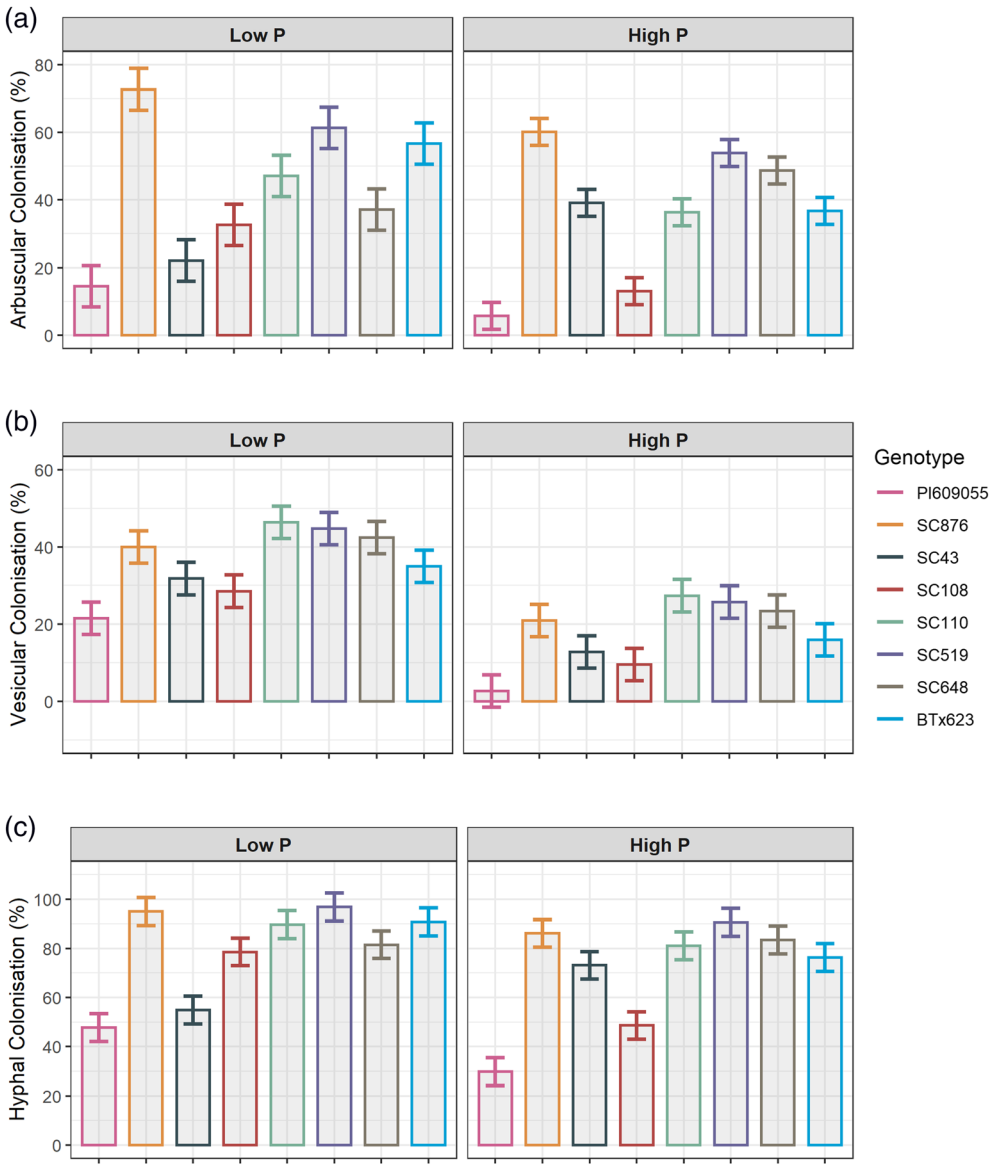

FIGURE 4 Arbuscular (a), vesicular (b) and hyphal (c) AM colonisation of roots at harvest of eight *Sorghum bicolor* genotypes inoculated with the AMF *Rhizophagus irregularis*, grown at Low or High plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi

contents were higher in all genotypes when colonised by AM fungi (Figure 5a).

Grain Fe concentration was similarly variable between sorghum genotypes and was only higher in the AM BTx623 plants than the non-AM plants (Figure S6b). However, grain Fe contents were higher in all genotypes when colonised by AM fungi at Low P (Figure 5b). Grain P concentrations were higher when there was more available soil P, and when colonised by AM fungi in the SC110-14E and SC519-14E genotypes (Figure S6c). Grain P contents were higher in the AM plants of all genotypes except SC108-14E at Low P (Figure 5c).

Grain phytate concentration was generally greater in the grain derived from AM plants, which was likely a direct consequence of the increased grain mass and P contents of these plants (Figure 6a). However, the estimated bioavailability of Zn and Fe (based on the molar ratio of phytic acid to Zn or to Fe, respectively) was higher in the AM plants, due to the higher concentrations of Zn and Fe in the AM plants (Figure 6b,c). While SC519-14E had the greatest concentration of grain phytate (at both P treatments), SC876-14E had the most bioavailable Zn (i.e., lowest molar ratio of phytate to Zn) and BTx623 the most bioavailable Fe (i.e., lowest molar ratio of phytate to Fe).

4 | DISCUSSION

The high-throughput phenotyping revealed that effects of AM fungal colonisation on sorghum growth are highly temporal in nature. Capturing shoot biomass data only at harvest can miss the temporary effects of AM fungi on growth that occur prior to harvest. We also report that AM fungi have a strong effect on the grain yield of sorghum, through allocation of resources to grain rather than vegetative biomass (harvest index), or dependence of the sorghum on AM colonisation (SC876-14E). The AM-colonised plants also accumulated greater amounts of P, Zn and Fe in the grain, without a decrease in the bioavailability of micronutrients. The results are now discussed in the context of the potential effects AM fungi could have to improve grain sorghum production.

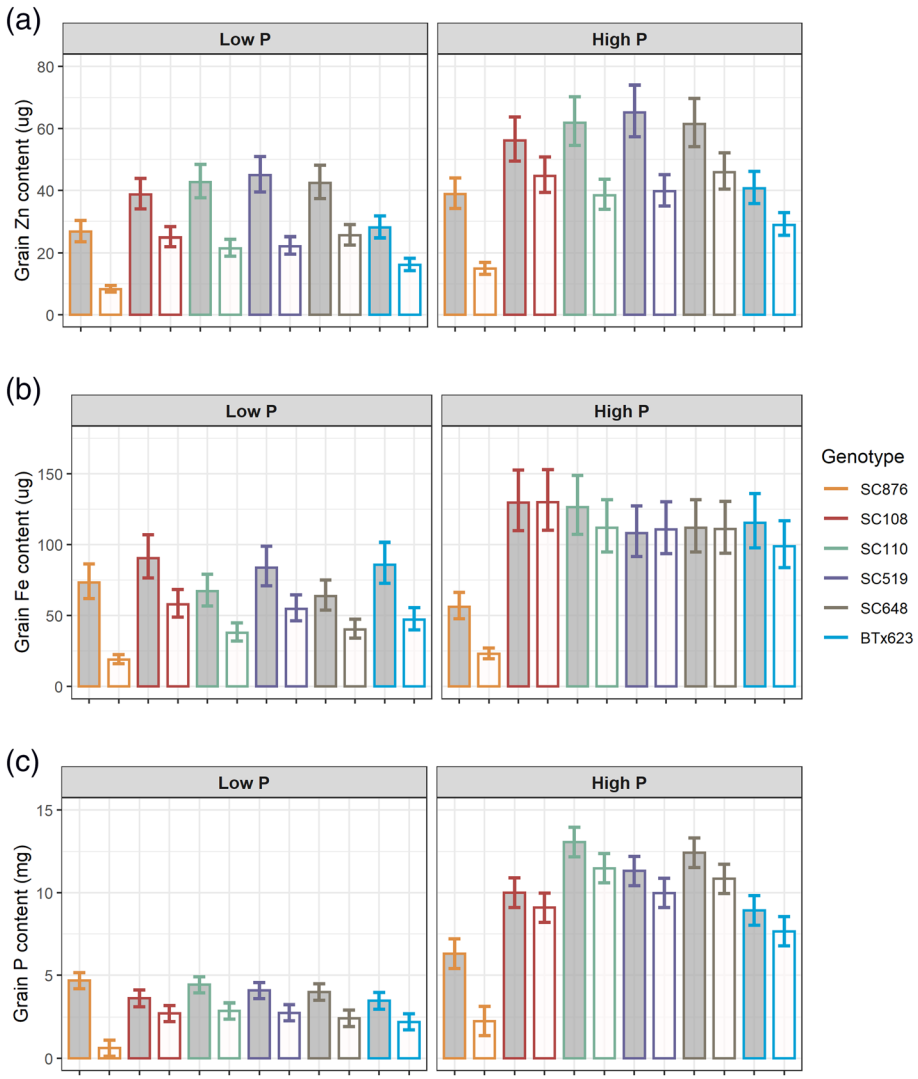

FIGURE 5 Grain phosphorus (a), zinc (b) and iron (c) contents at harvest of six *Sorghum bicolor* genotypes inoculated with the AMF *Rhizophagus irregularis* (grey bars) or mock-inoculated (white bars), grown at Low or High plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi

4.1 | Effects of AM colonisation on early sorghum growth and flowering time

In the early stages of sorghum growth, the non-AM sorghum plants had greater shoot growth rates and accumulated more shoot biomass than the AM plants. This AM-mediated growth depression early on may be due to a carbon (C) drain on the plant—a cost to establish the AM association, before there was activity of the mycorrhizal pathway(s) of nutrient uptake to compensate the drawdown. Early effects of AM colonisation on aboveground biomass have been demonstrated previously using this HTP system in other plant species, such as *Medicago truncatula*, tomato and barley (Tran, Cavagnaro, Jewell, et al., 2021; Watts-Williams, Jewell, et al., 2019). The shoot growth depressions observed in response to AM colonisation continued until 50–55 DAP (depending on the genotype), before the response to AM inoculation became neutral. Importantly, the growth depressions were resolved in the later stages of growth when it was revealed that AM plants in general allocated more biomass to grain, compared to the non-AM plants.

At the stage where the sorghum plants began flowering (beginning at 54 DAP), the AM and soil P treatments had genotype-dependent effects on time of flowering. In SC876-14E and SC648-14E, the AM plants flowered slightly (but significantly) earlier than the non-AM plants, and higher soil P led to earlier flowering in SC108-14E, but later flowering in SC519-14E and SC648-14E. Previously, in rice and cowpea, application of P to the soil resulted in earlier flowering (Islam et al., 1980; Ye et al., 2019), and the inverse has also been reported: in a forward genetics study of maize, low P availability significantly delayed flowering (Ren et al., 2019). Likewise, inoculation of various plant species with AM fungi has also led to reduced time to flowering, for example in *Capsicum annum* L. (green pepper) (Ortas et al., 2011), *Abutilon theophrasti* Medic. (Lu & Koide, 1994), *Pelargonium peltatum* L'Her. (Perner et al., 2007) and *Medicago truncatula* Gaertn (Liu et al., 2018). It is generally concluded that the effect of AM fungal colonisation in reducing time to flowering works by the same mechanism as that of soil P fertilisation, whereby increased P uptake by the AM pathway improves resource accumulation or plant size, thus contributes to the initiation of flowering (in Koide, 2010;

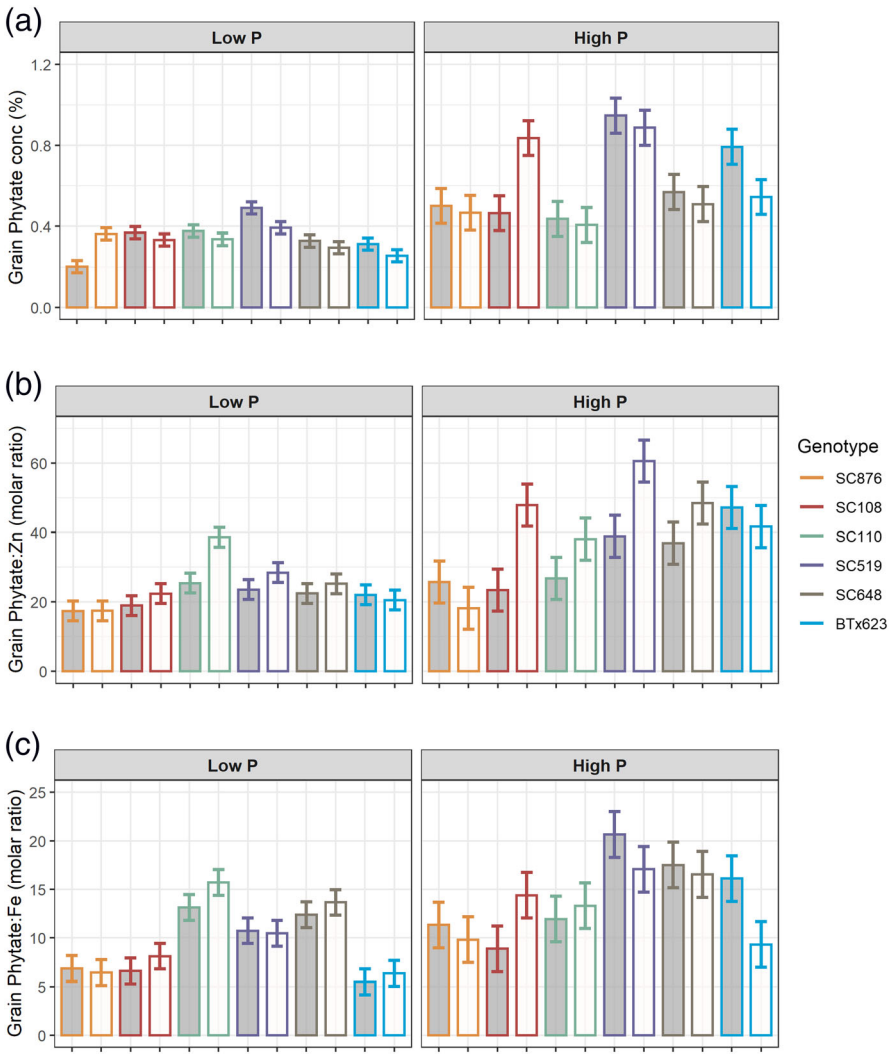

FIGURE 6 Grain phytate concentration (a), molar ratio of phytic acid to zinc (b) and phytic acid to iron (c) at harvest of six *Sorghum bicolor* genotypes inoculated with the AMF *Rhizophagus irregularis* (grey bars) or mock-inoculated (white bars), grown at Low or High plant-available (Colwell) soil P concentrations (6.6 or 28 mg P kg^{-1}). The bars correspond to predictions ± ½ LSD (5%). See Table S2 for statistical results. AMF, arbuscular mycorrhizal fungi

Schemske et al., 1978; Wyatt, 1981). This effect may, in turn, lead to greater reproductive success when the consequence of earlier flowering is greater number of flowers (and thus grain) (Sreenivasulu & Schnurbusch, 2012; Sukumaran et al., 2016). Evidence of improved plant fecundity in AM crop species has been reported in tomato (Bryla & Koide, 1998), which is a crop that is commonly non-responsive to AM colonisation when in the vegetative growth phase (Watts-Williams et al., 2014). When grown in the field, an AM-colonised tomato genotype produced more fruits than its non-AM control (Bowles et al., 2016; Tran et al., unpublished).

4.2 | Enhanced allocation to grain yield compared with vegetative biomass in arbuscular mycorrhizal plants

Although grain yield was enhanced by AM colonisation in sorghum, the overall aboveground biomass was not affected, demonstrating an effect of partitioning and allocation of resources to grain as a result of AM colonisation. Cobb et al. (2016) grew three landraces and three commercial hybrids of sorghum and observed positive response to AM colonisation in both the vegetative and grain portions only in the landraces, and only without fertiliser amendment. Although the commercial hybrids showed no vegetative biomass response, they did produce a small amount of grain in comparison to the non-mycorrhizal controls which did not produce grain (Cobb et al., 2016). The effect of AM colonisation on partitioning observed in the current study is likely an indirect one, whereby the colonised plants experienced enhanced nutrient uptake (and possibly other benefits related to abiotic/biotic stress tolerance) and were thus able to meet nutrient requirements for earlier maturity and greater yield. It suggests that an AM-colonised sorghum plant can capitalise on the soil nutrients available to it faster than the equivalent non-AM control plant.

4.3 | Mycorrhizal dependence in sorghum is linked to arbuscular mycorrhizal colonisation

In general, the AM sorghum genotypes grown in the Low P soil had an advantage over the non-AM plants. The AM plants accumulated

significantly more P, Zn and Fe in the grain, and had greater grain yield and harvest indices. This was also observed by Watts-Williams, Emmett, et al. (2019) who grew 18 sorghum lines in a low available P soil to vegetative stage and found 16 lines had a positive mycorrhizal growth response, and eight had significantly more P in the shoots. Furthermore, Cobb et al. (2016) found positive relationships between root AM colonisation (%) and grain contents of several mineral nutrients (including Zn and Fe) across diverse sorghum genotypes in a non-fertilised soil.

All of the sorghum genotypes that produced grain demonstrated some degree of positive response to AM colonisation, but there was genotypic variation in the magnitude of the positive response. There was a range of mycorrhizal dependence of the different sorghum genotypes, from very low (14%) to very high (84%; a MD value of 100% corresponds to the non-AM plants unable to produce grain). SC876-14E was almost unable to produce grain without AM inoculation, even at High P, and had up to 78% root length colonised by arbuscules. On the other hand, the two genotypes that did not produce grain had extremely low arbuscular colonisation of between 15% and 22% root length colonised. In general, there appears to be a stronger dependence on AM associations in sorghum than in other cereal crops, which may be due to C_4 crops being more responsive to AM colonisation than C_3 crops, more generally (Frew, 2019; Hetrick et al., 1990).

Here we focused on the effects of sorghum genotypic diversity, but we acknowledge the limitation of conducting the study using one, albeit common, species of AM fungi. Fungal identity (species, isolate) also plays an important role in determining the effect of the AM symbiosis in sorghum. When a panel of diverse sorghum lines were challenged with four different AM fungal species, seven sorghum lines responded positively (MGR) to just one AM fungus, one line to two fungi, five lines to three fungi, while two lines responded positively to all four AM fungal species (Watts-Williams, Emmett, et al., 2019). Furthermore, three different AM fungi were the 'preferred partner' across the seven lines that responded to just one AM fungal species each. Sorghum has also been shown to respond differently depending on whether it's inoculated with a single AM fungal species, multiple species, or the native soil AM fungal community (Frew, 2019, 2020). Clearly, the outcomes for sorghum biomass and nutrition are dependent on the identity of both the plant and the colonising AM fungus, and this should be kept in mind when interpreting the results of this work.

4.4 | No reduction in grain micronutrient bioavailability in mycorrhizal sorghum

Here we studied AM effects on grain Fe and Zn bioavailability in sorghum, an important crop for human caloric and nutrient intake in many parts of the world. The ability for crops to exploit the association with AM fungi is particularly relevant to subsistence farming systems that are often low-input by circumstance rather than choice, but is still highly dependent on management practices (Cobb & Wilson, 2018). In other cereal crops, AM fungi imparted a negative effect on the estimated bioavailability of micronutrients, presumably because of the increased uptake of P via the AM pathway, which is converted to more phytate in the grain (Tran et al., 2019; Tran, Cavagnaro, Able, & Watts-Williams, 2021).

In this study, when soil P availability was low, the effect of AM colonisation on the bioavailability of micronutrients was minimal. Specifically, there were no differences in estimated bioavailability in any genotypes except SC110-14E, which had more bioavailable Zn and Fe when colonised by AM fungi. This suggests that although grain P and phytate concentrations were generally higher in AM sorghum grain than non-AM, this did not lead to less bioavailable Zn and Fe. This is likely also due to Zn and Fe that were transported in greater amounts in the AM plants, offsetting the increased P and phytate accumulation in the grain. Future studies that examine the location and concentration of Zn and Fe in the aleurone layer of sorghum grain, the primary storage location of grain phytate, will be of interest.

Crop genetic modification and plant breeding, as well as changes to post-harvest processing practices, have been considered for their ability to minimise phytate accumulation in sorghum (Kayodé et al., 2006; Kruger et al., 2012). Of these approaches, genetic modification has been particularly successful, demonstrating significantly improved bioaccessibility of Zn in an animal model after feeding with a low-phytate sorghum (Kruger et al., 2013). Selection of sorghum genotypes that have reduced phytate accumulation as well as favourable associations with AM fungi (Cobb et al., 2021; Gemenet et al., 2016) may provide a solution for production on low P soils of greater sorghum grain, which has more bioavailable micronutrients.

5 | CONCLUSIONS

In grain sorghum, AM colonisation affected early stages of growth, flowering time, harvest indices, grain yield and nutrition. Generally, the effects of AM colonisation on sorghum grain yield and nutrition were positive. In a low soil P system, AM fungal colonisation increased the yield of six diverse grain sorghum genotypes (relative to a non-colonised control). Although yield may not have reached that of a P-fertilised soil, the benefit is that the negative effects of decreased bioavailability that can come with P fertilisation may be avoided, as well as the cost of fertiliser. Where access to plentiful P fertiliser is not an option, it will be important for sorghum growers to ensure their management practices enhance the potential for AM fungi to colonise their crops and provide benefits (Bowles et al., 2017).

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AUTHOR CONTRIBUTIONS

SJWW, NJ, CJB, BB, TG and TRC conceptualised the study and designed experiments. BTTT, EM, AWC and DRJ contributed materials or knowledge critical to the experiments. SJWW and ARG performed experiments. SJWW, NJ and CJB analysed data. SJWW wrote the manuscript. All the authors have read and edited the final manuscript.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data and images from high-throughput phenotyping are available at: https://zegami.com/collections/public-6077ed5261cd67c23c445998. The data from plant harvest that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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