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The genotypic variation in the positive response of sorghum to higher sowing density is linked to an increase in water use efficiency

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ABSTRACT

In semi-arid tropical areas, sorghum is sown at very low planting densities. Hence, increasing plant density represents an opportunity to improve productivity. However, assessing the expected increase in water needs is critical prior to testing higher densities under rainfed conditions. This was tested with a panel of elite cultivars in field and lysimiter experiments, and testing the effects of two density treatment, high (HD, 22 plants.m^{-2}) and low (LD, 11 plants.m^{-2}), on grain and biomass yield and on water use and water use efficiency (WUE). Doubling the conventional sowing density significantly increased biomass and grain yield, with a genotypic variability in the biomass response. No link was found between the response to density and the maintenance of the tillering capacity, whereas the response to density was somewhat explained by a differential increase in the leaf area index under high density ($r=0.43$ $P<0.05$). Lysimeter experiments showed that, compared with the conventional density, the high-density treatment had 62% increase in biomass vs a 38% increase in water use, resulting in a 17% higher WUE on average of the genotypes tested. There was an appreciable genotypic variability in this degree of WUE increase under high density. The most striking result was the very tight positive link between the biomass response to density and the differential increase in WUE in the dry season ($r=0.91$ $P<0.0001$), whereas in the wet season this link was negative ($r=-0.48$ $P<0.02$). This work shows that intensifying sorghum production by increasing sowing density is possible, in the short term using cultivars that show the largest WUE increase under high density, in the longer term by breeding high-density adapted cultivars, targeting plant traits that explain the tight link between higher WUE and higher yield under high density.

1. Introduction

Sorghum is a staple food in semi-arid regions (Proietti et al., 2015) and is the main source of income for small-scale farmers (Tabo et al., 2007). Intensifying sorghum production could be a means to improve food security, which is an absolute necessity in the current demographic context of developing countries (Godber and Wall, 2014; Thurlow et al., 2019). Traditionally, fodder and grain sorghum have been sown at low or very low densities, e.g. 12 plant m^{-2} as recommended in India (Silva et al., 2017), and even lower in Africa. However, higher densities (40–50 plant m^{-2}) increase yield in fodder sorghum crops under temperate latitudes (Corleto et al., 1990). Extension services in Europe recommend densities ranging from 26 to 35 plant m^{-2}, depending on the soil properties (Arvalis, 2020). Here, we tested whether increasing plant density would increase sorghum production in semi-arid tropical regions. Such benefit was observed in other species in dry areas such as sunflower, soybean and maize (Andrade et al., 2002; Hatfield et al., 2001) and in pearl millet (Pilloni et al., 2022).

However, evapotranspiration is usually high in semi-arid tropical regions and would increase under high density because the leaf area index would likely increase. A higher evapotranspiration could deplete soil water faster and before the critical grain filling period that requires water in sufficient amount (Vadez et al., 2013). Therefore, we first need to measure how much more water is needed to grow sorghum under high density in a semi-arid tropical climate, to gauge the potential and the feasibility of modifying this agronomic practice. On the positive side,

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increasing plant density increased water use efficiency (WUE) in maize (Barbieri et al., 2012; Hernández et al., 2020), and sunflower (Echarte et al., 2020), and this would have compensated, at least partially, the higher evapotranspiration. Higher plant density also increased WUE in pearl millet and the vapor pressure deficit (VPD) was lower in the high- than in the low-density canopy (Pilloni et al., 2022). This was consistent with the higher WUE data in the high-density canopy, since WUE and VPD are inversely correlated (Sinclair et al., 2017). Increasing the sowing density could then be an agronomic management that could help the crop escape the high VPD conditions of semi-arid tropical climate and increase WUE. In some other crops such as soybean and wheat, when water was not limiting and evaporative demand was low (temperate areas), there was no change in the total water use upon an increase in sowing density (Eberbach and Pala, 2005; Mason et al., 1982; Reicosky et al., 1985). Therefore, we also need to assess whether WUE would increase under high density in sorghum in a semi-arid tropical climate. The key in the success of increasing the sowing density is then to find a positive tradeoff between the extra water cost of the intensification and the water savings from a putatively higher WUE, toward a higher biomass and/or grain yields. Here, we evaluated the amount of water required to support an increased plant density, and measured WUE and yields. In these initials steps, we chose to carry out the trials under fully irrigated conditions in order to establish evapotranspiration, WUE, and yield benchmarks at the full potential of the crop. Once these benchmarks established and the feasibility of increasing the sowing density of sorghum in semi-arid tropical regions assessed, subsequent steps would be to test this agronomic management under rainfed conditions.

Another risk associated with increasing plant density is the competition for light resource. This could decrease tiller number (Casal et al., 1986; Munir, 2002), although a large genetic variability was observed for this effect (Blanc et al., 2021). Less tillering would reduce the number of fertile tillers at flowering and could eventually decrease grain yield. The tradeoff between tillering reduction due to light competition and the gain in production thanks to an increased density was therefore something important to also pay attention to.

Hence, we quantified trade-offs between plant water use, WUE, leaf area, tiller number and biomass accumulation and yield in response to an increase in plant density in a panel of elite cultivars commonly used by farmers in India. We hypothesized that cultivars which, putatively, would have the most positive responses to plant density would be those able to optimize these tradeoffs. The work involved two field and two lysimeter experiments with plants grown in two planting densities under contrasting evaporative demand. In the field we investigated the consequences of an increased plant density on grain yield, biomass accumulation, tillering ability, and leaf area index. In the lysimeters we measured total water used, WUE, and vegetative biomass in a dry and a rainy season and its relationship to the biomass response to density. In both experimental setups, we analyzed the genotypic variability in these responses.

2. Material and methods

The biological material consisted in a panel of 20 elite high-yielding sorghum genotypes, including hybrids from seed companies and hybrid parents and varieties from ICRISAT breeding program. Experiments were carried out in the field and at the lysimeter facility (LysiField – see Vadez et al., 2011 for details) of the ICRISAT campus (Hyderabad, India, 17°30 N; 78°16 E; altitude 549 m). Two field experiments were carried out in 2017 and 2018, both during the dry seasons (February-May) characterized by a high evaporative demand and radiation. Two lysimeter experiments were carried out in 2018, one during the dry season (and simultaneous to the 2018 field experiment), and one during the rainy season, characterized that year by limited rains with moderate solar radiation and VPD (Fig. 1). All experiments were fully irrigated to avoid any water deficit. Lysimetric experiments aimed at measuring the effect of high density on soil evaporation, plant water use and water use efficiency (WUE) from an early stage and until maturiy.

2.1. Genotypic response to density in the field

Each of the twenty sorghum genotypes was sown in a high density and low-density conditions. Irrigation was provided at regular intervals, usually every 7–10 days, to ensure the crop faced no water deficit at any time of the crop cycle, with no irrigation differences between the high and low density treatments, and applying between 30 and 40 mm at each irrigation. Under high plant density (HD), row-to-row distance was 30 cm, vs 60 cm under low plant density (LD). Plant-to-plant distance within rows was 15 cm in both density treatments, giving a density of 11 plants m^{-2} and 22 plants m^{-2} in LD and HD respectively. The design was a randomized complete block with density as the main factor and genotypes randomized three times within each density block. A replicate consisted in a plot of 4 and 8 rows for the LD and HD treatments respectively, each row being 4 m long. Seeds were manually sown (24th

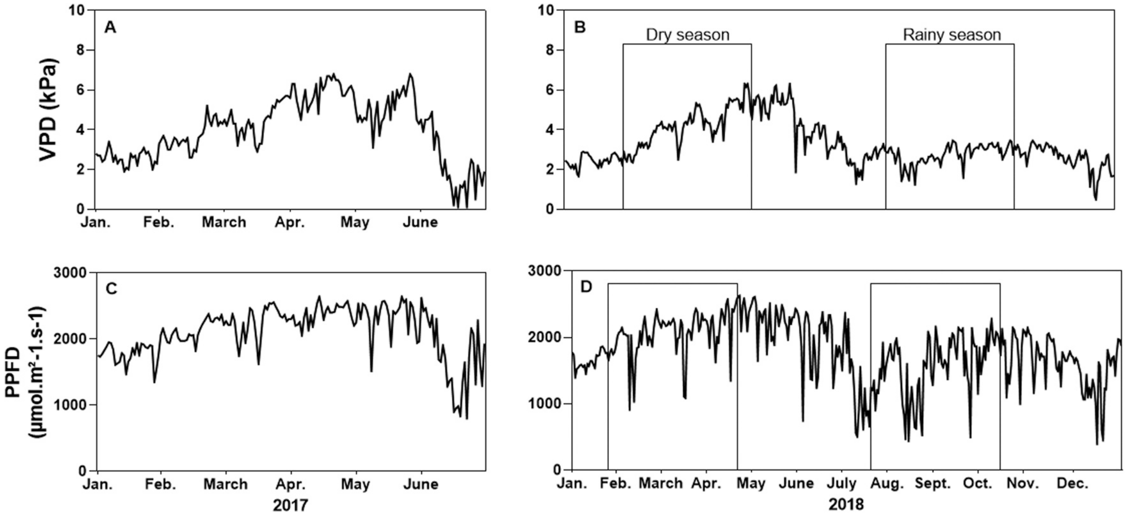

Fig. 1. – **Weather data**. Daily vapor pressure deficit (VPD) (A, B) and Photosynthetically active Photon Flux Density (PPFD) (C, D) from the ICRISAT meteorological station during the 2017 dry season (A, C) and during the dry and rainy seasons experiments in 2018 (B, D). Empty boxes correspond to the periods of the lysimetric measurements in 2018.

February 2017 and 22nd February 2018), and plants were thinned down to the right plant density after emergence. Care was taken to avoid the presence of any weeds. Fields were fertilized with di-ammonium phosphate before sowing at a rate of 100 kg ha^{-1}. A top dressing was applied at a rate of 100 kg ha^{-1} urea four weeks after sowing. A four meters wide border was set up around the field, while a border of two rows of plants separated the two density blocks. Yields (grain or vegetative biomass) were measured from the entire plots, i.e. from an area of 9.6 m^2, and then converted in g/m^{-2}. In 2017, the experiment lasted until maturity and vegetative dry biomass, grain yield, and the sum of both representing the aboveground dry biomass were measured. The number of panicles was also counted at harvest as an estimate of the number of fertile tillers in the two treatments. In 2018, a storm at 79 days after sowing (DAS) forced us to harvest the entire field before maturity, allowing only a measurement of aboveground dry biomass.

2.2. *Leaf area index measurement in the field*

Leaf area index (LAI) was measured in 2018 using a 1-meter long ceptometer (AccuPAR LP-80), 40 days after emergence in both high and low densities, i.e. at a phenological stage close to anthesis. In each plot, two measurements from above the plants assessed the incident radiation on that particular time. Then, four measurements were taken from the ground, below the canopy, in different locations of the plot, placing the ceptometer diagonally between two rows. The ceptometer measured the amount of photosynthetically active photon in μmol/s/m^2 and converted the light quantity into a leaf area index using the following formula:

$$LAI = \ln\left(\frac{I}{Io}\right) \Big/ k$$

where *I* is the incident light above the canopy *Io*, the light at ground level and *k* a crop extinction coefficient, with a value of 0.6 for sorghum (Kim et al., 2010).

2.3. *Water use and WUE response to density in lysimeter trials*

Two experiments were carried out in 2018 on a lysimetric platform with the same 20 genotypes during the dry (February to May) and rainy seasons (August to October). Lysimeters consisted of PVC tubes filled with alfisol collected from the ICRISAT farm. Each tube was 1.2 m long and 20 cm in diameter. The tubes were placed in a pit over which a scaffold equipped with a pulley system could move for lifting the lysimeters. Each tube had a steel collar at its top. A S-type load cell (Mettler-Toledo, Geneva, Switzerland) hanging from the scafold allowed the weighing of lysimeters by lifting them with a block chain pulley (see Vadez et al., 2011 & 2014 for details). The twenty genotypes of sorghum were sown with four replicates. Each replicate consisted of four tubes. For the HD treatment, there was one plant in each tube (4 plants in total for each replication). For the LD treatment, only two tubes out of four were planted, one plant per tube, while the other two tubes remained empty (2 plants in total for each replication). Hence, final plant density was 10 and 20 plants m^{-2} in LD and HD, respectively, similar to those in the field experiments (11 and 22 plants m^{-2}). The design was a randomized complete block with density as the main factor and genotypes as a sub-factor randomized within each density block with four replications. Fifteen days after sowing, tubes were watered and left to drain for 24 h to reach field capacity. Subsequently, plants were weighted weekly. The experiment was carried out under fully irrigated condition. This was done by re-watering after each weekly weighing, using the initial weight at field capacity (Weight_{FC}) as a benchmark to calculate the water needed in each tube such as: Water to add on day n = $\text{Weight}_{FC} - \text{Weight}_{\text{day n}} - 2$

Removing '2' from the subtraction allowed to bring back tubes to 2 L below field capacity, thereby avoiding possible drainage (should we have wanted to reach exactly field capacity) and reaching a tube water availability of about 80% field capacity. During the dry season, tubes were not covered with plastic beads so that the tube weight differences between weighings gave measurements of the evapotranspiration. During the rainy season experiment, within a replication of four tubes, half of the tubes were covered with plastic beads in order to prevent soil evaporation, while the other half was not. In this way, transpiration (Tr) and evapotranspiration (ETr) could also be measured from the tubes with and without beads, to measure soil evaporation (See suppl. Fig. 1 for details of replications designs). Tr and ETr were expressed in mm.

Plants were harvested 79 and 81 days after sowing (DAS) in the dry and rainy season respectively. The total aboveground biomass was dried for one week in an oven at 60°C. This allowed calculation of water use efficiency (WUE) for each replicate (*WUE = Total biomass (g) / Total Water Use (kg)).*

2.4. *Statistical analysis*

The statistical analysis (Analysis of variance, simple linear regressions) presented in this study was performed using GraphPad Prism version 9.4.1 for Windows, GraphPad Software, San Diego, California USA, (www.graphpad.com). Broad sense heritability (H^2) was computed as in Falconer et al., 2005 with $H^2 = \frac{\sigma g^2}{\sigma p^2}$ where σp^2 is the phenotypic variance and σg^2 the genotypic variance plus residuals obtained through two-way analysis of variance.

3. Results

3.1. *High density increased the biomass production in most genotypes, with no appreciable effects of tillering nor leaf area index*

Grain yield and biomass was higher under high than under low density, both in 2017 and 2018. In 2017, the increase in grain yield with plant density ranged from 8.4% to 93%. Despite this large range of response, genotype-by-density interaction for grain yield was not significant (Fig. 2 A, Table 1 & Table 2), indicating that, statistically, grain yield responded similarly in all genotypes. The increase in aboveground biomass with plant density ranged from 5.0% to 67% in 2017 and from 10% to 56% in 2018 (Fig. 2 B and C). This wide range of response was consistent with the significant genotype-by-density interactions in both years (Table 1), indicating that biomass responded more to high density in certain genotypes than in others. The broad sense heritability for biomass was 0.70 in 2017 and 0.25 in 2018.

Plant density increased the panicle number for all genotypes, with a ratio between high and low density ranging from 1.6 to 2.0 for all except three genotypes, then showing a slight decrease in tillering under high density. However, the ratio panicle number HD / panicle number LD, which represented the relative change in panicle number between LD and HD, had no significant relationship to the equivalent ratio for biomass (Fig. 3 A). This showed that the yield increase under high density was not driven by the change in panicle number. There was no significant genotype-by-density interaction for panicle number, indicating that the panicle number of all genotypes responded the same way to the increase in density.

The leaf area index (LAI) ranged from 0.92 to 2.39 in LD and from 1.67 to 3.08 in HD (Table 2) with no significant genotype-by-density interaction on LAI (Table 1 and Table 2). Higher LAI correlated with higher biomass. However, the ratio LAI HD / LAI LD, which represented the relative change in LAI between LD and HD, showed only a weak, yet significant, relationship to the equivalent ratio for biomass ($r=0.43$ $P<0.05$; Fig. 3B). This showed that the biomass increase under high density was driven in part by the increase in LAI.

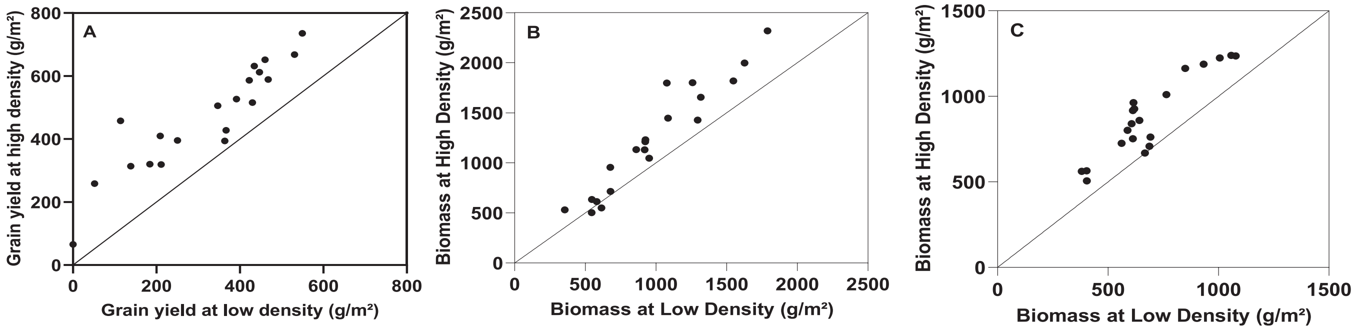

Fig. 2. - **Yield and biomass response to density**. Grain yield (g/m^2) during the dry season field trial 2017 (A), and vegetative biomass accumulation (g/m^{-2}) in the 2017 (B) and the 2018 field trial (C), in 20 genotypes grown under high and low density. Data points are the genotypic means of three replications in each density treatment. The distance to the 1:1 line of each datapoint shows the degree of response of each genotype to the density treatment.

Table 1

Analysis of variance of the different traits measured: biomass and grain yield, panicle number m^{-2}, leaf area index (LAI), water use efficiency (WUE), across the different trials (field and lysimeter) and year of experiment. Broad sense heritability (H^2) is provided at the bottom.

	Field trials				Lysimeter trials		
Two-way ANOVA	Biomass Yield 2017	Grain Yield 2017	Biomass Yield 2018	Panicle no. Per m^2 2017	LAI 2018	WUE Dry season 2018	WUE Rainy season 2018
Source of Variation							
Genotype	****	****	****	****	****	*	****
Density	****	****	****	****	****	****	****
Genotype x Density	***	ns	*	ns	ns	***	**
Heritability (H^2)	0.7	0.22	0.25			0.67	0.78

Table 2

Genotypic means of the grain yield, vegetative biomass yield (stover) for both 2017 and 2018 field trials, water use efficiency (WUE) in the two 2018 seasons of lysimetric experiment, and leaf area index (LAI) in the 2018 field trial, for the 20 genotypes studied. Grand mean (Mean), maximum and minimum values (Max, Min), standard deviation (SED), Wald statistic (F-value) and probability (Prob) for genotype (G), density (D) and interaction (GxD) of each traits are presented. Values are the genotypic means of the three (field trials) or four replications (lysimeter trials).

Genotypes	Grain yield 2017 (g/m$^{2)}$		Stover yield 2017 (g/m$^{2)}$		Stover yield 2018 (g/m$^{2)}$		WUE 2018 dry season (g.kg-^{1})		WUE 2018 rainy season (g.kg^{-1})		LAI	
	Low Density	High Density	Low Density	High Density	Low Density	High Density	Low Density	High Density	Low Density	High Density	Low Density	High Density
CSH 16	530.7	668.2	925	1231	606.5	840.4	2.3	2.4	8.0	9.8	1.28	2.02
ICSB 404	208.2	409.5	355	531	403.2	564.4	2.3	2.2	9.0	11.9	1.49	2.59
ICSH 14002	422.5	586.1	1086	1446	615.5	963	2.5	2.9	8.0	9.2	1.81	2.41
ICSH 28001	429.9	515.9	1295	1428	764	1010.2	2.8	3	8.0	9.6	2.39	3.08
ICSR 101	460.1	651.8	860	1132	612.4	752.6	2.2	2.3	8.1	11.0	1.39	2.17
ICSR 14001	447.1	612.3	952	1047	692.3	761.8	2.5	2.9	8.0	9.1	2.32	2.63
ICSR 196	434.6	631.4	920	1130	588.4	801.60	2.1	2.6	8.1	10.1	1.87	2.73
ICSR 89058	391.9	526.7	545	502	404.1	505.8	2.6	2.3	8.4	11.2	0.92	1.67
ICSV 112	347.1	505.8	547	634	561.7	725.9	2.2	2.8	8.4	10.1	2.08	2.34
ICSV 15013	363.8	394.1	679	715	642.2	859.2	2.6	3	8.3	8.5	2.05	2.77
ICSV 25302	51.8	258.5	1548	1819	933.1	1188.3	2.7	3	8.0	8.4	1.6	2.47
ICSV 25308	184.2	320.1	1627	1997	1057.7	1239.2	2.5	3.1	7.9	9.1	1.97	2.49
ICSV 25316	0.0	65.7	1789	2318	1078.4	1235.8	2.5	3.2	8.4	9.8	1.74	2.73
ICSV 745	250.4	395.7	1318	1655	612.3	916.8	2.5	3	9.1	10.5	1.51	2.30
ICSV 93046	211.3	318.9	1077	1798	1006.3	1224.6	2.6	3.1	8.6	9.4	1.58	2.13
Isiap Dorado	NA	NA	677	956	667.1	668.4	2.8	2.7	9.0	10.2	1.87	2.65
MR 750	549.5	735.3	614	550	380.6	561.6	2.5	2.6	8.7	11.7	1.41	2.33
NTJ-2	138.3	314.1	1259	1800	849.2	1163.7	2.5	2.7	9.3	9.7	1.69	2.58
PVK 801	468.0	589.0	924	1211	687.6	708.2	2.4	2.4	7.6	9.1	1.92	2.03
S 35	367.1	427.5	581	614	619.4	927	2.7	3.1	9.1	10.1	1.36	2.31
Mean	318.6	469.2	978.90	1225.70	689.10	880.93	2.49	2.77	8.40	9.93	1.71	2.42
Min	51.8	65.7	355.00	502.00	380.60	505.80	2.10	2.20	7.60	8.40	0.92	1.67
Max	549.5	735.3	1789.00	2318.00	1078.40	1239.20	2.80	3.20	9.30	11.90	2.39	3.08
SED	159.7	169.6	395.73	544.93	204.54	235.60	0.19	0.31	0.49	0.97	0.36	0.32
G F-value	17.41		67.92		41.59		1.98		117.5		3.32	
Prob	<0.0001		<0.0001		<0.0001		0.0102		<0.0001		0.0001	
D F-value	77.21		95.57		164.8		993.6		93.19		80.93	
Prob	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
G x D F-value	0.73		3.19		2.008		1.95		1.93		0.53	
Prob	0.73		0.0002		0.017		0.0003		0.0015		0.9389	

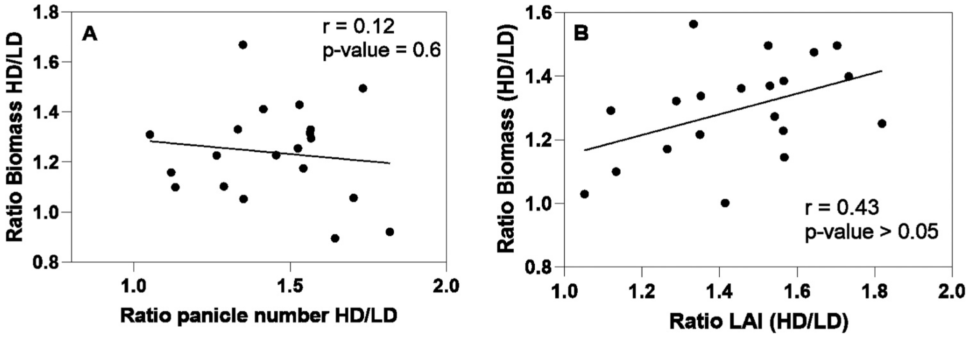

Fig. 3. – **Tillering and LAI effect on the density response** – Scatter plots of the ratios of the biomass under high density to the biomass under low density (ratio biomass HD/LD) as a function of similar ratios for panicle number (A, Ratio panicle number HD/LD) and for leaf area index (LAI) (B, Ratio LAI HD/LD) in high and low density. Data points are the genotypic means of three replications in each treatment. Data are from the 2017 field trial.

3.2. WUE increased under high density and correlated with the biomass responses of genotypes

Water use was higher under HD than under LD: 607 ± 13 mm and 431 ± 5 mm in HD and LD respectively during the dry season (Fig. 4 A) and 166 ± 8 mm and 122 ± 3 mm in HD and LD treatment respectively during the rainy season (Fig. 4B, two-way ANOVA, P value <0.0001) mean of of the 20 genotypes studied). Biomass also significantly increased under HD in both seasons. However, the increase in water use was lower than the increase in biomass (41 vs 65% for the dry season, 35 vs 59% for the rainy season). Therefore, WUE was higher under HD than under LD ($p<0.0001$) in both the seasons (Fig. 4 C&D), Table 2).

During the dry season, direct soil evaporation in the LD canopy (measured in the empty tubes) was 42 mm, vs 431 mm for total evapotranspiration (Fig. 5 A). During the rainy season, the treatment with beads on the top of the lysimeters did not show significant differences in the total water use compare to the lysimeters without beads (Fig. 5 B). However, in both treatments WUE of the plants grown with beads on the top of the lysimeters was significantly higher (p-value <0.0001) than WUE measured in the lysimeters without beads (i.e. 9.2 $g.kg^{-1}$ vs 8.4 $g.kg^{-1}$ in low density and 11.25 $g.kg^{-1}$ vs 9.9 $g.kg^{-1}$ in high density) showing a positive effect of soil covering on WUE (Fig. 6).

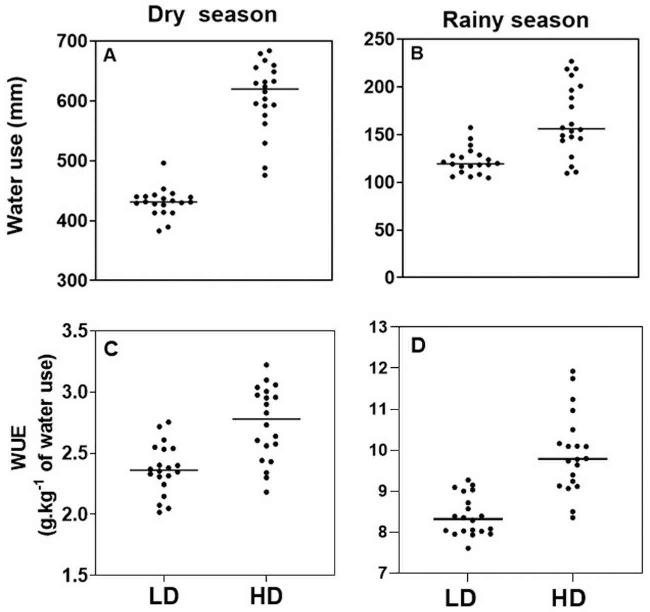

Fig. 4. – **Water use and water use efficiency (WUE).** Water use (mm) in the dry (A) and the rainy (B) season, and WUE (g kg^{-1} water use) in the dry (C) and the rainy (D) season for each of the 20 genotypes tested in India in both high (HD) and low (LD) density treatments. Data points are genotypic means of four replications in each treatment and genotype. Horizontal bars represent the grand means across the 20 genotypes in each of the two density treatments.

There was also a genotypic variation in the degree of increase in WUE under high density, shown by the significant genotype-by-density interaction effect of density on WUE during both seasons (Table 2). This genotype-by-density interaction effect (GxD) on WUE had a similar F-value than the genotypic effect (G) on WUE in the dry season, indicating that WUE variations were both due to a genotypic effect and to a genotype response to density effect in the dry season. By contrast, in the rainy season, while the genotype-by-density interaction effect on WUE was significant, the magnitude of the F-value was much lower than the F-value for genotypic effect on WUE. Hence, the ratios of WUE in LD vs HD ranged from 0.96 to 1.39 in dry season, and only from 1.03 to 1.36 during the rainy season ($p< 0.0001$) (Suppl. Table 1). Interestingly, the ratio of the biomass in HD vs LD, which represented the degree of response of biomass to the HD treatment, showed a strong and positive correlation (p value <0.0001, $r = 0.91$) with the ratio of WUE under HD and LD during the dry season (Fig. 7 A), which represented the degree of response of WUE to the HD treatment.

In other words, genotypes that showed a strong WUE increase under HD also showed a strong biomass response to the HD treatment in the dry season. Hence, an increase in biomass under HD was not followed by a proportionally similar water cost in the dry season, whereas the opposite was observed in the rainy season, with a significant and negative correlation between the WUE ratio and the biomass ratio (p value $=0.02$, $r = - 0.48$) (Fig. 7 B). In other words, in the rainy season, an increase in biomass came at a relatively higher water cost since WUE and biomass progressed in opposite directions.

4. Discussion

Our results showed that grain and biomass yield increased under high sowing density for most genotypes. These results contrast with those in another study (Carmi et al., 2006), where only a single genotype was used, instead of 20 in our case, which may explain such differences. The biomass production also showed genotypic variation in the degree of response to an increase in density, i.e. there were significant genotype-by-density interaction, indicating that some genotypes responded more to the higher density than others did. This result then suggests it would be possible to breed cultivars for high-density response in sorghum. So far, no breeding program has purposely taken the tolerance to higher density as a breeding target and the existing literature on the reaction to density for sorghum dealt with much lower densities than the one in the present paper (Berenguer and Faci, 2001; Tang et al., 2018). A positive response to an increased plant density has been shown in other species like maize and sunflower (Barbieri et al., 2012; Hernández et al., 2020). However, these studies did not test the genotypic variation in the response to density treatments. In high tillering cereals, the ability to maintain tillering was necessary for a good

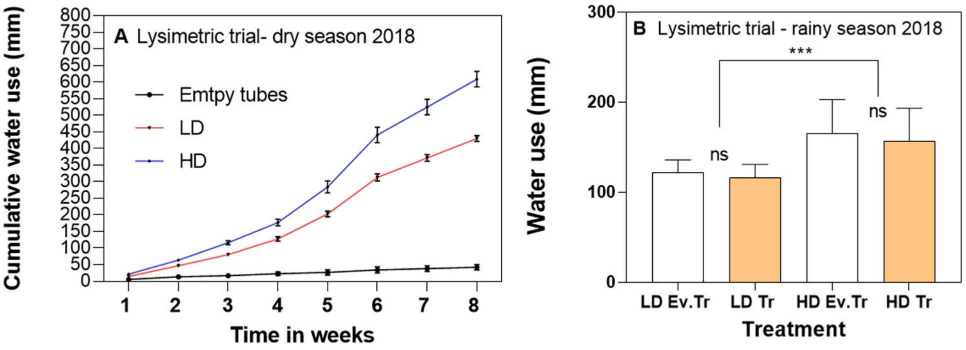

Fig. 5. – **Water use dynamics and effect of protecting the soil surface.** (A) Cumulative water use (mm) of the high-density (HD) (one replication consisting in 4 tubes and one plant in each tube), the low-density (one replication consisting in 2 tubes and one plant in each tube) (LD) and the empty tubes from the low density treatment (one replication consisting in 2 empty tubes), up until harvest during the dry season trial. Cumulated water use data were measured each week and were the means +/- SE of the means for each of the 20 genotypes. Each individual genotypic mean was that of four replications in each genotype and treatment. (B) Total water use of the crops in both the high density (HD) and the low density (LD) treatments during the rainy season, distinguishing when lysimeters were not covered with beads to prevent soil evaporation (WU = evapotranspiration, Ev.Tr, white bars) or when beads covered the top of the lysimeters (WU = transpiration, Tr, orange bars). Data are the means +/- SE of the water use means of 20 genotypes, each of these genotypic means being those of four replications in each genotype and treatment.

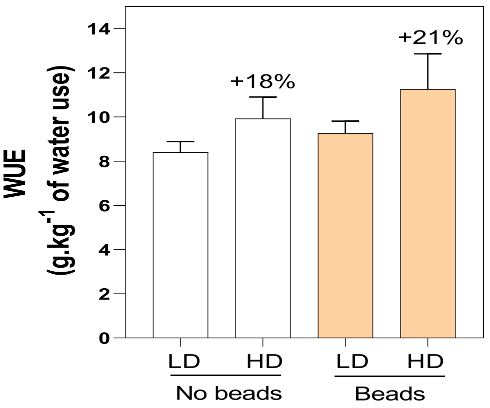

Fig. 6. - **Water use efficiency**. WUE (g kg^{-1} water use) measured in the pots without bead at the top of the tubes (white bars) and beads at the top (orange bars) under both high and low density during the 2018 rainy season lysimetric trial. Data are the means +/- SE of the WUE means of 20 genotypes, these means being those of four replications in each genotype and treatment.

performance under high-density conditions (Lloveras et al., 2004; Munir, 2002). Maintaining tiller number being a marker of tolerance to the competition (Bastos et al., 2020) we took that aspect into account. Nevertheless, our results showed no influence of tillering in the positive response to higher sowing density.

The biomass response to high density in the lysimeters also showed genotypic variation and confirmed the field results. Biomass under high plant density also increased proportionally more than water use, resulting in a higher WUE in the HD treatment. This is consistent with recent results in maize (Hernández et al., 2020). However, in that maize study the densities were lower than in our experiments, their high densities corresponding to our low densities (10 plants/m^2). Another recent study in sunflower also showed an increase in WUE under higher sowing density (grain and oil), which was interpreted as an effect of the protection of soil from solar radiation (Echarte et al., 2020). Here, we showed that the part of water saved by soil coverage was also substantial, contributing to 10% of total water use in the dry season. Unfortunately, we did not have a proper factorial in this trial to allow us measuring the WUE gain from adding beads. During the rainy season trial, in contrast, there was no significant difference in water use whether cylinders were covered with beads of not. Yet, a higher WUE was found when the lysimeters were covered with beads at the top of the pots, both for the HD and the LD treatments (Figure 5B). We may interpret that, although non-significant, the slight reduction in soil evaporation in cylinders covered with beads was still sufficient to trigger an increase in WUE. However, the impact of soil covering represented only a small proportion of the gain in WUE caused by the HD treatment, with an increase of 18% without beads and 21% with beads.

There was a positive relationship found between the increase in biomass and the increase in WUE in high density during the season characterized by high VPD and radiation conditions (Figure 7 A). This suggests a positive trade-off between biomass production and water consumption benefitting dense canopies in the high evaporative demand

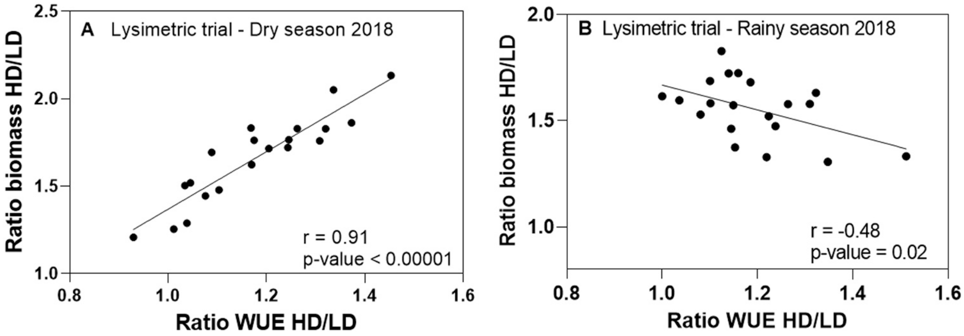

Fig. 7. – **Relative change in biomass versus relative change in WUE.** Simple linear regression between the ratio of the biomass under high density to the biomass under low density (biomass HD/LD) and the ratio of the WUE under high density to the WUE under low density (WUE HD/LD) during (A) the summer season 2018 (r= 0.91, p-value <0.0001) and (B) during the 2018 rainy season (r= - 0.48, p-value <0.0001). Data are the means of four replications in each treatment.

season. It fits with assumptions described in recent works in maize and sunflower, of a better light interception in sunflower to explain the increase in WUE (Echarte et al., 2020). However, these earlier reports did not consider the possibility that a higher WUE could have been related to differences in the light distribution within the canopy. This hypothesis would agree with our recent results showing an increase in WUE in genotypes that also allowed light to penetrate deeper inside the crop canopy, where VPD was lower than in the air (Pilloni, 2022), and where photosynthesis would be less water-expensive than in leaves exposed to air VPD. This could also explain the smaller density effect observed in the 2018 field trial, which was stopped prematurely soon after canopy closure, and when the possible genotypic differences in light penetration would start being the strongest.

Finally, our water benchmarking showed that water use was approximately 30% higher under high density treatment, whereas WUE increased by about 20%, so that the extra water cost was about 10% short from being compensated by the WUE increase. This is without counting a likely benefit from a lower soil evaporation under high density, which we did not measure in the present work, and which could not be measured accurately in lysimeters. Therefore, we may speculate that similar results would have been obtained under rainfed conditions, and this should be the next step. In any case, these results are very promising and open the door for a possible intensification of this crop in semi-arid regions, especially since this increase in biomass accumulation under high density received the same management practices. In the short term, this would be possible by promoting higher sowing density with the existing cultivars, possibly after testing those that would be the most responsive to an increased sowing density. Genotypes ICSV112, ICSV25308, ICSV25316, ICSV745 are a few such potential candidates. In the longer term, this could be from breeding cultivars adapted to high density planting.

5. Conclusion

Our results showing a positive yield and biomass response to higher sowing density open the possibility to intensify sorghum production of existing varieties that most positively responded to an increased density. The positive effect of high density on WUE also brings an interesting value on this crop management practice in dryland conditions, showing that the extra water use of higher density stands would be in a large part compensated by this increase in WUE. The finding of genotypic variations in the degree of biomass and WUE response to high density also open the possibility to breed density-adapted cultivars, which might also have higher WUE. Density-responsive variants were found, even in the small panel of cultivars that was tested. This calls for a larger screening of density response variants, which could be used for the identification of density response traits in sorghum and for the breeding of density tolerance cultivars in sorghum.

CRediT authorship contribution statement

Tardieu François: Writing – review & editing. **Kholova Jana:** Writing – review & editing, Supervision. **Aparna Kakkera:** Investigation. **Pilloni Raphaël:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Vincent Vadez:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare they have no conflict of interest

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2024.127207.

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