# Sowing summer grain crops early in late winter or spring: Effects on root growth, water use, and yield

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**Research Article**

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# Sowing summer grain crops early in late winter or spring: Effects on root growth, water use, and yield

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## Highlights

- Sorghum grown in cooler than recommended environments will transfer water use from vegetative to reproductive stages increasing water use efficiency.
- Electromagnetic induction indices of root activity have potential to develop high throughput root phenotyping applications.
- To adapt to warmer climates, there is a need for breeding to consider cold tolerance in sorghum as a target.

## Abstract

CONTEXT. Drought and extreme heat at flowering are common stresses limiting the yield of summer crops. Adaptation to these stresses could be increased by sowing summer crops early in late winter or spring, to avoid the overlap with critical crop stages around flowering. Though little is known about the effects of cold weather on root growth, water use and final grain yield in sorghum.

OBJECTIVE. To research the effects of cold conditions in early sowing sorghum on crop and root growth and function (i.e., water use), and final grain yield.

METHODS. Two years of field experiments were conducted in the Darling and Eastern Downs region of Qld, Australia. Each trial consisted of three times of sowing (late winter, spring, and summer), two levels of irrigation (i.e., rainfed and supplementary irrigated), four plant population densities (3, 6, 9 and 12 pl $m^{-2}$), and six commercial sorghum hybrids. Roots and shoots were sampled at the flag leaf stage on three times of sowing, two levels of irrigation, and three replications, for a single hybrid and a single plant population density (9pl $m^{-2}$). Crop water use and functional root traits were derived from consecutive electromagnetic induction (EMI) surveys around flowering. At maturity crop biomass, yield and yield components were determined across all treatments.

RESULTS. The combinations of seasons, times of sowing and levels of irrigation created large variations in growth conditions that affected the growth and production of the crops. Early sowing increased yield by transferring water use from vegetative to reproductive stages increasing water use efficiency (kg $mm^{-1}$ available water). The larger yields in the early and spring sown crops were associated to larger grain numbers, particularly in tillers. Cold temperatures in the early sowing times tended to produce smaller crops with smaller rooting systems, smaller root-to-shoot ratios, and larger average root diameters. Total root length and root length density increased with increasing pre-flowering mean air temperatures up to 20°C. Linear relationships were observed between an EMI derived index of root activity and the empirically determined values of root length density (cm $cm^{-3}$) at flowering.

CONCLUSIONS. Sowing sorghum, a summer crop, early in late winter or spring transferred water use from vegetative stages to flowering and post-flowering stages increasing crop water use efficiency. The higher grain numbers in early sown crops were related to higher grain numbers in tillers. Root length and root length density were reduced by pre-flowering mean temperatures lower than 20°C, indicating a need to increase cold tolerance for early sowing. The EMI derived index of root activity has potential in the development of high throughput root phenotyping applications.

## 1. Introduction

Sorghum (*sorghum bicolor* (L.) Moench) is a major dryland crop across Australia's northern grains region, where droughts and extreme heat are common abiotic stresses that limit grain yield (Clarke et al., 2019; Rodriguez et al., 2023). Across the region, and for conventional sorghum sowing times, there is a high likelihood of heat stress events at flowering (Singh et al., 2017). Even though heat stress affects multiple physiological processes i.e., photosynthesis, respiration, and transpiration (Prasad et al., 2017), the most yield sensitive phase in sorghum is concentrated around a narrow window i.e., 10–15 days around flowering (Singh et al., 2016). A short duration of high-temperature episodes coinciding with this window, will cause pollen damage (flattened and collapsed pollen) leading to reduced pollen viability and pollen germination on the stigmatic surface (Li et al., 2015). This causes fertilization failures and reduced seed set resulting in lower grain numbers and grain yield (Singh et al., 2017). Terminal drought stresses after flowering may also affect grain filling by reducing grain weight and quality (Prasad et al., 2015; Impa et al., 2019).

Ongoing climate change is increasing global surface temperatures and the frequency and intensity of extreme heat and drought events (IPCC, 2021). Pathways to increase adaptation to heat and drought stress include improved genetic tolerance and agronomic avoidance (Prasad et al., 2015). Genetic tolerance to heat stress has been shown for both, the threshold at which pollen viability starts to be affected, and the response of pollen viability to increases in temperature above that threshold (Singh et al., 2015). In-silico assessments of the likely benefits of genetic tolerance to heat stress have shown yield gains between 5-8% and 13-17% under baseline and climate change projections, respectively (Singh et al., 2014). Clearly, in the long haul, plant breeding should be able to contribute to crop adaptation in warmer and drier environments (Nguyen et al., 2013), though in the meantime, agronomy might be used to avoid the likelihood of heat stress damage (Prasad et al., 2015). Agronomy practices such as early sowing (in late winter or spring), could advance flowering dates so that the overlap between times of the year of a high likelihood of the stresses and sensitive crop stages are avoided (Rodriguez et al., 2023). Ealy-sowing sorghum will develop during periods of the year of lower atmospheric demand, and flower before yield-limiting summer heat waves, reducing the impact of heat and terminal water stresses (Raymundo et al. 2021). However, sowing sorghum into soil temperatures lower than 16°C will slow the rate of metabolic activation enzymes in the seed (Patanè et al., 2021), leading to poor emergence, seedling establishment, and reduced plant stands (Rutayisire et al., 2021). Chilling temperatures after crop emergence can also reduce photosynthesis rates and shoot and root growth. A poorly developed root system might limit access to soil water and nutrients (Aroca et al., 2001), further reducing crop growth and production. Here we present results from a two-season field experiment in which we aim to i) answer whether sowing sorghum early i.e., in late winter or spring affects crop and root growth and function (i.e., water use), and final yield, and ii) study the relationships between ambient temperature, root traits, root function, shoot biomass, yield, and yield components.

## 2. Materials and Methods

### *2.1. Field trials*

Field trials were conducted at a commercial farm in Nangwee, Qld Australia (27°34'2.73" S, 151°18'34.36" E) during the 2019/20 and 2020/21 Southern Hemisphere summer growing seasons. The climate in the region is semi-arid subtropical with an average of 621 mm rainfall per annum and mean annual maximum and minimum temperatures of 27.0 °C and 12.0 °C, respectively (Bureau of Meteorology, 2023). Each season the trial covered an area of ~ 3.2ha (82m × 384m) of a uniform black, self-mulching cracking clay, characterized as a Vertosol soil (Isbell, 2016), with a clay content larger than 60%.

The trials included the factorial combination of three times of sowing (TOS, referred to as late winter, spring and summer), two levels of irrigation i.e., rainfed and supplementary irrigated, four plant densities (3, 6, 9 and 12 pl $m^{-2}$) and six commercial hybrids coded as A (A66), B (Agitator), C (Cracka), D (HGS114), E (MR Buster) and F (Sentinel). Each season, there were 432 plots with each 4m wide (4 rows) × 10m long. Further details of the experiment layout can be found elsewhere (Zhao et al., 2022). In 2019/20, crops were sown on 14 August, 11 September and 10 October. In 2020/21, crops were sown on 11 September, 6 October, and 5 November, respectively. Even though sowing was targeted to take place on soil temperatures ranging between 13°C (low) and above 16°C (recommended) at sowing depth, this was not always possible due to wet weather conditions. The supplementary irrigation treatment was imposed by laying drip irrigation pipes along each row after sowing. The objective of the supplementary irrigation treatment was to create additional growing environments, though water availability was limiting during the first season. Crops were fertilised following commercial sorghum production practices of the region and were kept free of weeds, pests and diseases.

An automatic weather station and soil temperature probe were installed before sowing to monitor daily minimum and maximum temperature, soil temperature at seed depth, total radiation, and rainfall. The normalised photo-thermal quotient (NPTq) was calculated using daily climatic records during flowering period (Rodriguez and Sadras, 2007). Initial plant available water (PAW) was measured gravimetrically at each time of sowing (one core per replicate down to 1.5 m).

*2.2. Measures of root growth and function*

Time-lapse EMI surveys were conducted to infer spatiotemporal variability of the plant available water (PAW, mm) and crop water use (mm) throughout the growing season. A DUALEM-21S (Dualem Inc., Milton, ON, Canada) instrument was used to collect soil apparent electrical conductivity ($EC_a$), which is a function of soil moisture content. The instrument was towed 3m to the right of a four-wheel all-terrain vehicle that traversed the field along the transect in the middle of each plot. In the first season fewer EMI surveys were taken, though during the second season surveys were conducted at fortnightly intervals. A detailed description of the method used to calibrate $EC_a$ to PAW in this study site is in Zhao et al. (2022). The crop water use down to 1.5m was determined between every two consecutive EMI surveys using eq. 1:

$$Crop\ water\ use = \Delta S + P + I \quad \text{eq. 1}$$

Where $\Delta S$ (mm) is the change of PAW in the 0-1.5m soil profile between the two consecutive EMI surveys, $P$ is precipitation (mm) and $I$ irrigation (mm). Crop water use was divided into pre-flowering, post-flowering, and total crop water use. Water use efficiency (WUE, kg $mm^{-1}$) was calculated as the ratio between grain yield (kg $ha^{-1}$) and total crop water use (mm).

In addition, in the 2020-2021, a root activity factor was calculated around flowering to represent the presence and activity in each studied soil depth as in Zhao et al. (2022) (eq. 2). Briefly, eq. 2 assumes that water use from an $i^{th}$ soil layer can be represented by the plant available water (mm) of that $i^{th}$ soil layer, a term representing the size of the canopy, and a factor we call *root activity factor* ($R_i$) (eq. 2). Another assumption is that given the large volume of soil surveyed, all treatments were affected by the same environmental conditions, and as all plots are measured within a small-time window (~2hs), therefore, changes in atmospheric demand can be expected to be small. The root activity factor was then calculated for the 0.3-0.5m, 0.5-0.8m, 0.8-1m, 1-1.3m, and 1.3-1.5m layers as in Zhao et al., (2022).

$$\boldsymbol{Root\ activity\ factor_i} = \frac{\boldsymbol{Crop\ water\ use_i}}{\boldsymbol{Plant\ water\ availibility_i \times Canopy\ size}} \qquad eq.\ 2$$

In eq. 2, the *Root activity factor$_i$* can be considered a functional proxy for root presence and activity in the *ith* layer (Zhao et al., 2022); *Water use$_i$* is the change in water content (mm) in the *ith* soil layer between two consecutive EMI surveys around flowering and permanent crop wilting point; *Plant water availability$_i$* is the plant available water (mm) in the *ith* layer at the start of the measurement period; and canopy size as main determinant of crop water demand. The Normalized Difference Vegetation Index (NDVI) was used as a proxy to account for canopy size. In this study, NDVI around flowering for each plot was derived from satellite images from PlanetScope (Planet Labs Inc, 2020).

### *2.3. Root and shoot growth*

The industry standard genotype (i.e., E, MR Buster) at one plant density (9 pl m$^{-2}$) was selected to conduct roots and shoots sampling. The sampling was conducted at the flag-leaf stage for three times of sowing, the two irrigation levels and three replications, resulting in 18 plots sampled each season. The shoots of twelve plants per plot were also sampled and oven-dried at 65 °C until constant weight. After sampling the shoots, the root system was sampled using a narrow tubular soil auger (44 mm diameter) down to a soil depth of 2.1 m. At each sampled plot, six cores were taken, two taken in the row and four in the interrow (Fig. S1). Each core was cut into eight depths of 0-0.3, 0.3-0.5, 0.5-0.8, 0.8-1, 1-1.3, 1.3-1.5, 1.5-1.8 and 1.8-2.1m. Corresponding depths of the six cores from each plot were bulked to give eight composite samples per plot, one from each depth. The samples were then soaked in water with a softening agent. The solution was then rinsed over a sieve in a root washing facility and the roots were collected with tweezers and stored in a 60-70% ethanol solution at 5°C. The root samples were then scanned using a digital scanner (Epson Expression XL 10000) with a resolution of 400 dpi. The scanned root images were analysed using the WinRHIZO® software, Regent Instruments Inc., Quebec, Canada (Trachsel et al., 2011). The root length (cm), average root diameter (cm), root surface area (cm$^2$), and root volume (cm$^3$) at each depth were calculated from WinRHIZO as in Rose (2017) and converted to per core basis. The root length density (cm cm$^{-3}$) and specific root length (cm g$^{-1}$) at each depth were calculated by considering the sample soil volume and root dry weights.

The total root length, total root surface area, total root dry weight, and total root volume at plot level were then calculated by summing the corresponding root traits across the soil profile (0-2.1 m). The average root diameter at plot level was determined from the total root length and total root volume. Similarly, a plot level average root length density (cm $cm^{-3}$), average specific root length (cm $g^{-1}$), and the root length to shoot dry weight ratio (cm $g^{-1}$) were calculated.

### *2.4. Dry matter production, yield, and yield components*

Yield and biomass data were measured on samples taken at physiological maturity from eight plants in the central rows of each plot; areas showing uniform plant density were selected. Each sample was oven dried to a constant weight at 65 °C to determine the above-ground biomass. Panicles were then separated and threshed to determine yield components including grain number (grains $m^{-2}$), grain weight (g per 1000 grains), and grain yield (t $ha^{-1}$). Seed set (%) was calculated for a period 10-15 days around anthesis, i.e. a period of 150°Cd, starting 50°Cd before anthesis and using maximum daily temperatures as in Singh et al., (2017). Yield components were partitioned into main stems and tillers. The harvest index was estimated as the ratio of grain yield to total biomass.

### *2.5. Statistical analysis*

Root traits were analysed using a linear mixed model (LMM) framework for each season at both plot and across depths levels. At the plot level, the LMMs included fixed effects for TOS, irrigation, and the interaction between TOS and irrigation. Replicate was included as random effects.

Across depths, the LMMs were used to test the effects of TOS, irrigation, depth, and their interactions on root traits. The residual variance model was upgraded in stages, to test for heterogeneity of residual variance between depth intervals, as well as residual correlation models across depth intervals. The most parsimonious model for each measure was selected using the Akaike Information Criterion (Akaike, 1998). Moreover, the values of root traits (i.e., root length, root surface area, root dry weight and root volume) were weighted on a "per 10 cm" basis to account for the differing widths of the depths.

Grain yield and its components (i.e., grain number and grain weight) and water use (i.e., pre-flowering, post-flowering, total crop water use and WUE) were also tested with LMMs. The season, TOS, irrigation, plant population, and genotype levels and their interactions were used as fixed factors and season×replication interactions were taken as random. Separate residual variances were fitted for each season by a separate scaled column×row variance structure.

All LMMs were fitted using the 'ASReml-R' statistical package (Butler et al. 2017), whereby variance components were estimated using residual maximum likelihood (Patterson and Thompson 1971) in R (R Core Team 2022). The fixed effects were tested using Wald tests (Kenward & Roger

1997), and Empirical Best Linear Unbiased Estimates (eBLUEs) were generated from the models for significant effects. Significant differences between pairs of treatments were determined using Fisher's least significant difference (LSD) (Welham et al. 2014), and all significances were assessed at the 5% level.

To explore the environmental effects of TOS on root growth, root function (i.e., crop water use), yield components and harvest index, a principal component analysis (PCA) was performed including environmental covariates (Table 1) based on 'stats' package in R. Conditional inference trees and random forest models were performed to untangle the G×E×M effects on yield in R using 'partykit' and 'randomForest' packages. In addition, the relationships between plot level root traits and pre-flowering mean air temperature and between WUE and yield components were also fitted in JMP 17 based on the least squares function.

## 3. Results

The effects of early sowing on the avoidance of heat stresses around flowering is described in full in a previous article that used results from a multi-environment (n=33) network of GxExM trials and includes the sites in this manuscript (Rodriguez et al., 2023). In this manuscript we focus on the effects of early sowing of sorghum on crop and root growth and function (i.e., water use), and final grain yield.

### *3.1. Environments, yield and yield components*

The combination of season, TOS and supplementary irrigation exposed the crop to a highly diverse range of growing conditions (Table 1). In the first season, soil temperatures for the late winter sown crop were well below the recommended 16°C at sowing depth, though in the second season they were close to 16°C. The early sown crops were also exposed to chilling ambient temperatures (<15°C) between emergence to flowering.

**Table 1.** Environmental conditions for the late winter, spring, and summer sown sorghum in the 2019/20 and 2020/21 growing seasons at Nangwee, Queensland, Australia.

| Environmental variables | Late winter | 2019/20<br>Spring | Summer | Late winter | 2020/21<br>Spring | Summer |
|---|---|---|---|---|---|---|
| Sowing-emergence average soil min T (°C) | 10 | 12.8 | 15.4 | 15.7 | 18.9 | 20 |
| Emergence-flag leaf average soil min T (°C) | 15.1 | 17.8 | 19.6 | 18.6 | 20.4 | 22.3 |
| Mean T (°C) | 17.4 | 19.5 | 22.5 | 20.7 | 21.7 | 22.8 |
| Pre-flowering average min T (°C) | 7 | 8.2 | 11.3 | 12.7 | 14.8 | 16.8 |
| Post-flowering average min T (°C) | 12.5 | 14.6 | 17.3 | 17.7 | 17 | 15.9 |
| Pre-flowering average max T (°C) | 25.4 | 27.5 | 30.4 | 26.7 | 28.1 | 29 |
| Post-flowering average max T (°C) | 31.2 | 33 | 36 | 28.4 | 27.6 | 29.4 |
| NPTq (MJ $m^{-2}$ $°C^{-1}$ kPa) | 0.96 | 0.79 | 0.55 | 0.90 | 1.38 | 1.40 |
| Pre-flowering rainfall (mm) | 16 | 10 | 28 | 60 | 83 | 111 |
| Post-flowering rainfall (mm) | 19 | 30 | 37 | 85 | 82 | 107 |
| Initial PAW (mm) | 105 | 102 | 104 | 145 | 171 | 228 |
| Pre-flowering irrigation (mm) | 102 | 119 | 94 | 136 | 137 | 160 |
| Post-flowering irrigation (mm) | 28 | 28 | 0 | 52 | 25 | 0 |
| Total plant available water (mm) | 271 | 289 | 263 | 478 | 496 | 603 |
| Seed set (%) | 91.5 | 95.7 | 91 | 89 | 92 | 87.6 |

T, NPTq and PAW indicate temperature, normalised photo-thermal quotient and plant available water, respectively.

There was a significant five-way interaction on grain yield between season, time of sowing, irrigation, plant population density and hybrid (Fig. 1 and Table S1). We used conditional inference trees and random forests on G, E and M variables, to further untangle these interactions. Fig. 1a and b show that total plant available water, a measure of heat stress around flowering i.e., Seed set (%) (Singh et al., 2017), and hybrid were the most important variables yields classifying grain yields within the whole data set (both seasons together). The highest yields were obtained with values of total plant available water higher than 340mm, and values of seed set higher than 88%. Higher yields were also associated to hybrids A, B and D, while plant population was the least important variable (Fig. 1b).

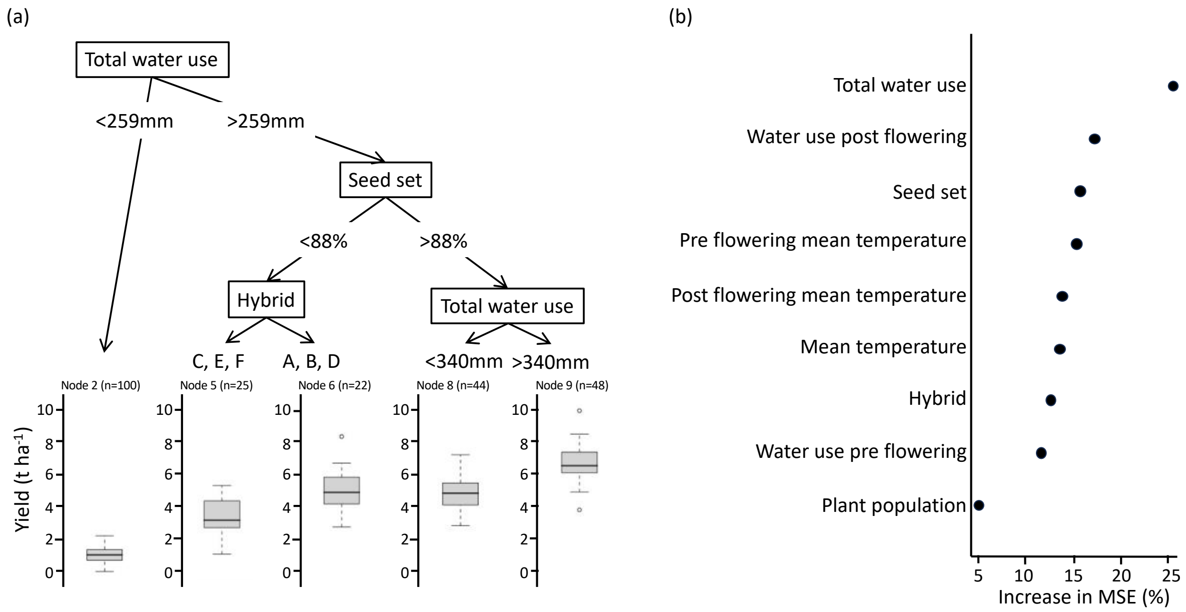


**Figure 1.** Conditional inference tree for environmental (i.e., time of sowing, irrigation, and season), genotype and management factors the interaction terms on grain yield in Table S1 (a), and (b) variable importance represented as the percent increase in the mean squared error for attributes assigned by a random forest. For the mean increase in accuracy the most relevant descriptors either relate to the total plant available water, water use after flowering and a measure of heat stress around flowering (Seed set, %) calculated as in Singh et al., 2012.

In both seasons, spring sown sorghum had larger or similar yields than the late winter sown crop, and the summer sown crop always had the lowest yields (Fig. 2a). Grain yields were associated to grain numbers, with the late winter and spring sown crops having a larger contribution of grain numbers from tillers (Fig. 2b and Fig. S2)

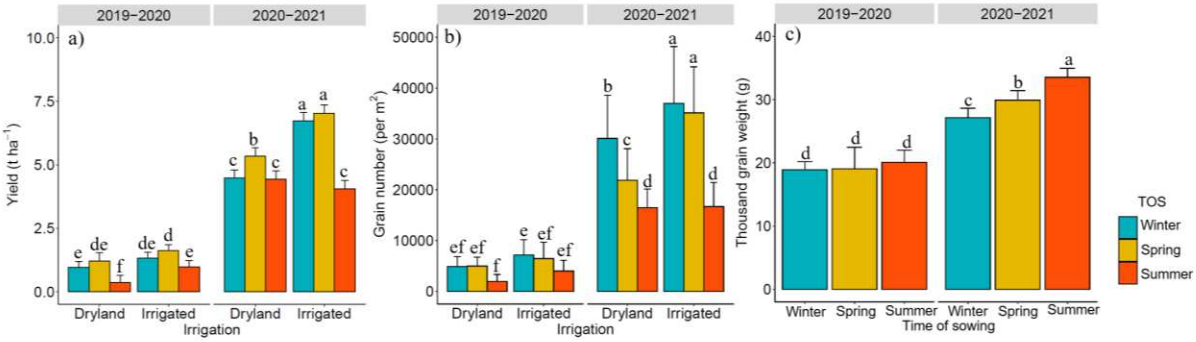


**Figure 2.** Effects of time of sowing (TOS, i.e., late winter, spring, and summer) or TOS by irrigation (i.e., dryland and irrigation) on the (a) grain yield, (b) grain number, and (c) grain weight across the 2019/20 and 2020/21 seasons. Different lowercase letters indicate a significant difference at $p \leq 0.05$. Error bars represent standard errors of the estimations.

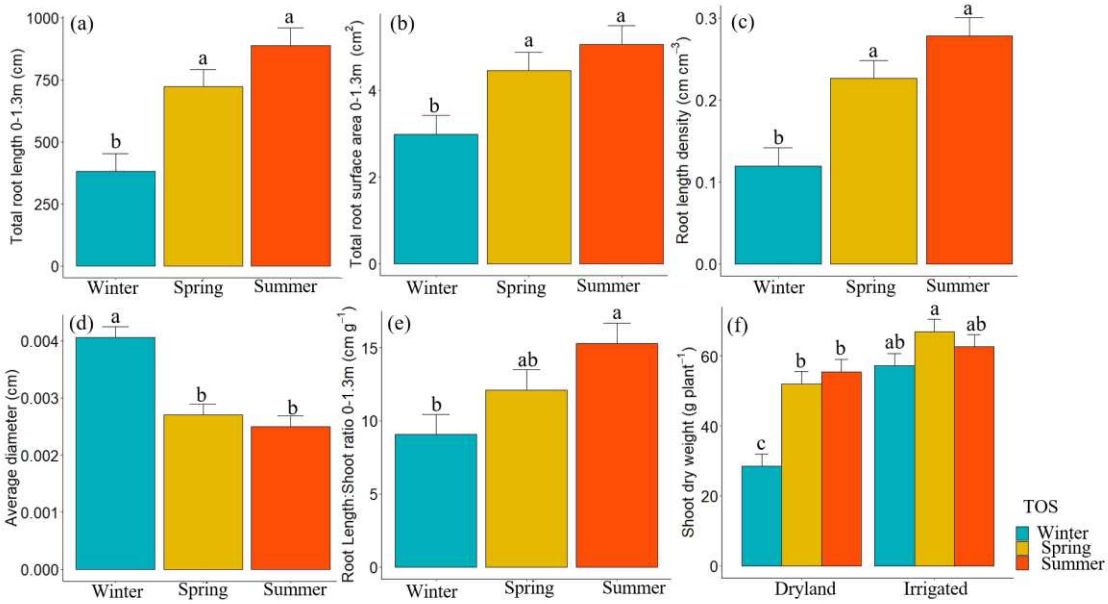


**Figure 3.** Effect of time of sowing (TOS) including late winter, spring, and summer on (a) total root length, (b) total root surface area, (c) root length density, (d) average root diameter, (e) total root length to shoot weight ratio and the effects of TOS by irrigation on (f) shoot dry weight at plot level at the flag leaf stage in the 2019/20 season. Different lowercase letters indicate a significant difference at $p \leq 0.05$. Error bars represent standard errors of the estimations.

*3.2. Root traits*

Differences between treatments on root traits were affected by the contrasting environmental conditions between both seasons of trials (Table 1). However, from the collective analysis, the wide range of environmental conditions across seasons and times of sowing, allowed to develop functional relationships between environmental co-variates (Table 1) and the studied traits (Fig. 3). In the drier and cooler 2019/20 season, the late winter sown crop had a significantly smaller rooting system, i.e., smaller total root length, total root surface area, root length density and shoot dry weight (Fig. 3 a to f). Conversely, the roots of the late winter sown crop were thicker (Fig. 3d). Compared to spring and summer sown crops, late winter crops were smaller (Fig. 3f), particularly under dryland conditions. Similarly, late winter crops had a smaller total root length to shoot dry weight ratio (Fig. 3e). In the wetter and warmer 2020/21 season, the value of the root traits was generally larger than in 2019/20, although there were no significant differences between treatments (Table S2).

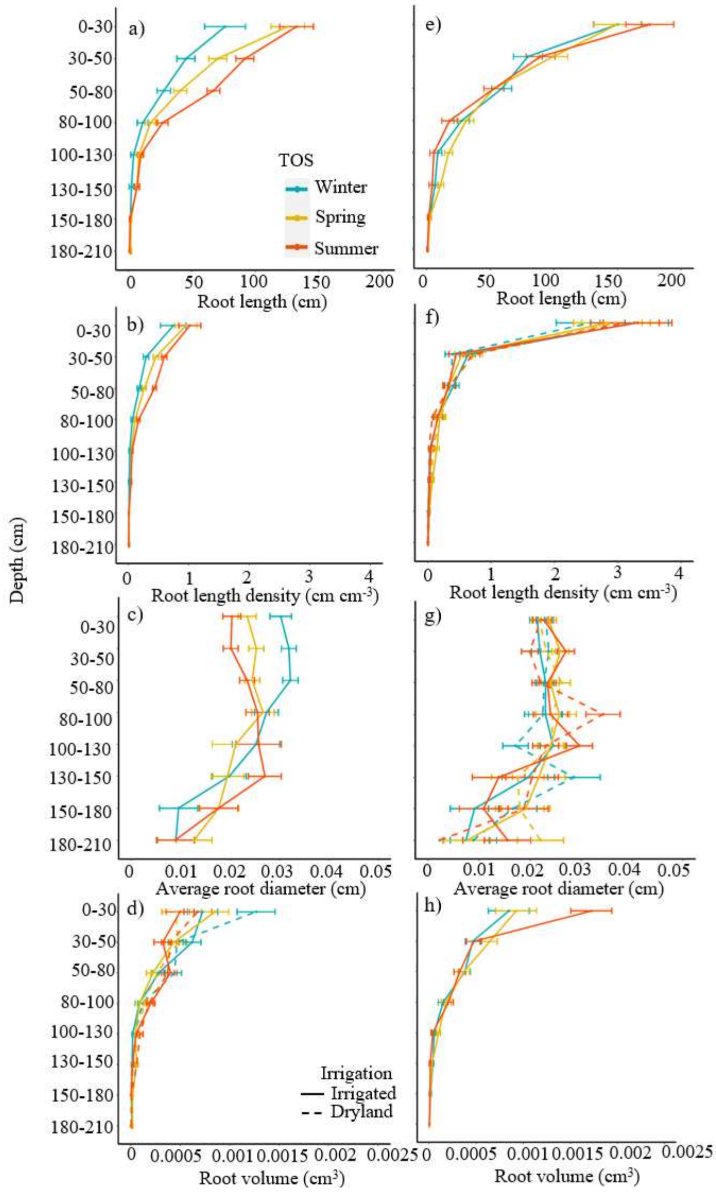


**Figure 4.** Effects of depth by the time of sowing (TOS) or depth by TOS by irrigation on root length, root length density, average root diameter, and root volume in 2019/20 (a, b, c, and d, respectively) and 2020/21 season (e, f, g and h, respectively). Values were the means for the three replicates. Error bars represent standard errors of the estimations.

Fig. 4 and 5, and tables S2 and S3, show root traits (eBLUEs) from the LMM for the Depth×TOS or Depth×TOS×Irrigation interactions. In 2019/20, the cold conditions of the winter sown crop significantly affected all root traits. The late winter sown crop had significantly smaller root length (Fig. 4a) and root length density (Fig. 4b) at each soil depth. Whereas the opposite was true for the average diameter (Fig. 4c) in which late winter sown sorghum significantly increased the average root diameter in the 0-0.8 m soil profile. This was also the case for the root volume (Fig. 4d), especially in dryland treatments. In contrast, late winter sowing reduced the surface area (Fig. 5a), root dry weight (Fig. 5b), and specific root length (Fig. 5c) across the soil profile, though differences between TOS were not significant.

The warmer and wetter conditions during the second season of trials, reduced the differences between sowing times, though as in the first season summer sown sorghum had a significantly larger root dry weights in the topsoil (Fig. 5e, and Table S3). In the second season, there were no significant effects of TOS, irrigation, or their interactions with depth observed for the other root traits (Table S2).

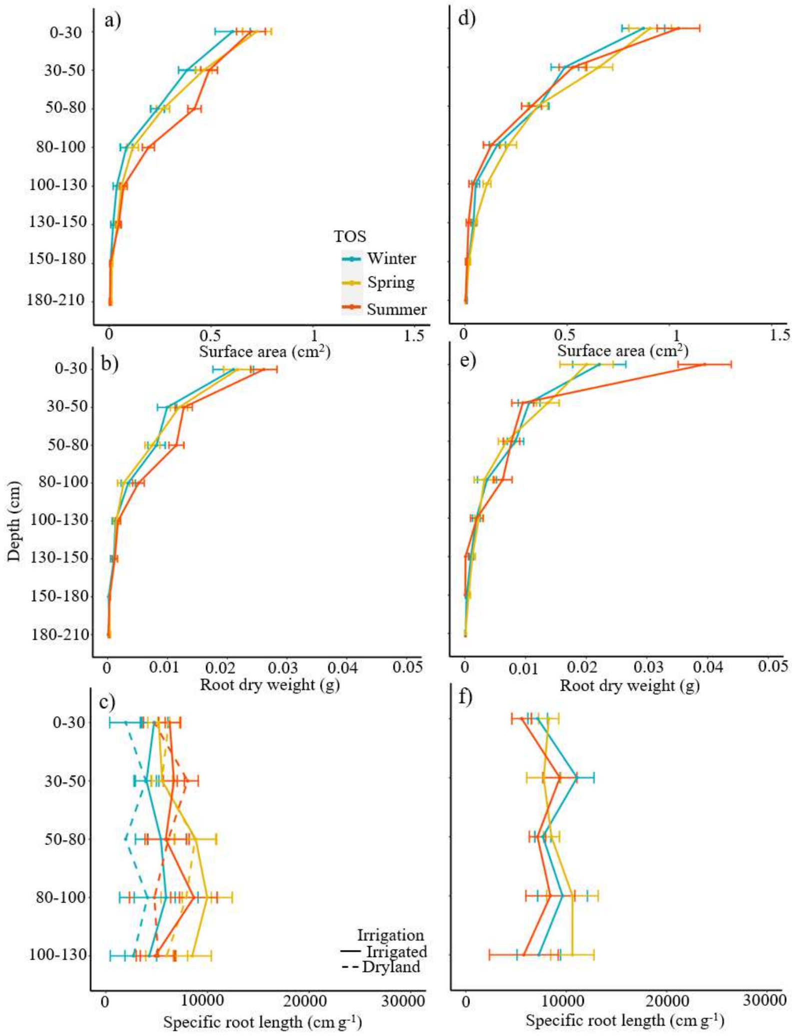


**Figure 5.** Effects of depth by the time of sowing (TOS) or depth by TOS by irrigation on the surface area, root dry weight, and specific root length in the 2019/20 season (a, b and c, respectively) and 2020/21 season (d, e, and f, respectively). Values were the estimated means. Error bars represent standard errors of the estimations.

Irrespective of the contrasting time of sowing, the root activity factor (R) calculated using eq. 2 was linearly related to the measured root length density (RLD) (Fig. 6), this is, the larger the root length density the larger the root activity factor. Fig. 6 also shows that for similar values of root length density, the dryland plots had a larger values root activity than the supplementary irrigated plots. In the dryland plots, the relationship did not hold for the topsoil layer (0.3-0.5m) as in the top layers the main limiting factor to water uptake was plant available water.

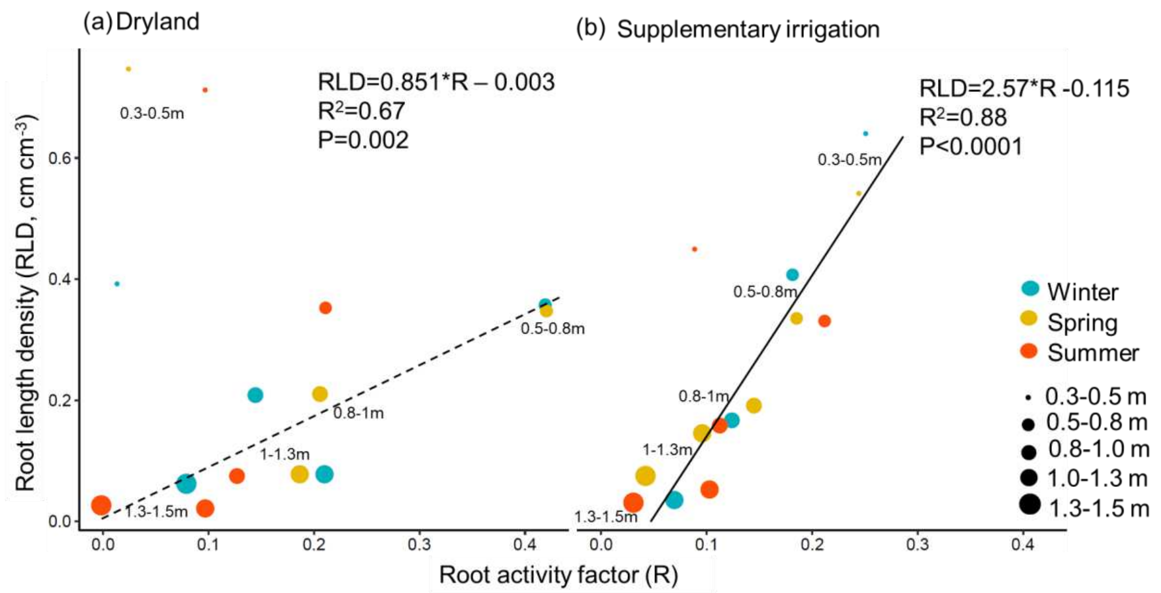


**Figure 6.** Relationship between the root length density (RLD, $cm^3$ $cm^{-3}$) and root activity factor (R) at flowering for the rainfed/dryland (a) and irrigated (b) plots. The data is for genotype E, sown at 9 pl $m^{-2}$ in the 2020-2021 season. Blue, orange, and red dots indicate the late winter, spring, and summer sown crops, respectively, and the size of the points indicate the soil layer. The linear relationships were not fitted to the data from the 0-0.3m and 0.3-0.5m depths, as those layers were close to wilting point, particularly in the rainfed treatment.

*3.3. Plant available water (PAW) and water use*

Plant available water was highly contrasting between the two seasons and three times of sowing. (Table 1). Across both seasons early and spring sown crops tended to have less pre-flowering water use and larger post-flowering water use than the summer sown crops (Figs. 7 and 8). Even though similar values of total plant available water across times of sowing i.e., within the dryland and irrigated treatments in the first season (Fig. 7c), during both seasons the values of water use efficiency were larger for the early and spring sown crops comparing to the corresponding summer sown crops (Fig. 7 d and h).

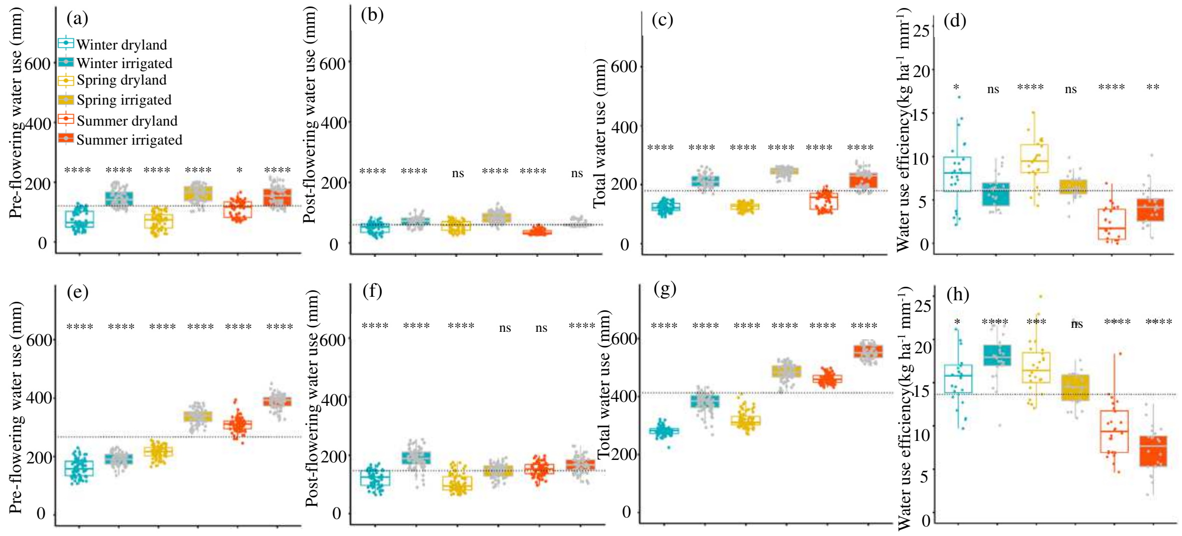


**Figure 7.** Cumulative crop water use (mm) derived from the electromagnetic induction surveys during (a) pre-flowering, (b) post-flowering, and (c) the whole crop cycle during 2019/20 (a, b, c, d, respectively), and 2020/21 (e, f, g, h, respectively). Significance tests are for the treatment mean versus the overall mean.

Particularly during the second, wetter season, larger PAW values were observed for the early sowing crops at flowering stage (Fig. S3). For example, in 2020/21 the irrigated late winter sowing had 272 mm PAW at flowering compared to the summer sown crop (211 mm). During the second season lower plant populations (3 and 6pl $m^{-2}$) showed larger values of plant available water at flowering displaying a difference of up to 61mm compared to higher populations and left more water in the soil profile by maturity, particularly in the early sown crops.

*3.4. Relationships between root traits, water use, yield components, and environments*

In both seasons (Fig. 8), PC1 explained ~45% of variations in the dataset, which was largely attributed to differences in root traits and environmental conditions, while PC2 was primarily associated to yield and yield components, shoot biomass, and crop water use. In general, the larger yield and harvest index values of the early sown crops were associated to a higher value of post-flowering water use resulting in higher water use efficiency values (WUE). Fig. 8 also shows an association between the root length, root weight, root surface area, root length density, and specific root length, in the summer sown crop with mean temperature and solar radiation.

Irrespective of the season, positive linear relationships between WUE and the total grain number (Fig. 9a). As expected, different relationships were observed between the irrigated and dryland treatments. The larger values of WUE in the early sown crops were also associated to a larger grain number contribution from tillers (Fig. 9b), and a larger fraction of water use after flowering (Fig. 9c).

As shown in Fig. 8, root traits were related to the temperature environment. Fig. 9d and e, show that root length and root length density responded positively to increasing pre-flowering mean air temperatures ranging between 16 and 20°C, but there was little further response above 20 °C. In addition, in the first cooler season, the late winter sown sorghum crops had thicker roots. Irrespective of the season or irrigation treatment, the mean root diameter fitted quadratic relationships with pre-flowering mean air temperatures, with the smallest values observed at around 20 °C (Fig. 9f).

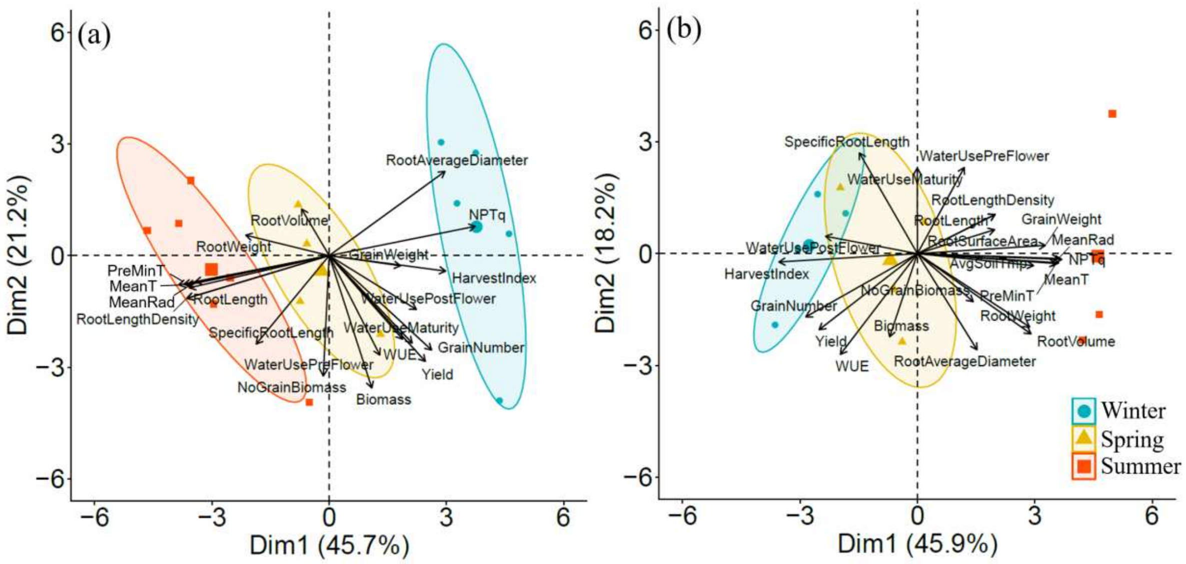


**Figure 8.** Principal component analysis of root traits, biomass, harvest index, yield components (i.e., yield, grain number, and grain weight), crop water use (i.e., pre-flowering water use – WaterUsePreFlower, post-flowering water use – WaterUsePostFlower, total water use – WaterUseMaturity) and environmental variables (i.e., mean temperature – MeanT, mean pre-flowering minimum temperature – PreFlwMinT, mean radiation – MeanRad, Normalised photo-thermal quotient – NPTq) across the (a) 2019/20 and (b) 2020/21 seasons. Each time of sowing was identified by a different symbol and a 68% confidence limit ellipse.

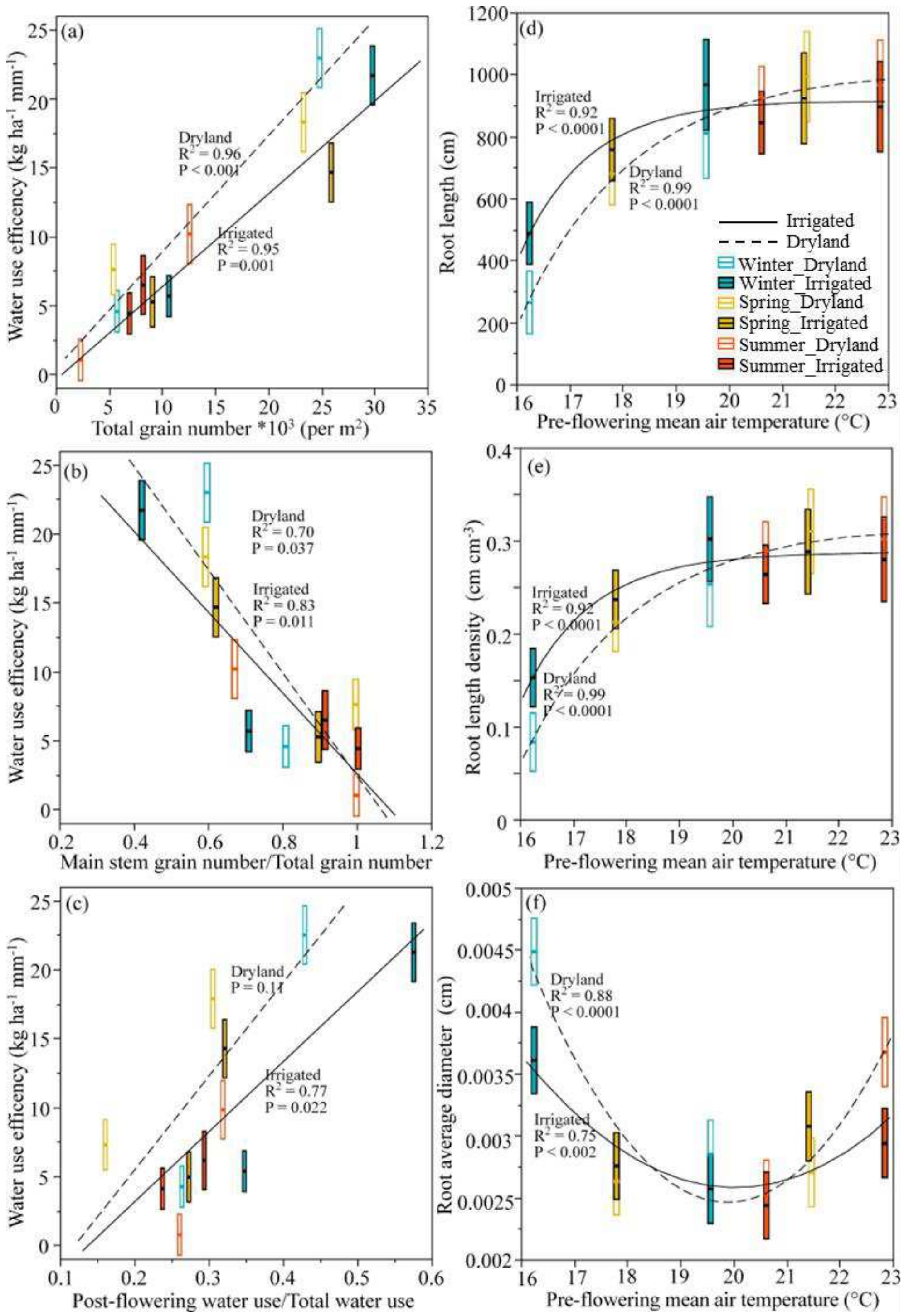


**Figure 9**. Relationships between (a) water use efficiency (kg $ha^{-1}$ $mm^{-1}$) and total grain number (per $m^2$), (b) the ratio of main stem grain number to total grain number and (c) ratio of post-flowering water use to total water use and between plot-level (d) root length (cm), (e) root average diameter (cm) and (f) root length density (cm $cm^{-3}$) and pre-flowering mean air temperature (°C), respectively, across the two study seasons for the three times of sowing (i.e., late winter, spring and summer) and two water levels (i.e., dryland and irrigated). The horizontal line in each box is the estimated mean with upper and lower bounds for standard errors of the estimation.

## 4. Discussion

Tolerance to heat stress, escape, and agronomic avoidance, have long been proposed as opportunities to increase adaptation to heat stresses around flowering in summer crops (Jagadish, 2020; Prasad et al., 2017; Reynolds et al., 2016). Though avoidance requires sowing sorghum, a summer crop, early during late winter or early spring (Carcedo et al., 2021), at soil and air temperatures lower than optimum. In this study we explored the effects of cold weather in early sowing sorghum, on crop and root growth and function (i.e., water use), and final grain yield. We showed that early sowing increased yield and water use efficiency by transferring crop water use from vegetative to reproductive stages. The larger yields in the early sown crops were associated to larger grain numbers in tillers. In general terms, root length and root length density responded positively to increasing pre-flowering mean air temperatures ranging between 16 and 20°C, but there was little further response above 20 °C. The linear relationships observed between an EMI derived index of root activity and the empirically determined values of root length density (cm $cm^{-3}$) show potential to be used in the development of high throughput functional root phenotyping applications.

### *4.1. Soil water dynamics and crop yield*

Crop production in terminal water stress and hot environments is primarily determined by the interactions between crop phenology, seed set, and water use dynamics before and after flowering (Siddique et al., 2001; Nguyen et al., 2013). In these environments, using genotypes that show stay-green phenotypes (Borrell et al., 2000), wide or skip row configurations and low plant populations (Whish et al., 2005), can transfer water use from vegetative to reproductive stages stabilising grain yields (Clarke et al., 2019; Carcedo et al., 2021). While avoiding air temperatures higher than 33°C during a 10–15-day window around flowering (Singh et al., 2017) will limit seed set losses due to pollen sterility (Prasad et al., 2017). Most of these principles are relevant when summer grain crops are sown early in late winter or early spring. Our results agree with these results to show that earlier sowing i.e., late winter and spring, tended to reduce pre-flowering water use, particularly in the dryland treatments (Fig. 7 and 8). The relatively larger availability of soil water during reproductive stages (Fig. S3), the lower total water use (Fig. 7), the larger grain yield contribution from tillers (Fig. S2i), and larger grain yields (Fig. 2), resulted in higher values of water-use efficiency for the early sown crops (Fig. 7d, h). Higher values of seed set (%) i.e., cooler temperatures around flowering, were also associated to higher yields (Fig. 1a), while seed set was also an important variable associated to grain yield in the random forest analysis (Fig. 1b). Thus, sowing sorghum, a summer crop, early into cold soils and chilling temperatures, can be expected to have little negative impact on grain yields, providing adaptation options to the expected increase in intensity and frequency of heat waves and drought events (IPCC, 2021).

In the long term, breeding can be expected to contribute to improving genetic tolerance to heat stresses (Singh et al., 2014), though in the meantime, early sowing can play an important role in improving crop adaptability to future climates before well-adapted cultivars are available (Munaro et al., 2020). However, sorghum is sensitive to cold temperatures (Rooney, 2004) requiring soil bed temperatures higher than 18 °C for germination and seedling establishment (Shroyer et al., 1998; Ostmeyer et al., 2020). In this and previous studies (Ostmeyer et al., 2020), cold soil temperatures and chilling temperatures significantly limited root growth and development in early sown crops, indicating that to increase sorghum adaptation to heat stress breeding should seriously consider breeding cold tolerance traits during crop germination, emergence, and vegetative stages. Recent studies have identified promising candidate genes putatively conferring germination (Upadhyaya et al., 2016), seedling emergence and survival (Parra-Londono et al., 2018), and seedling vigour. Traits related to the capacity of tissues to maintain photosynthetic capacity in cold conditions (Moghimi et al., 2019; Vennapusa et al., 2021). Related studies suggest that under cold stress the development of the root system determines the success or failure seedling establishment (Enns et al., 2006; Farooq et al., 2009).

*4.2 Root growth and function*

Our results showed that sowing sorghum into a soil temperature lower than 16°C produced thicker roots, and significantly reduced total root length, root length density and root volume. In general, cold soil temperatures are known to limit root growth and branching by reducing the availability of sugars to the roots (Kaspar and Bland, 1992; Nagel et al., 2009), and increase the mean diameter of roots (Miyasaka and Grunes, 1990; Farooq et al., 2009; Hassan et al., 2021; Zhou et al., 2021). In sorghum, known effects of cold soil temperatures and chilling stresses early in the season include impaired metabolism and photosynthesis, carbon assimilation, and stomatal control (Abbas, 2012; Bekele et al., 2014; Casto et al., 2021). Low temperatures in the root meristems can also be expected to affect the production of growth substances, and or reduce the uptake of diffusion of nutrients such as potassium and phosphorus (Koevoets et al., 2016; Zhou et al., 2022).

Our root activity factor (Zhao et al., 2022) was not calculated during the first season of trials due the lack of enough EMI surveys around flowering. For the second season of trials though, the calculated root activity factor was closely related to root length density (RLD) across most of the soil profile. The decline in root length density with soil depth was previously related to a lack of time for the rooting system to explore deeper soil layers (Robertson et al., 1993). Though, irrespective of the time of sowing, the larger the RLD the larger the values of the root activity factor (Fig. 6). Figure 6 shows that different relationships were evident for the irrigated and dryland treatments. For the same value of RLD, dryland plots had larger values of the root activity factor than the irrigated plots, while in the top layer of the dryland plots root activity was limited by water supply irrespective of the presence of roots. The differences in slope between the dryland and irrigated treatments might be related to a stress adaptation e.g., an increase in root hair and length, or in root hydraulic conductivity in water-limited environment (Calleja-Cabrera et al., 2020; Schneider, 2022). The linear relationship between RLD, and its consistency between times of sowing are highly encouraging and highlights opportunity to use EMI techniques to develop high throughput functional root phenotyping tools for breeding and agronomy. In Fig. 9 we showed that root length and root length density were both correlated with pre-flowering mean air temperatures. Both traits followed a typical temperature response curve in which the root length increased with the increasing temperature to an optimal temperature of 20°C (Kaspar and Bland, 1992). Which also explained the lack of statistical differences on root traits between times of sowing during the second season of trials.

## 5. Conclusion

Sowing sorghum, a summer crop, early in late winter or spring transferred water use from vegetative stages to flowering and post-flowering stages increasing crop water use efficiency. The higher grain numbers in early sown crops were related to higher grain numbers in tillers. Root length and root length density were reduced by pre-flowering mean temperatures lower than 20°C. Our results suggest that in the race to increase crop adaptation to hotter climates, breeders should consider cold tolerance during crop germination, emergence, and vegetative stages as a target.


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## CRediT authorship contribution statement

**Dongxue Zhao:** Formal analysis, writing - original draft, writing - review & editing; **Daniel Rodriguez**: Project leadership, conceptualization, methodology, formal analysis, writing & editing; **Peter deVoil**: Data management, review & editing; **Bethany Rognoni:** statistical data analysis & editing; the rest of the authors contributed by running field experiments and collecting field data.

## Declaration of Competing Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data is available upon request to the corresponding author and approval from the funding body (GRDC).

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