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AGRONOMICAL AND PHYSIOLOGICAL STUDY OF THE RESPONSE OF SORGHUM AND PEARL MILLET CROPS TO HIGHER SOWING DENSITY IN THE SEMI-ARID TROPICS.

ASSESSEMENT OF THE OPPORTUNITY FOR SUSTAINABLE INTENSIFICATION AND CONSEQUENCE FOR THE TRANSPIRATION RESPONSE TO EVAPORATIVE DEMAND OF THE CROPS.

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Abstract

Sorghum and millet are a key source of income and calories for an estimated 250 million people in the semi-arid tropics. The climatic and demographic context of India and the Sahelian regions, where these cereals are widely grown, calls for a sustainable intensification of these staple food crops, as it has been done elsewhere and in the past for other crops, notably in Western intensive maize systems. Increasing the sowing density is an important factor in agronomic management and resulting increases in yield are described in the literature in several crops. In semi-arid regions, sorghum and millet are grown at very low densities. The objective was to identify a potential new strategy to increase the production of these important small grains through an increase in sowing density. Through field and lysimeter trials carried out in India and Senegal this work showed the possibility to increase significantly the biomass and grain yield in both sorghum and pearl millet crops with the same irrigation regime, and could explain the mechanisms involved in this response. The high-density treatment reduced the vapor pressure deficit (VPD) in the canopies, both in the field and in the lysimeter experiments, resulting in a significantly higher water use efficiency (WUE) of high plant stand canopies in both crops. Indeed, a proportionally higher biomass accumulation than total water use was measured in several lysimetric trials. We linked the genotypic variation for the increase in WUE to an increase in biomass and more so in high evaporative demand season. While both species responded very positively to the increase in density, there were also large specie differences in the genotypic variation of the response to density, namely a strong genotype x density interaction in sorghum for biomass and WUE, but none in pearl millet. The genotypic variation in the degree of WUE response found in sorghum and its link with biomass accumulation led to investigate putative differences in the transpiration response of the crops to the evaporative demand. Indoor experiments with individual plants indeed often indicate that transpiration rate can be limited under high vapor pressure deficit (VPD) and follows a nonlinear response in some genotypes, and this may increase water use efficiency (WUE). We tested this hypothesis outdoors with canopy-grown sorghum plants in field and lysimeter experiments. The response of the evapotranspiration was measured against the evaporative demand here, and not VPD alone as in previous studies. This response was linear and, with WUE, showed large genetic variation. WUE was surprisingly higher in genotypes with the highest transpiration response to the evaporative demand (Penman-Monteith). These genotypes were also those that allowed maximum light penetration into the canopy. The large variability in transpiration response to the evaporative demand and WUE opens the prospect of exploiting these differences for breeding, possibly in relation to canopy architecture characteristics. The same approach was implemented on the pearl millet varieties used in this study. No genotypic variation was found in pearl millet for the response of the evapotranspiration to the evaporative demand. Unlike sorghum, no relationship between this response and WUE regardless of seasons and locations tested. These results are coherent with the absence of genotype x density interaction for the density response found in millet, both for biomass increase and WUE. This work opens the door to intensification, in the short term by increasing sowing density in drylands using sorghum and pearl millet cultivars that show a strong response to density, and in the medium term by selecting sorghum cultivars adapted to high density.

Résumé en Français

Le sorgho et le mil représentent une source essentielle de revenus et de calories pour environ 250 millions de personnes dans les régions tropicales semi-arides. La plupart des agriculteurs qui produisent ces céréales vivent en dessous du seuil de pauvreté et gèrent des systèmes agricoles de petites tailles. Le contexte climatique et démographique de l'Inde et des régions sahéliennes, où ces céréales sont largement cultivées, appelle à une intensification durable de ces cultures vivrières de base, comme cela s'est fait ailleurs et auparavant pour d'autres cultures, notamment dans les systèmes intensifs occidentaux sur le maïs par exemple.

La densité de semis est un facteur important de la gestion agronomique qui fait référence au nombre de plantes ciblées pour une surface donnée. C'est un facteur majeur qui affecte la croissance et le développement des cultures chez les espèces cultivées, aussi bien annuelles que pérennes, car la densité modifie l'entourage des plantes individuelles et détermine la quantité de lumière disponible pour chaque plante. La modification de la densité influence également la quantité d'eau et de nutriments disponibles par individu. Les principaux facteurs environnementaux qui affectent le rendement sont le rayonnement solaire, la disponibilité en eau du sol, la nutrition minérale et la température. Une augmentation du peuplement végétal sur une même surface modifierait évidemment la qualité et la quantité de lumière disponible pour chaque plante, ce qui pourrait à son tour affecter le rendement.

La densité de semis dans les systèmes agricoles de maïs à haut niveau d'intrants est passée de 3 plantes/m² dans les années 1930 à 9 à 11 plantes/m² dans les années 2000. Aujourd'hui encore, il a été démontré que les rendements du maïs peuvent être augmentés par des pratiques agronomiques adaptées, comme la densité de semis. Suite aux grands épisodes de sécheresse dans la région du Sahel dans les années 70, les recommandations agronomiques dans ces régions ont poussés les agriculteurs à éviter une trop grande surface evaporative des feuilles exposées (i.e. le nombre de plantes par m²) afin d'éviter de faire face à un stress hydrique.

Ce travail étudie donc séparément la réponse du sorgho et du mil à une augmentation de la densité de semis dans le contexte des tropiques semi-arides en tenant compte de ce qui a été fait précédemment sur les céréales cultivées des systèmes agricoles occidentaux comme le maïs. A ce jour aucun travail de ce type n'ayant été entrepris dans cette voie. L'objectif est d'identifier une nouvelle stratégie potentielle permettant d'augmenter la production de ces petites céréales importantes, car elles sont cultivées à de faibles densités dans les régions tropicales semi-arides.

Ce manuscrit se compose d’un premier chapitre proposant un bilan de l'état de l'art concernant la densité de semis et les mécanismes agronomiques et physiologiques qui sont impliqués dans la réponse des cultures. Le deuxième chapitre présente une analyse de la variabilité génétique de la réponse des deux cultures dans différentes conditions de densité. Un troisième chapitre présente une approche physiologique, particulièrement axée sur le sorgho, décrira la réponse spécifique des plantes aux demandes d'évaporation élevées lorsqu'elles sont étudiées dans des conditions de canopée dense. La même approche, plus succincte et en mettant en évidence une réponse spécifique au mil est également décrite.

Travaux sur le mil :

Le mil est la principale culture de subsistance des systèmes de petits exploitants agricoles des régions semis arides. Il y est cultivé à faible densité (environ 3 plantes/m²). L'intensification de la culture du mil au travers d’une augmentation de la densité de plantation pourrait augmenter la productivité, mais il n'est pas trivial que le stress hydrique engendré ne devienne pas un problème. En effet, l'augmentation de la densité de plantes par unité de surface augmenterait la surface foliaire et le budget hydrique de la culture. Cependant, des canopées plus dense pourraient également créer un microclimat intra-couvert plus favorable au bénéfice de l'efficience de l'utilisation de l'eau (WUE) des cultures. Le premier objectif était donc de tester la réponse du rendement des variétés populaires de mil dans densité augmentée et ce dans différents endroits (Sénégal et Inde), et d'évaluer les possibles variations génotypiques dans cette réponse. Le deuxième objectif était de mesurer l'utilisation de l'eau et la WUE de la culture dans différentes densités, en utilisant des lysimètres. Le principal résultat de cette étude est que la densité de semis plus élevée a augmenté de manière significative le rendement de tous les génotypes lorsque les essais ont été réalisés dans des conditions de forte demande evaporative. Il n'y a pas eu d'interaction génotype x densité dans ces essais, ce qui suggère l'absence de variation génotypique dans la réponse à l'augmentation de la densité. Le traitement à haute densité a également réduit le déficit de pression de vapeur (VPD) dans les canopées, tant au champ que dans les expériences au lysimètre. Bien que le traitement à haute densité ait augmenté le budget total en eau, l'augmentation de la biomasse qui en a résulté était proportionnellement plus élevée, augmentant ainsi la WUE des cultures dans tous les génotypes sous haute densité. L'augmentation du rendement sous haute densité était étroitement liée à l'augmentation de la WUE, bien que ce lien ait été plus étroit dans les saisons à forte demande evaporative que dans celles à faible demande evaporative. Ceci a confirmé un fort effet environnemental sur la réponse à la densité de tous les génotypes testés. Ces résultats soulignent la possibilité d'améliorer le rendement du mil en augmentant la densité, en ciblant spécifiquement les zones à forte demande evaporative, bien qu'ils n'aient pas ouvert la voie à la sélection de cultivars tolérants à la densité.

Travaux sur le sorgho :

Dans les zones tropicales semi-arides, le sorgho est également une nourriture de subsistance pour des millions d'habitants de ces régions. Tout comme le mil, il est traditionnellement semé à de faibles densités de plantes par unité de surface mais des résultats préliminaires générés en Inde suggèrent la possibilité d'une intensification durable via une densité plus élevée. Cependant, une évaluation de l'utilisation supplémentaire d'eau associée à cette option d'intensification est une condition préalable. Il est apparu au cours de ces travaux que le doublement de la densité de semis conventionnelle des cultivars commerciaux a augmenté de manière significative les rendements en biomasse et en grains lors d'essais menés en plein champ. De manière intéressante il a été démontré une variabilité génotypique dans le degré de réponse. Ces résultats ont été obtenus en appliquant la même quantité d'engrais et d'irrigation dans les deux traitements de densité. Aucun lien clair avec le maintien de la capacité de tallage et l'augmentation de l'indice de surface foliaire n'a été trouvé. Les expériences au lysimètre ont montré que les plantations à haute densité avaient une biomasse 62% plus élevée et une utilisation de l'eau seulement 38% plus élevée, résultant en une efficacité d'utilisation de l'eau (WUE) 17% plus élevée, liée à l'augmentation de la biomasse. Il y avait une variabilité génotypique appréciable dans le degré d'augmentation de la WUE. Ce travail ouvre la porte à l'intensification, à court terme en augmentant la densité de semis dans les zones arides en utilisant des cultivars qui montrent une forte réponse à la densité, et à moyen terme en sélectionnant des cultivars de sorgho adaptés à la haute densité.

Réponse physiologique des plantes en conditions de canopées :

Les expériences menées en intérieur sur des plantes individuelles indiquent souvent que le taux de transpiration est limité en cas de déficit de pression de vapeur (VPD) élevé et suit une réponse non linéaire chez certains génotypes, ce qui peut augmenter l'efficacité de l'utilisation de l'eau (WUE). Nous avons testé cette hypothèse en extérieur avec des plants de sorgho cultivés en canopée dans des expériences de terrain et lysimétriques. La réponse de l'évapotranspiration à une demande d'évaporation croissante a été linéaire et, avec WUE, a montré une grande variation génétique. La VPD était plus faible dans la canopée que dans l'air, et les différences étaient plus importantes pour les canopées avec un indice de surface foliaire (LAI) élevé que faible. Les différences de VPD entre l'air et la canopée se sont produites principalement au-dessus de 2,5 kPa et au-dessus des valeurs de LAI de 2,5-3,0. WUE était étonnamment plus élevée chez les génotypes présentant la plus forte réponse de la transpiration à la demande evaporative (Penman-Monteith). Ces génotypes étaient également ceux qui permettaient une pénétration maximale de la lumière dans la canopée. Nous interprétons que la WUE élevée malgré une transpiration élevée était due à une plus grande disponibilité de lumière pour les feuilles dans la canopée, qui bénéficiaient d'un VPD plus faible que dans l'air. La grande variabilité de la réponse de la transpiration à la demande evaporative et à la WUE ouvre la perspective d'exploiter ces différences pour la sélection, éventuellement en relation avec les caractéristiques de l'architecture de la canopée.

La même approche a été mise en place sur les variétés de mil utilisées dans cette étude. Il a été mis en évidence que le mil ne répondait pas à la demande évaporatoire de manière génotypique (ou peu, lors d’expérimentations menées sur des variétés communément utilisées en Afrique de l’Ouest). Contrairement au sorgho, il n’a pas été possible de mettre en évidence une quelconque relation entre le degré de réponse des plantes à la demande évaporatoire et l’efficience d’utilisation de l’eau, et ce dans toutes les saisons et localités testées. Ces résultats sont en accord avec le peu de variabilité génétique pour la réponse à la densité mis en évidence chez le mil, tant au niveau de l’augmentation de biomasse que de l’efficience d’utilisation de l’eau.

L’hypothèse du rôle de l’architecture des couverts pourrait ici encore expliquer ces résultats. En effet il est possible de penser qu’un découplage air-canopée (VPD plus faible au cœur des couverts) intervienne également chez le mil, permettant le gain pour l’efficience d’utilisation et *in fine* l’accumulation de biomasse. Cependant, l’absence de variabilité génotypique pour les traits susmentionnés suggère que les différences d’architecture chez le mil soit bien moins grande que chez le sorgho. De manière intéressante, cela suggère également que d’autre mécanismes entre en jeu pour expliquer la variabilité génétique intrinsèque à l’espèce (i.e. sans facteur densité) mise en évidence dans cette étude et déjà montrée dans la littérature.

Cette thèse, à l'interface entre l'écophysiologie et l'agronomie, montre l'interconnexion entre ces deux disciplines et le lien étroit entre l'effet de la gestion des cultures et la réponse physiologique des plantes aux conditions environnementales. Elle met en évidence la possibilité d'influencer positivement et significativement de nombreux caractères d'intérêt agronomique en faisant varier un seul paramètre de la gestion des cultures, dans ce cas la densité de semis. Les résultats de rendement, replacés dans le contexte climatique des régions arides de semis, semblent indiquer l'application directe possible de l'augmentation de la densité de semis dans les zones les plus susceptibles de rencontrer des conditions de forte demande evaporative. Il semble que le matériel répondant favorablement à ce changement de densité soit déjà entre les mains des agriculteurs qui dépendent de ces cultures vivrières. Les aspects socio-culturels et techniques sont maintenant les leviers à actionner pour rendre ces résultats effectifs sur le terrain. Une porte génétique a également été ouverte par ce travail avec la possibilité de sélectionner la tolérance à la densité, qui pourrait être une cible pour les futurs programmes de sélection. La compréhension des paramètres affectant la réponse des cultures à ces changements de densité devrait également permettre d'ajuster les sorties des modèles de culture afin de tester différents scénarios de densité dans des zones contrastées pour les conditions climatiques, principalement le VPD et le rayonnement solaire. Ces travaux appellent de nouvelles expériences pour décrypter avec une plus grande résolution les mécanismes physiologiques impliqués dans la réponse des plantes à l'augmentation de la densité. Plusieurs outils, impliquant plusieurs domaines et technologies, y compris la physiologie, l'imagerie 3D, la génétique et l'étude agronomique pourraient travailler ensemble dans ces futures recherches.

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List of figures and tables captions

Chapter 1

Figure 1.1: Schematic adapted from Calonnec et al., 2013, representing the link between different plants features and crop management related positively (arrows) to the susceptibility of crops to pests. Additional circles and arrows in red were insert to the figure to highlight features potentially related to the crop response to high density and discussed in the above sections.

Chapter 2.1

Figure 2.1.1: Mean temperature, mean relative humidity, and rainfall recorded at the ICRISAT station (A and B) and at the Bambey (C and D) meteorological station during the field trials. The arrows in each panel correspond to the sowing and harvest dates in each crop cycle.

Figure 2.1.2: Grain yield under high density (HD) as a function of grain yield under low density (LD) in 2017 (A), total aboveground biomass under high density (HD) as a function of total aboveground biomass under low density (LD) in 2018 (B) in India (A, B). Grain yield under medium and high density (D2 and D3) as a function of grain yield under low density (D1) in 2019 (C) and 2020 (D) in Senegal. Data are means of four replicated plots in India and three replicated plots in Senegal, for each genotype-by-density combination.

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Figure 2.2.1: Daily vapor pressure deficit (VPD) and Photosynthetically active Photon Flux Density (PPFD) from ICRISAT meteorological station in the 2017 dry season (A, C) and the dry and rainy seasons of experiment in 2018 (B, D). Empty boxes correspond to the periods of lysimetric measurements in 2018.

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Figure 2.2.5: (A,B) biomass accumulation (grams of biomass per replicate) (C, D) total water use (E, F) water use efficiency (WUE, grams of biomass per kilograms of water used) under high (HD) and low (LD) density in dry and rainy season for the 20 genotypes grown on the lysimeter platform in 2018. Values are the genotypic means of the four replications.

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Chapter 3.1

Figure 3.1.1: Daily vapor pressure deficit (VPD) and solar radiation data from the ICRISAT meteorological station in 2018 (A and B) and the Niakhar meteorological station located 25km south of the trials location in 2021 (C and D). Empty black boxes correspond to the periods of lysimetric measurements.

Figure 3.1.2: Measured evapotranspiration plotted against the reference evapotranspiration (ETref), for 20 genotypes in the lysimeter platform in India (A, C) and 27 genotypes in the lysimeter platform in Senegal (B, D). A and B: point cloud regressions for 4 contrasting genotypes, C and D regression lines for all studied genotypes (one line per genotype). Data are means of 4 replications per genotype and treatment (P-values < 0.0001).

Figure 3.1.3: Frequency distribution of the water use efficiency (WUE) values measured on the lysimeter platforms for the 20 genotypes from panel A India during the 2018 dry (A) and rainy seasons (B), and for the 27 genotypes from panel B in Senegal during the 2021 dry season (C).

Figure 3.1.4: Water use efficiency (WUE) plotted against the slopes of the evapotranspiration response to ETref (see figure 2). (A) 20 genotypes of panel A in the dry season, India (r = 0.64, p-value = 0.0001). (B) 27 genotypes of panel B in the dry season, Senegal (r= 0.54, p-value = 0.004). (C) 20 genotypes of the panel A in the wet season, India (r = - 0.65, p-value < 0.01). Data are means of 4 replications per genotype and treatment (P-values < 0.0001).

Figure 3.1.5: (A) Vapor pressure deficit (VPD) measured in the air and within canopies with high and low densities (12 and 24 plants/m² respectively, HD and LD), as a function of time after sowing in the dry season field trial in Senegal. Black arrows in (A) represents dates at which LAI was measured (xx and 53 days after sowing), and stars indicates significant differences between HD and LD (paired t-test, p-value <0.001). (B) Daily time course of VPD during the 4th week of the same field experiment from 7am to 7 pm, where stars indicates significant differences between HD and LD (paired t-test, p-value <0.001). (C) Photosynthetically active Photon Flux Density (PPFD) at 4 time points across the same day in the two densities. Stars indicates significant differences between HD and LD (paired t-test, p-value <0.0001). Each data point is the average of sensor data collected in three plots for each of the densities.

Figure 3.1.6: Water use efficiency (WUE) measured in the lysimeters plotted against the amount of light measured in the canopy in the adjacent field trial during the Senegal dry season, at (A) 45 DAS (ground level) and (B) 60 DAS (mid-canopy level). Data are the mean of four replications for WUE and three replications for light measurements.

Suppl. Fig 3.1.1: Example of the polynomial regressions using both VPD (grey lines) and ETref (red lines) in x axis for two genotypes from the panel A. Genotypes MR750 showed significant quadratic term with both variables with a concave relationship (A). Genotype NTJ-2 showed non-significant quadratic term with VPD and significant one with concave relationship with ET ref.

Suppl. Fig 3.1.2: Leaf area index (LAI) measured at 33 (A) and 40 (B) days after sowing in the 2018 dry season field trial as a function of the slope of the transpiration response to evaporative demand (ETref) from the adjacent lysimetric experiment. (India, Panel A).

Suppl. Fig 3.1.3: Linear regression of the slopes values generated with Kc method as a function of the values of the slopes generated with APSIM software for the dry season experiment in India (r= 0.99) (A). Water use efficiency (WUE) as a function of the slope generated by the regression of the measured evapotranspiration against the ETref calculated with the Penman-Monteith corrected with a crop constant (Kc) for the 20 genotypes of the panel A in the dry (B) and rainy (C) season in India.

Suppl. Fig 3.1.4: Water use efficiency (WUE) plotted against the slope of the time course of transpiration rate during the 3 hours preceding the maximum transpiration in the glasshouse experiment (Montpellier, France, 9 genotypes from panel B).

Suppl. Fig 3.1.5: Vapor pressure deficit (VPD) measured in air and within canopies with high and low densities (12 and 24 plants/m² respectively, HD and LD), as a function of time after sowing in the glasshouse experiment (Montpellier, France, 2 genotypes from panel B). Stars indicates significant differences between air and HD VPD.

Suppl. Fig 3.16: Normalized biomass and evapotranspiration (ET) plotted against the slopes generated by the regression of measured evapotranspiration and ET_{ref} during the dry (A) and wet (B) seasons in India.

Table 3.1.1: Values of slopes, R², significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for all genotypes from the panel A tested in India. Regressions are ETr values from the lysimeters as a function both ETref (calculated via

APSIM software) or VPD (Calculated with T° et RH data from the adjacent meteorological station). *, p-value <0.05 , **, p-value <0.01, *** p-value <0.001, ****, p-value <0.0001.

Table 3.1.2: Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms in all genotypes from the panel B. Regressions are ETr values from the lysimeters as a function of Etref calculated via the Penman-Monteith equation corrected with a crop constant (Kc). *, p-value <0.05 , **, p-value <0.01, *** p-value <0.001, ****, p-value <0.0001.

Table 3.1.3: ANOVA table showing the genotype variability for the water use efficiency (WUE) in the dry and rainy season experiment in India and the dry season experiment in Senegal. *, p-value <0.05, **, p-value <0.01, *** p-value <0.001, ****, p-value <0.0001.

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Table 3.2.1: Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for the 20 genotypes tested in India. Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software)

Figure 3.2.1: Measured evapotranspiration (ETr) plotted against the reference evapotranspiration (ETref), for 20 genotypes in the lysimeter platform in India (A) and 30 genotypes in the lysimeter platform in Senegal (B). regression lines for all studied genotypes (one line per genotype). Data are means of four and three replications per genotype and treatment in India and Senegal respectively (P-values < 0.0001).

Table 3.2.2: Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for the 30 genotypes tested in Senegal . Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software)

Figure 3.2.2: Leaf area index (LAI) measured 33 days (A) and 40 (B) days after sowing in both high and low density in the 2018 dry season field experiment as a function of the slope of the transpiration response to evaporative demand (ETref) from the adjacent lysimetric experiment. (India).

Figure 3.2.3: Water use efficiency (WUE) plotted against the slopes of the evapotranspiration response to ETref (see figure 1). (A) 20 genotypes of genotypes tested in the dry season, India.

(B) 20 genotypes in the wet season, India. (C) 30 genotypes in the dry season, Senegal. Data are means of four replications per genotype and treatment.

Table 3.2.3: Synthesis of the differences between sorghum and pearl millet crops to density regarding the traits measured in the different experiment and seasons.

General Introduction

-Le paysan qui laboure au soleil mangera à l'ombre-

(Proverbe Sérère)

Sorghum and pearl millet are key source of income and nutrition for 250 million persons in the semi-arid tropics (Chaturvedi et al., 2022). Most of the farmers producing these cereals live below poverty threshold and manage smallholder farming systems (Sanchez & Denning, 2009). The climatic and demographic context of India and the Sahel regions, where these cereals are largely grown, call for a sustainable intensification, as it has taken place elsewhere for other crops.

Sowing density (i.e. the number of plants targeted for a given soil area) is a major factor affecting crop growth and development in cultivated species in both annuals and perennials (Ricaurte et al., 2016), because it affects the amount of light, water and nutrients available to each plant (Podolska, 2016). An increase in plant number on the same soil area also changes the quality and quantity of light available to each plant, which could in turn affect yield. Sowing density in higher-input maize systems increased from 3 plant/m² in the 1930's to 9 to 11 plant/ m² in the 2000's (Duvick et al., 2004; Lee & Tollenaar, 2007). Still, nowadays, yield in maize can be increased through adapted sowing density (Morales-Ruiz et al., 2016). Following the drought episodes in the Sahel region in the 70's, agronomic recommendation in the Sahel pushed farmers to reduce plant density to avoid water deficit.

This work investigates the response of sorghum and pearl millet to an increase in sowing density in the context of the semi-arid tropics, taking into account what was done earlier in cultivated cereals of western farming systems such as maize. The objective was to identify new strategies to increase production, as they are cultivated at low densities in the semi-arid tropic regions.
The first chapter of this manuscript reviews the state of the art regarding the consequences of sowing density and the involved agronomical and physiological mechanism. It considers what has been done in different farming systems and to what extent plant density affects physiological processes and yield. The second chapter presents an analysis of the genetic variability of the response of pearl millet and sorghum to different plant densities. The third chapter examines the responses of sorghum genotypes to high evaporative demands in a range of environmental conditions and plant densities conditions in both crop species. These chapters are based on manuscripts submitted to academic journals.

Chapter 1

Literature review

Unpublished

Sowing density and yield

Plant density is an essential component of crop yield across regions and species (Bednarz et al., 2000). It decreases the aboveground biomass of individual plants, but increases that per unit area in several crops such as maize, soybean, and barley. Farmers grow crops at high target sowing densities in order to maximize yield (Středa et al., 2016). The response of yield to plant density follows a linear relationship up to a critical value, beyond which yield does not increase anymore because of negative effects such as lodging or an over allocation of biomass in the stems at the expense of leaves (Weiner & Freckleton, 2010). The optimal sowing density is environment dependent and can be adapted according to the different climates or soils, the crops are exposed to (Guzman et al., 2019). Such management is known to impact the global water usage of the crops at it was described in Whish et al (2005) and must be taken in consideration in dryland areas.

Tillering: Physiological processes and importance in the response to density.

Tillers are secondary stems that can be fertile or sterile according to conditions sensed by plants. The probability of fertility is higher if tillers develop before they are shaded by the canopy, meaning that tillers developing under high light competition have lower chance to be fertile (Escalada & Plucknett, 1975). The proportion of initiated tillers that reach maturity is also controlled by temperature and light quality, with a genetic variability (Deregibus et al., 1983; Kim et al., 2010). In sorghum, tiller emergence is determined by carbon balance in source leaves (Kim et al., 2010). Light availability decreases inside the canopy when plant density increases, and an adaptation in many cereals crops to this competition for light is to reduce the tillers number (Evers et al., 2006). This is well documented in barley (Munir, 2002; Soleymani et al., 2011) and wheat (Casal et al., 1988) but also in sorghum (Lafarge and Hammer, 2002). At very high densities, the number of tillers can decrease drastically, even causing the death of tillers through self-thinning phenomenon (Fraser & Dougherty, 1977). However, the tillering capacity of different genotypes was weakly involved in the final yield of sorghum: above a density of 12.5 plants.m², the contribution of tillers to grain yield was not significant (Gerik & Neely, 1987). The strong plasticity of the size of the main panicle indeed compensates for the variations in tillering.

Based on genetic and environmental controls presented above, we considered tillering as a potential component of the plant response to plant density.

Light distribution and response to plant density.

Plants can detect changes in quantity and quality of light, thereby causing changes in physiological processes and growth patterns (Aphalo et al., 1999; Ballaré et al., 1994; Dorn et al., 2000; Lewis & Smith, 1983). One of the typical response to high density is shade avoidance through stem elongation. In maize, leaves turn narrower with a preferential biomass allocation to the stem. The angle of attachment with respect to the stem decreases, leading to a change in the leaf area exposed to solar radiation (Gou et al., 2017). This is mainly controlled by the phytochrome-mediated 'shade-avoidance' response of plants to competition from neighbors, in which plants show increased stem extension growth and therefore an increased risk of lodging (Sawers et al., 2005), at the expense of yield (Boccalandro et al., 2003).

Carbon assimilation depends on how much incident light is intercepted and, at a leaf level, is associated with the nitrogen content (Evans, 1989; Yin & Struik, 2015). According to their position in the canopy, leaves are exposed to different light conditions, with a degree of shading increasing from top to base, leading to a progressive light extinction in the canopy. This gradient in light availability increase as LAI rises. The literature on rice suggests that LAI value over 3 (corresponding to a fully expanded rice canopy) associated with horizontal leaf angles in lowest parts and vertical in the upper part allows optimal light interception and use efficiency of the intercepted light (Ku et al., 2012; Tollenaar & Wu, 1999).

Crop photosynthetic activity and, *in fine*, yield, is related to photosynthesis of the whole canopy rather than that of top leaves (Ort et al., 2011; Zhu et al., 2012). Indeed, top leaves exposed to full incident light may receive light in excess of their needs (SP Long, Nugawela & Farage, 1987), while lower leaves, only receive diffuse light and do not photosynthesize at their maximum capacity. Some authors proposed that shaded leaves (i.e. receiving diffuse light and not the direct radiation) account for more than 70% of the total leaf area (Song et al., 2013) but less than half of the total canopy photosynthesis activity and carbon gain. The relationship between photosynthesis and stomatal conductance is not linear, so that leaves receiving high radiation are also likely to have stomata largely open, losing water with only little added carbon fixation. Therefore, the vertical distribution of light in a crop canopy affects the whole canopy carbon gain, and eventually biomass production and yield (Yin & Struik, 2015). Light reaching within-canopy leaves is also important for these leaves to keep their function of nitrogen storage (Sinclair et al., 1999). According to Niinemets (2010), the traits that most control radiation efficiency at plant level are the angle distribution (i.e. how leaves angles change along the z axis) and the foliage spatial aggregation (i.e. how the leaves are inserted with respect to the stem and shade each other).

Many studies have shown a link between plant architecture and radiation interception efficiency. The angle of attachment of leaves on the stem can influence how the light resource is shared vertically along the plant canopy. In sorghum, an allele of a gene encoding for a p-glycoprotein involved in polar auxin transport is responsible for change in the leaf inclination angle by up to 34°. The presence of this allele has the effect of changing the light distribution pattern and of increasing radiation use efficiency (RUE), and this leads to a higher productivity (Truong et al., 2015). According to Perez et al., 2019, the increase in RUE during the last century of breeding is in part due to the spatial rearengement of leaves. An increase in density, accompanied by changes in light interception, allowed modern maize hybrids to increase LAI from 2.4 m^2m^{-2} to 4.8 m^2m^{-2}. This increase in LAI resulted in a 20% increase in light interception and up to 14% increase in yield, and was shown to result from smaller leaf angles (Lee & Tollenaar, 2007).

Other studies have demonstrated that light penetration in the canopy increases radiation use efficiency (Duursma et al., 2012; Falster & Westoby, 2003). In rice, several authors showed positive correlations between high yields, better ability to intercept light and higher photosynthesis with more erect leaves (Ito & Hayashi, 1968; Kumagai et al., 2014; Morinaka et al., 2006; Erik H. Murchie et al., 1999). Erect leaves allow, when the light is saturating, to protect the plants against the photo inhibition phenomenon and thus to maintain a higher level of photosynthesis (Kumagai et al., 2014; E. H. Murchie et al., 2009). Song et al., 2013 demonstrated *in silico* that it was possible to significantly increase the photosynthetic activity of a crop by improving its architectural characteristics. Their model demonstrated that, for a given canopy structure, the estimated CO_2 canopy uptake was over-estimated by 25% when considering the ‘average’ canopy light conditions over the light period rather than light conditions at individual points in the canopy.

Therefore, we have considered in this study the role of light penetration in the response of biomass accumulation to plant density.

Different strategies to face high VPD and maintain water use efficiency.

The difference between air and leaf temperatures affects yield, directly or indirectly, in rice. Indeed, water deficit during the grain filling period leads to higher differences in temperature and is correlated to the yield , with a genotypic variability (Meng et al., 2020). In well-watered plants, transpiration represents the most effective way of leaf cooling. Under water deficit, with essentially closed stomata, transpiration only occurs via cuticle and remaining stomatal aperture. This ‘cuticular’ transpiration increases exponentially with rising temperature due to the increase in the water permeability of the cuticle and in VPD (Chaves et al., 2016; M.Keller, 2015).

In crops exposed to high evaporative demand, a first strategy can be to maintain a high level of transpiration ensuring an appreciable biomass accumulation, at the risk of encountering a terminal drought that severely impacts the grain filling stage (Kholová et al., 2010). An opposite second strategy is to limit transpiration: some genotypes reduce transpiration through stomatal closure in response to high VPD (Vadez et al., 2014), with a considerable genotypic variation (Gholipoor et al., 2012; Kholová et al., 2010). There are two possible benefits for this second strategy. The first is a transitory slowdown of the transpiration flow during very hot and dry episodes, leading to a higher water availability for the grain filling period (Kholová et al., 2014; C. D. Messina et al., 2015; Vadez et al., 2013). The second benefit is an increase in water use efficiency (WUE) (C. D. Messina et al., 2015; Sinclair et al., 2005), which is an important variable in the ability to yield (Condon et al., 2004; Passioura, 1977). The above studies attempted to deal with the high VPD conditions to which the leaves are exposed. However, none considered whether these VPD conditions could be altered through changes in crop management such as plant density.

Plant transpiration is driven by their hydraulic capacity to transport/extract water from the soil to the atmosphere, resulting in changes in stomatal conductance (Comstock, 2002; Kholová, et al., 2010), thereby affecting the gradient of water potential from the rhizosphere to stomata during daytime (Cowan, 1965). This gradient increases with stomatal conductance, VPD, wind and solar radiation which affect evapotranspiration predicted by the Penman Monteith equation (Zotarelli et al., 2014).

In view of their consequences on plant growth, we have explored in this study the relationship between these two, opposite, strategies and the plant response to plant density.

Effect of plant density on the canopy microclimate.

An increase in leaf area increases the global water budget of the crop, but also affects light, temperature, relative humidity, and air movements sensed by leaves located deep in the canopy, thereby avoiding atmospheric drought for these leaves. This effect has mostly been ignored in the analyses of the responses to plant density. However, reducing row spacing in maize and sunflower has a small effect on soil water depletion but WUE for grain was significantly increased in several studies, thereby suggesting a role for the intra-canopy microclimate.

For Di Matteo et al., 2016 the positive response in maize was due to the increased number of kernels produced per unit area, increasing with density. In Hernández et al., 2020, authors concluded that a higher light interception by the crops, decreasing the soil evapotranspiration, may explain the benefit of higher density for WUE, as also reported in cotton (Yang et al., 2014). The same response of crops to density was also reported by the same group in sunflower (Echarte et al., 2020). In the study of Barbieri et al., 2012, authors assumed nitrogen availability as the main driver of the increase in WUE. They discussed the possibility of a better root distribution in the inter row when nitrogen is limiting, the roots tending to go deeper, avoiding the competition due to higher density. Such hypothesis was already proposed by Sharratt & McWilliams, 2005. Few years before, it was also shown that plant density was a management practice with the potential to maximize WUEg (grain) (French & Schultz, 1984; Hatfield et al., 2001). A group working on cotton demonstrated that an optimum density was reached when the microclimate was the most favorable, especially when associated with a high light transmission through the canopy (Yang et al., 2014). Interestingly, a recent study from a group in Venezuela have highlighted that cotton varieties improved in the USA in the 1960s to increase light interception were the ones that responded best to increased plant density in terms of yield but no attempt was made to measure the microclimate characteristics of the canopies generated by the different densities (Guzman et al., 2019). According to different environments, soil conditions or cultural practices of crop management, there are different sowing geometries leading to different sowing densities (Huang et al., 2006) and that can affect the WUE (Hsiao et al., 2007; C. Messina et al., 2009). In these studies, it is described that high seeding rates and/or different crop management allow better soil coverage, and thus, reduce soil evaporation. In semi-arid regions of India, main cereals are sown in regularly spaced rows of about 60 cm leading to a density of about 10 plants/m². In western Africa, sowing in three-plants clusters (pocket) separated from each other by spaces of up to 90 cm in all directions of the space leads to a density of about 3 plants/m². Changing these geometries by adding plants may affect the air movements in the fields as well as the pattern of shading across the daylight period.

A working hypothesis of our study was that climatic conditions in dense canopies might affect the VPD and light sensed by leaves located inside the canopy. Consequently, this may also change the pattern of transpiration at the canopy level and affect the response of transpiration to the evaporative demand.

Possible interaction with pathogens

Plant density affects the interactions between hosts, pathogens and environments. Indeed, the dissemination of pathogens in a canopy is often linked to the frequency of contact points between organs, increased by an increased plant density (Costes et al., 2013). For the same reasons, the dissemination of the natural enemies of the pests can be affected. Randlkofer et al., (2010) showed differences in the behavior of grass pests that are related to the density, height and interconnectivity of the plants in the cover. According to Cintrôn-Arias et al. (2009) the transmission rate and the infection period are directly impacted by the distribution of plants or susceptible organs. The density and spatial arrangement of the canopy influences the volume and movement of air in the plant canopy and this has a direct influence on the pest population dynamics and on the canopy microclimate. Three of the microclimatic conditions can affect the development of pathogens. High irradiance generally decreases the risk of having higher population levels of harmful pathogens, the temperature, which favors the pathogens that do not depend on water, and finally the free water in the environment that facilitates the installation of water-dependent pathogens. Sowing density has a direct impact on these conditions (Calonnec et al., 2013; Yang et al., 2014) (Fig 1.1). In addition, the rate at which the organs come into contact within the canopy, leading to the closure of the canopy, can vary the sensitivity of the crop to pathogens. Indeed, young organs can be more easily infected. If connectivity between canopy organs occurs when the organs are more mature, the risk of epidemic spread is reduced (Calonnec et al., 2013). In a higher planting density, this leaf-to-leaf contact could occur at an early, sensitive stage and increase the risk of disease spread. In any case, it is understood that the risk of an increased pathogen pressure on the crops is in part linked to a higher humidity within the canopy. It is interesting to see how parameters such as the relative humidity in the canopy, that is linked to epidemic spread, are connected with traits we hypothesized to be at the basis of the response to density. On the Figure 1.1, the red arrows,

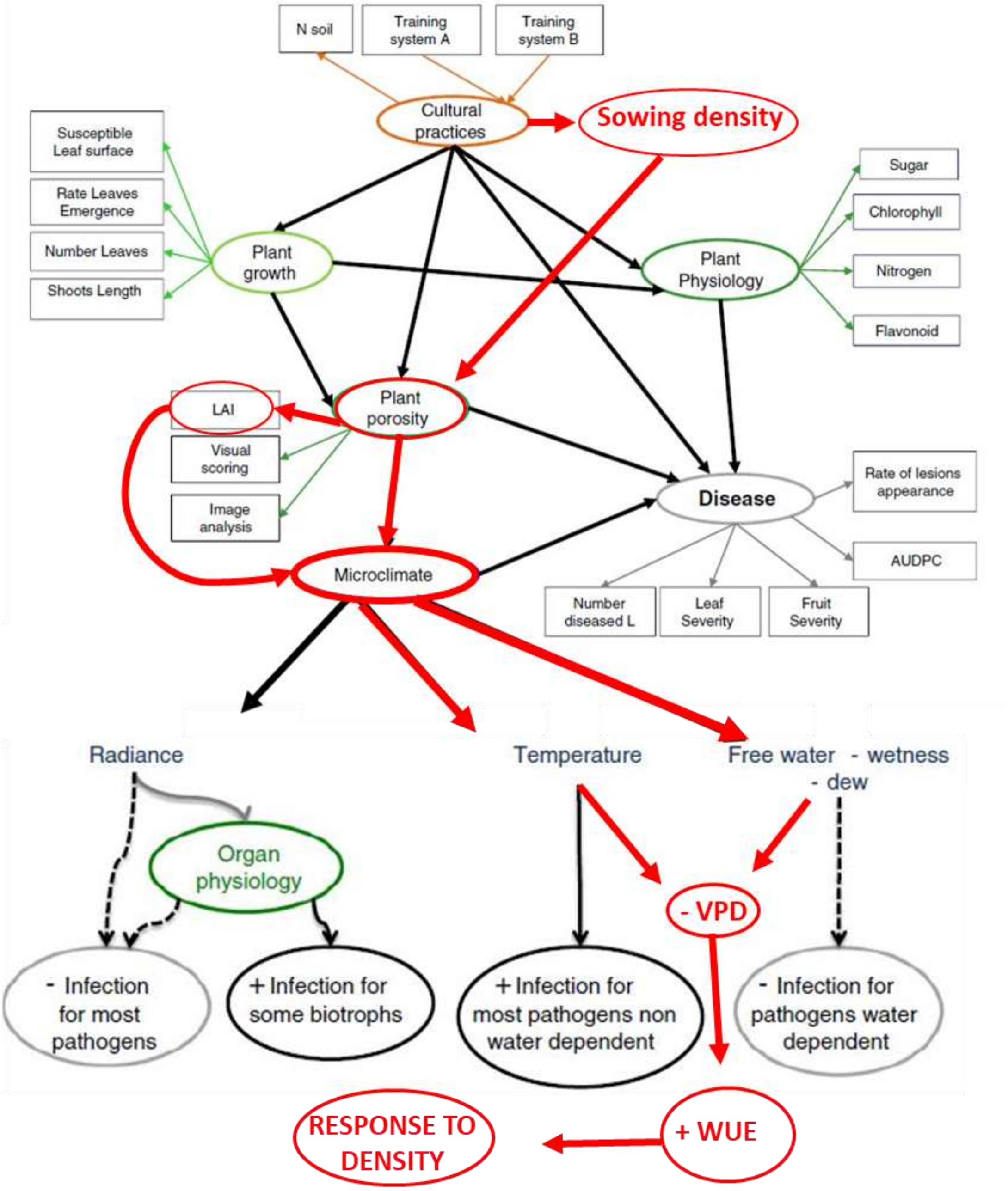

Figure 1.1: Schematic adapted from Calonnec et al., 2013, representing the link between different abiotic components, plants features and crop management related positively (arrows) to the susceptibility of crops to pests. Additional circles and arrows in red were insert to the figure to highlight features potentially related to the crop response to high density and discussed in the above sections.

circles and words were added to an existing figure, adapted from Calonnec et al., 2013. This figure highlights traits interconnected and putatively traits involved in both the density responses and susceptibility to epidemic. This interconnection between traits makes it important to consider potential pathogen issues in implementing this change in crop management (i.e. density).

Although we recognize that pathogen diffusion is an important trait that influences the responses to plant density, this aspect was not considered in our study because we did not observe obvious pest damages, probably because of the effects of high irradiance and VPD described above.

Working hypotheses and phenotyping strategy

The previous sections highlight that the response of yield to an increase in sowing density involves many traits. The first goal of this work was then to test whether a positive response to the change in density exists in some sorghum and pearl millet genotypes already in use in dry conditions of India and Senegal. The second goal was to test the relative contributions of possible processes in the overall responses to plant density, summarized at the end of each of the above paragraphs. We tested the hypothesis of the benefit of higher densities on the whole plant WUE and measured the effect of higher densities on the canopies' microclimate, but also systematically tested the genetic variability of responses to the environmental conditions studied here. Working hypotheses are described in more details in the second chapter for both sorghum (2.1) and pearl millet crops (2.2). To test the link between in-canopies conditions and the effect of the density on the WUE, an approach based on the plant response to the evaporative demand and variation for light interception under different densities was undertaken and presented in the third chapter of this document.

To this end, experiments were carried out during several years and in different seasons (ICRISAT and CNRA research stations in India and Senegal respectively) and in controlled conditions in Montpellier. Panels of sorghum and pearl millet genotypes were grown in different environmental conditions and plant densities to assess the increase the biomass and grain yield. We measured transpiration, water use efficiency and intra-canopy microclimate, considered as possible drivers of observed variability in responses between genotypes and environmental conditions. Because genotypic variability was a key feature of our study, we used genetic panels of both elite varieties from different ICRISAT breeding programs and commercial companies, or germplasm accessions (about 50 genotypes per species).

This study benefited of the progress of phenotyping, both in field conditions via the use of lysimetric platforms developed by ICRISAT and CNRA, adjacent to experimental fields, and in those allowing controlled conditions in Montpellier (IRD, CIRAD). This allowed us to test some of the working hypotheses presented above. A particular attention was paid, in the third part of this document, to measuring the water budget and microclimate of studied canopies under contrasting climatic conditions and plant densities. Methods and main hypotheses are described in more details in the introduction and material and methods of each section of this manuscript.

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Chapter 2.1

Higher sowing density of pearl millet increases productivity and water use efficiency in high evaporative demand seasons

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Abstract:

Pearl millet is the main subsistence crop for smallholder farmers systems where it is grown at low plant density (around 3 plants/m²). Intensifying pearl millet cultivation could boost productivity although it is unclear if water stress could become an issue. Indeed, increasing planting density would increase the leaf area and the related water budget. However, a denser canopy could also create a more favorable canopy microclimate to the benefit of the water use efficiency (WUE) of the crops. The first aim of this work was to test the yield response of popular pearl millet varieties to an increased density in different field locations (Senegal and India), and to assess possible genotypic variation in this response. The second aim was to measure the water use and the WUE of the crop in different densities, using lysimeters. The higher sowing density significantly increased yield in all genotypes when the trials were carried out in high evaporative demand conditions. There was no genotype x density interaction in these trials, suggesting no genotypic variation in the response to density increase. The high-density treatment also decreased the vapor pressure deficit (VPD) in the canopies, both in the field and in the lysimeter experiments. Although the higher density treatment increased water use, the resulting increase in biomass was proportionally higher, hence increasing WUE of the crops in all genotypes under high density. The increase in yield under high density was closely related to the increase in WUE, although this link was more tight in the high- than in the low evaporative demand seasons. This confirmed a strong environmental effect on the response to density of all the genotypes tested. These results highlight the possibility to improve pearl millet yield by increasing the density, targeting specifically areas facing high evaporative demand, although they did not open a scope for breeding density tolerant cultivars.

Keywords: Climate change, sustainable intensification, canopy architecture, semi-arid tropics, vapor pressure deficit.

Introduction

Pearl millet is a sustainable crop for the semi-arid tropics regions (SAT) where it is grown. In the Sahel region, it is a major staple food and represents the main economic supply for smallholder farmers. Around 45% of the world production of pearl millet takes place in Western and Central Africa (WCA) (FAO, 2020). In India, pearl millet is the fourth most produced cereal and therefore it also represents a major agronomic interest in this densely populated region (ICAR, 2020). In these small-farming areas, pearl millet is traditionally sown under low to very low density (Bationo et al., 1990). In other crops like maize, there has been a progressive increase in sowing density that has contributed to the yield increase of this crops over the years, without this being a specific breeding target (Di Matteo et al., 2016; Mansfield & Mumm, 2014; York et al., 2015). In our knowledge, no similar increase in the density of sowing of pearl millet has taken place, although a sustainable intensification of this crop is needed to cater for the increased demand of its grain and fodder, especially in the current tense situation of world cereal markets. Increasing the traditional sowing density could be a lever to increasing pearl millet productivity. Therefore, one hypothesis of this work is that there is room to increase the productivity of pearl millet and there may be genotypic variation is the degree of response to increased sowing density. One issue with crop intensification in Sahelian climates is water scarcity. As part of this problem, the vapor pressure deficit (VPD) is high in those climates and causes an atmospheric drought that will become even more acute in future climate (Vadez et al., 2012; Asseng et al., 2015; Kholová et al., 2010). This must be dealt with before proposing any recommendation. While indeed an increase in density of planting would likely increase the water demand of the crop, the increase in leaf area index from the increased density could also create a microclimate within the canopy with a milder VPD, as shown in sorghum (Pilloni et al, 2022, under review). This may mechanically increase the water use efficiency (WUE) of the crops (Fletcher et al., 2008; Grossiord et al., 2020) a trait of agronomic importance. Water use efficiency is indeed inversely related to the VPD that the plants are exposed to (T. R. Sinclair et al., 1984; Thomas R. Sinclair et al., 2005; Vadez et al., 2014), and then is higher at lower VPD’s. Our hypothesis is that an increase in sowing density may also increase the WUE of denser canopies. The objectives of this study were then to test whether pearl millet productivity could be increased by increasing its sowing density, whether there was genetic variation in this response, and how much this change in crop management could affect water use and WUE of the crops. To that end, we conducted several field and lysimeter experiments to quantify yield, water use and WUE in different cultivars and densities conditions. To increase the robustness of the results, our hypotheses were tested in two different locations, i.e. at the ICRISAT site in Patancheru in India and at the CNRA station in Bambey in Senegal, with two distinct panels of released cultivars.

Material and methods

Locations and biological material

Field and lysimeter trials were conducted during the 2017 and 2018 dry seasons and during the 2018 rainy season in India at the ICRISAT research station (Hyderabad, 17°31'01.3"N 78°16'33.4"E) and during the 2019 and 2020 rainy seasons and 2021 dry seasons in Senegal at the CNRA research station (Bambey, 14°42’ N ; 16° 28’ W). Soils at the ICRISAT farm are Alfisol while those at the Bambey farm are typical Sahelian sandy soil with more than 90% sand. The biological material used in this study consisted in two panels of genotypes. Panel 1 included 20 commercial elite varieties from the ICRISAT-India breeding program and from private companies, and was tested in India. Panel 2 included 30 elite varieties from the ICRISAT breeding program for West Africa along with regional elite lines, and was tested in Senegal. Temperature, hygrometry, and rainfall was recorded every day in both ICRISAT and CNRA stations and used to characterize the seasons of experiment (Figure 2.1.1).

Yield assessment in the field

In India, two field trials were carried out in the 2017 and 2018 summer season (February – May) characterized by high VPD. The field was mechanically tilled and 100 kg/ha DAP was applied before sowing and 100 kg/ha urea applied 15 days after sowing. The 20 genotypes from the panel 1 were manually sown in 8m² (4m long, 2m wide) plots of two different densities. A low-density treatment (LD) consisted in plot of 4 rows spaced at 60 cm intervals from each other and 15 cm between plants in the same row, leading to a density of 12 plants/m². High-density treatment consisted in plots of 8 rows spaced with 30 cm intervals from each other and 15 cm between plants within the row and leading to a density of 24 plants/m². The field trials were fully irrigated and received 40mm of irrigation every week from sowing to maturity. These trials followed a fallow period during the previous rainy season. Grain and vegetative biomass yield was harvested from the entire micro plot in 2017. A storm in 2018 forced the trial to be harvested soon after flowering, at the very beginning of grain filling. We measured total aboveground biomass in this case. Samples were dried 72h in the oven.

In Senegal, field trials were carried out during the 2019 and 2020 rainy seasons (June-September). The field was mechanically tilled and 150 kg/ha DAP (15-15-15) was applied 10 days after sowing and 100 kg/ha urea applied 15 days after sowing. The 30 genotypes from the panel 2 were manually sown in three different densities. A plot consisted in 3.25 m² (1.8m long, 1.8 wide). The standard density treatment (D1) consisted of lines of pockets, each thinned to three pearl millet plants, separated from each other by 90 cm in the row and between rows (3.2 plants/m²). Two increased density treatments were set up, consisting in adding either one pocket of three plants within the row (D2, 6.4 plants/m²), or both within in the row and between the rows (D3, 12.8 plants/m²). At grain maturity, panicles were harvested, sun dried and weighed to measure grain yield.

In both sites, the experimental design was a completely randomized block design, with density treatment as the main block and genotypes randomized three and four times in each main block in Senegal and India respectively.

Measurement of the leaf area index

The leaf area index (LAI) was measured in the 2018 field trial in India and in the 2020 field trial in Senegal, using a 1-meter long ceptometer (AccuPAR LP-80, Meter in India and Sun scan type SS1, Delta-T devices in Senegal). The aim was to compare the soil coverage allowed by the higher density and putative genotypic differences. In each plot, two measurements were taken from above the plants to assess the incident radiation at that particular time. The device was then placed diagonally on the ground, between two (Senegal) or four (India) rows according to the treatment and site, and four light measurements were done in each plot, at different locations of the plot. The ceptometer measured the amount of photosynthetically active photon in µmol.s^{-1}.$m^{2\ -1}$ and converted the light quantity into leaf area index using the following formula: $LAI = \ln(\frac{I}{Io})/k$ where I is the incident light above the canopy Io, the light at ground level and k a crop extinction equal to 0.6 for pearl millet crops.

Measurement was done at 33 and 40 days after sowing (DAS) in India and at 43 and 63 DAS in Senegal, close to sun zenith time.

Soil core to measure water content.

Water content of soil core was measured at harvest to assess how the different density treatments affected water content in the different layers of the soil profiles. Three and four micro plots in India and Senegal respectively were randomly selected in the different density treatments. Soil cores of 90 cm depth were taken in between two rows using a manual auger. The core was separated in layers of 30 cm and immediately weighed. The soil was then stored in metal boxes and put in the oven for 72h for complete drying. The samples were then re-weighed. The ratio of fresh weight to dry weight allowed the calculation of the water content (percentage) of the samples for each soil horizon studied using the following formula: $\text{soil moisture }(\%) = \frac{(P_h - P_S)}{P_S} * 100$ as proposed by Anderson & Ingram (1993).

Water use efficiency on lysimeter platforms

Three trials were carried out in both Senegal and India on similar lysimetric platforms. The platforms consisted of PVC tubes installed side by side in long trenches. The tubes were filled with soil from the fields adjacent to the platforms (Alfisol in India, sandy soil in Senegal). A pulley system associated with an S-type load cell (Mettler-Toledo, Geneva, Switzerland) allowed to weigh regularly each tube individually and to measure the water use of each plant through mass differences between consecutive weighing. The cylinders were brought to field capacity before sowing by watering cylinders abundantly and letting them drain. After sowing, cylinders received 500Ml on alternate days. Before starting the weighing, cylinders wereagain brought to field capacity, and the field capacity weight was taken as a benchmark for re-watering. At each subsequent weighing, water was added to each cylinder to reach field capacity weight minus a buffer of 2 kg to avoid possible drainage. A replication consisted in a set of four tubes, all being planted with one plant each in the high density treatment, whereas only two tubes were planted in the low density while the two others remained empty. The empty tubes' water losses were measured and were integrated in the total water use of the replication. The plants were harvested after a period of growth of 7 to 9 weeks according to the trials and total aboveground biomass was measured after drying for three days in an oven at 70ºC. The water use efficiency (WUE) was calculated by dividing the total biomass produced by the total water used, and expressed in g biomass per liter of water used by the crop (g L^{-1}). The experimental design was a completely randomized block design, with density treatment as the main block and genotypes randomized three and four times in each main block in Senegal and India respectively. Panel 1 was tested in two seasons contrasting for the evaporative demand (i.e. dry and rainy season 2018) in India. Panel 2 was tested during the 2021 post rainy season in Senegal (characterized by a high evaporative demand).

Canopies vapor pressure deficit assessment

Putative differences in the evaporative demand in the canopies of different density were measured through measurement of vapor pressure deficit (VPD). Temperature and relative humidity sensors were set up in both high and low density during 15 days of the 2018 high VPD season lysimeter trial in India and 6 days during the 2020 low VPD season field trial in Senegal (TinyTag ultra 2, TGU-4500, Gemini Datalogger Ltd, Chichester, UK). In India, the sensors were installed at 53 days after sowing (DAS) (corresponding to flowering time in most of the genotypes) and at 89 DAS in Senegal, corresponding to the last week before harvest at grain maturity . Data was recorded every 30 min from 7am to 7pm. Daily average of the VPD was calculated according the following formula:

VPD = (100 - %RH x SVP/ 1000) where SVP (saturated vapor pressure) is 610.7 x $10^{(7.5 \times T°C) / (273.5+T°C)}$.

Statistical analysis

The statistical analysis (Analysis of variance, t-test, 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

Results

Similar dry seasons in India but contrasting rainy seasons in Senegal

Weather data was continuously recorded on the meteorological station adjacent to the different trials in both India and Senegal. The average of daily VPD across the two seasons of 2018 when lysimeter trials were carried out differed and were 2.1kPa and 0.91 kPa during the high and low VPD season respectively. The 2017 and 2018 dry seasons when field trials were carried out in India were similar and characterized by high temperature and low relative humidity with no or very limited rainfall (3 days, 27mm and 7 days, 17, 4mm in 2017 and 2018 respectively (Figure 2.1.1 A and B). By contrast, the two rainy seasons when field trials were carried out in Senegal were different with a major difference in the rainfall distribution during the season and in the total rainfall. In 2019, it rained on 35 days during the growing season, for a total of 493.2mm (Figure 2.1.1C). By contrast, during the 2020 rainy season, it rained on 42 days for a total rainfall of 642.7mm (Figure 2.1.1D). Specifically, during the first 60 days of the crop cycle (before flowering), 2 rainy days only occurred in 2019 against 14 in 2020 over the same period.

(Figure 2.1.1C and D). Relative humidity was also lower by 5% on average of the season and 7% on the 60 first days of the crop cycle in 2019 compared to 2020. Light intensity, collected in a nearby weather station 25km apart, was also 10% higher in 2019 than in 2020 during the initial 60 days of the crop (data not shown).

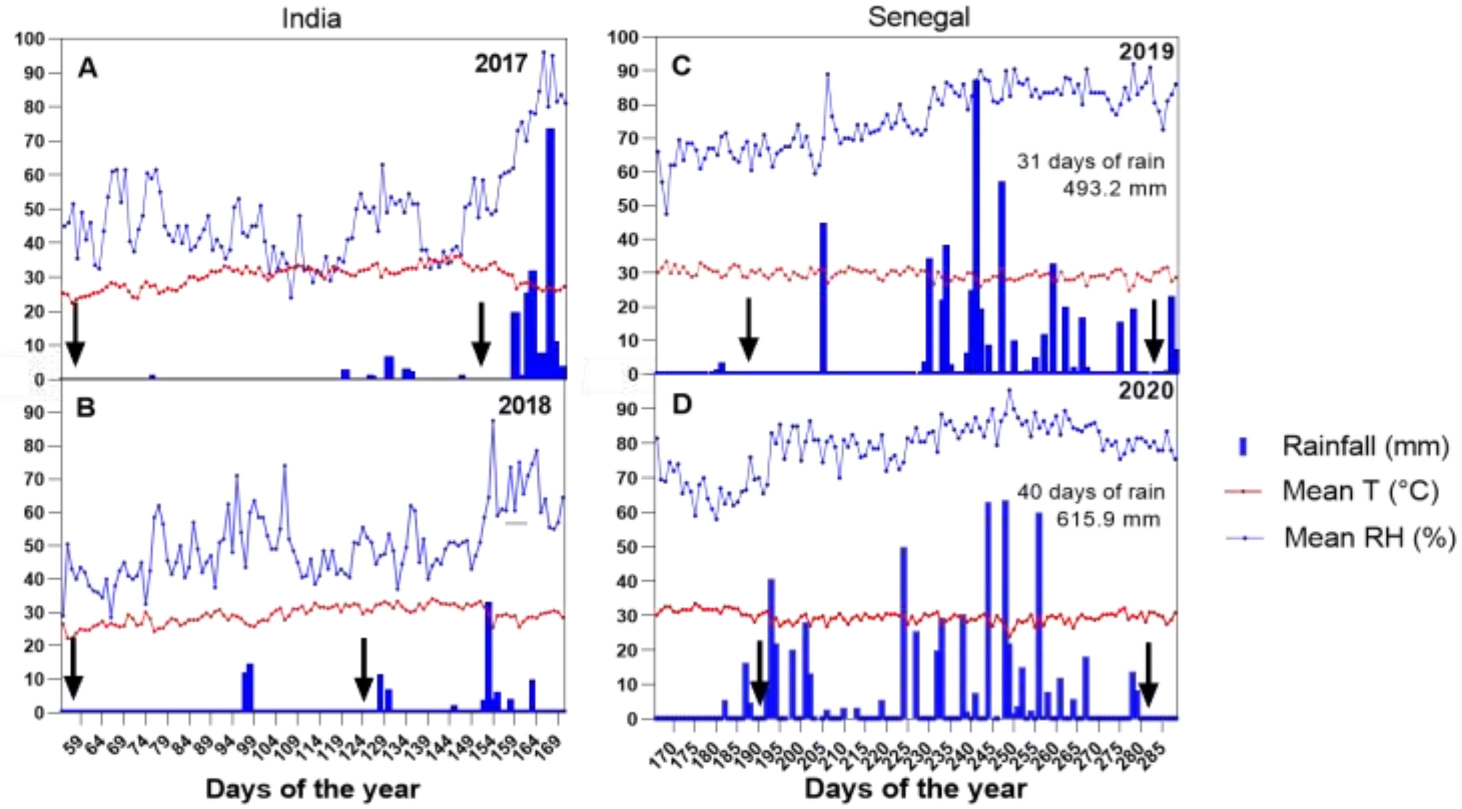

Figure 2.1.1: Mean temperature, mean relative humidity, and rainfall recorded at the ICRISAT station (A and B) and at the Bambey (C and D) meteorological station during the field trials. The arrows in each panel correspond to the sowing and harvest dates in each crop cycle.

High-density increases the yield differently across years and locations.

The high-density treatment increased yield significantly in the two Indian field trials. The average grain yield for the 2017 trial was 3.41 ± 0.42 t/ha in low-density and 3.92 ± 0.46 t/ha. (Fig 2.1.2A). A two-way anova highlighted a strong density effect on yield (Wald statistic, p-value=0.0001), a significant genotypic effect but no genotype-by-density interaction, indicating that no genotype responded better to the high-density treatment than another. The same trend was observed during the 2018 trial where the total aboveground biomass (vegetative biomass + emerging panicles) was used as a proxy for the yield response. There, aboveground biomass had an average of 5.74 ± 0.79 t/ha under low-density and 6.84 ± 0.58 t/ha under high density (Fig 2.1.2B). Again, a strong density effect was highlighted (Wald statistic, p-value=0.0001), with a significant genotypic response, and again no genotype-by-density interaction. In Senegal, the response of the panel of genotypes tested was drastically different across years, showing a positive yield response to density in 2019, and a slightly negative one in 2020. During the 2019 field trial, the grain yield significantly increased with density and was 1.45 ± 0.68 t/ha in the lowest density D1, 1.55± 0.60t/ha in the intermediate density D2, and 2.10± 0.88 t/ha in the highest density D3 (Fig 2.1.2C). The Anova analysis revealed a strong genotypic effect, explaining most of the variation, although the density effect was also highly significant (ANOVA, Wald statistic, Table 1). No genotype × density interaction was found on the three-density treatment dataset, although a slight but significant genotype x density interaction effect (p-value = 0.01) was found in a two-way ANOVA ran on D1 and D3 data from 2019. During the 2020 trial, the grain yield significantly decreased in the highest density treatment. Average yield data were the following in the three density conditions: 1.6 ± 0.55 t/ha in D1, 1.53 ± 0.56 t/ha in D2 and 1.3 ± 0.47 in D3. Analysis of variance on yield showed a genotypic effect (p-value <0.0001) and a slight density effect (p-value= 0.046) with no genotype-by-density interaction effect (Fig 2.1.2D).

High-density increases LAI.

In the different field trials, the leaf area index measured at two different times after sowing was always larger under higher density for all genotypes. In 2018 in India, the LAI measured at 33 DAS was in the range of 0.55 to 1.04 in LD and 1.14 to 1.75 and in HD. At 40 DAS, LAI varied between 0.94 and 1.61 in LD and 1.6 and 2.3 in HD. Two-way ANOVA showed a significant

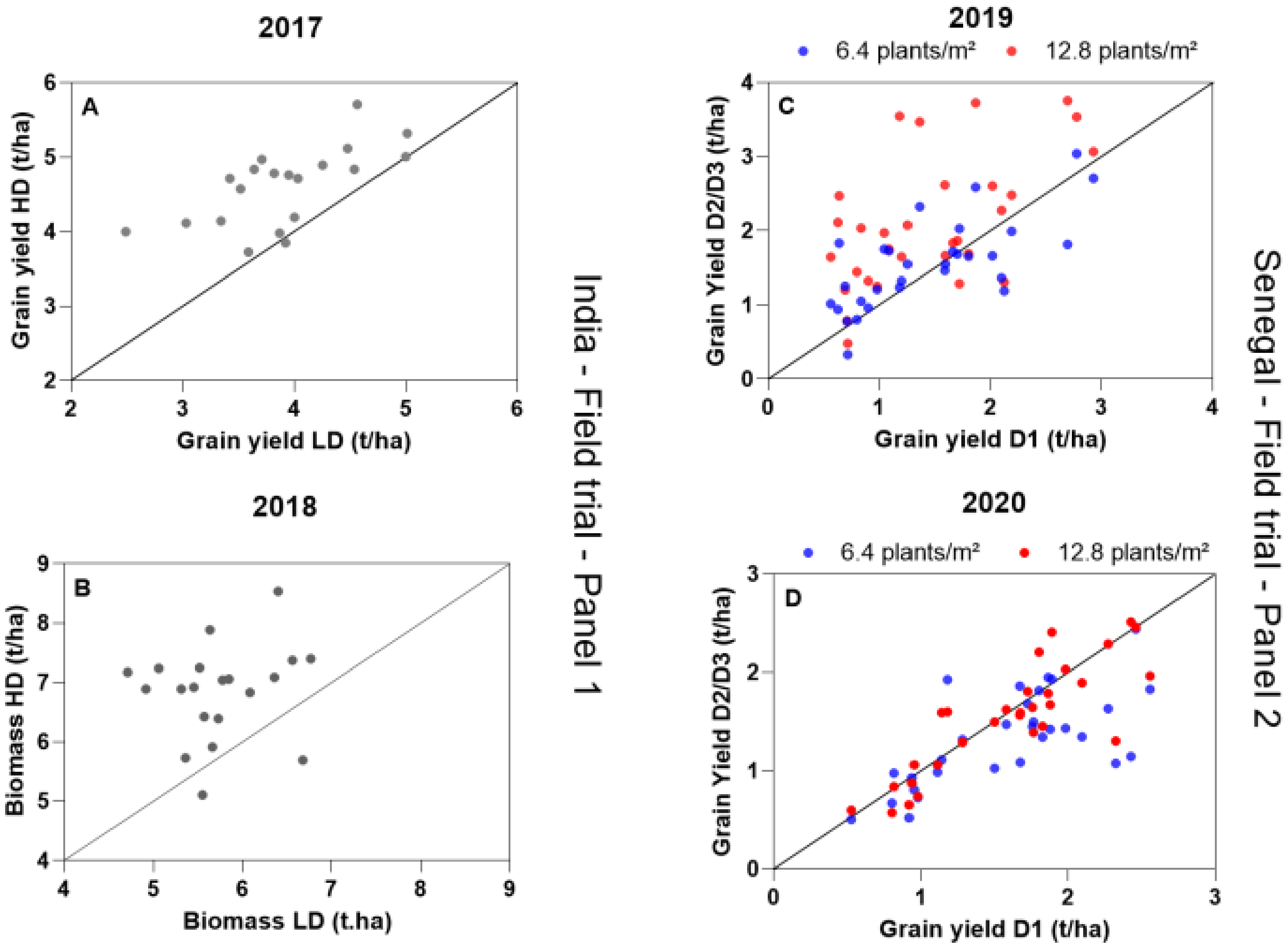

Figure 2.1.2: Grain yield under high density (HD) as a function of grain yield under low density (LD) in 2017 (A), total aboveground biomass under high density (HD) as a function of total aboveground biomass under low density (LD) in 2018 (B) in India (A, B). Grain yield under medium and high density (D2 and D3) as a function of grain yield under low density (D1) in 2019 (C) and 2020 (D) in Senegal. Data are means of 4 replicated plots in India and 3 replicated plots in Senegal, for each genotype-by-density combination.

density effect but no genotype or genotype-by-density interaction effect in the two dates of measurement (Fig 2.1.3A & B). In the 2020 field trial in Senegal, LAI measurement made at 43 DAS showed significant differences between different densities and genotypes while no genotype-by-density interaction was found. Measurements made at 43 DAS showed LAI values ranging between 0.9 and 2.0 in D1, 1.0 and 2.7 in D2 and between 1.3 and 3.1 in D3. At the second date of measurement (i.e. 63 DAS) LAI ranged between 1.23 and 2.93 in D1, between 1.37 and 3.2 in D2, and between 1.63 and 3.9 in D3. Strong and significant density and genotypic effect was found for LAI for the two dates of measurement (ANOVA, p-value <0.0001, Tuckey's multiple comparisons test) but no genotype-by-density interaction (Fig 2.1.3C and D). Genotypic values of LAI measured at the different dates, in the different trials were plotted against the yield, and did not show any significant relation (data not shown).

Denser canopies have lower VPD

In both the lysimeter and the field conditions, the high-density treatment reduced significantly the VPD within the canopies. Mean VPD during the 15 days of recording in India was 2.86 kPa in the HD treatment and 3.82 kPa in the LD treatment, i.e. a 0.96 kPa difference on average (paired t-test, p-value <0.0001) (Fig. 2.1.4A). In Senegal during the 2020 rainy season, VPD in the highest density treatment D3 was significantly lower (average VPD of 1.98 kPa) than in the lowest density treatment D1 (average VPD of 2.99 kPa in the first 4 days of the measurement) (paired t-test, p-value <0.01) (Fig. 2.1.4B). VPD differences were not significant between the two lowest densities D1 and D2 (Paired t-test, p-value = 0.11). On the days 5 and 6, ambient VPD dramatically decreased because of rainfall (2.02 kPa on day 5 and 1.76 kPa on day 6), leading to the loss of the density effect on the within-canopy VPD as non-significant differences were recorded for those two particular days, regardless of the treatments compared (Fig 2.1.4B).

Higher density increased water use efficiency in all genotypes.

In all trials, the HD treatment increased WUE significantly. On the panel 1 in India, average WUE was 1.25 g biomass kg^{-1} water transpired in LD and 1.50 g biomass kg^{-1} water transpired in HD for the summer season trial (Fig 2.1.5A), and 7.80 g biomass kg^{-1} water transpired in LD and 10.41 g biomass kg^{-1} water transpired in HD for the rainy season trial (Fig. 2.1.5B). However, a two-way ANOVA showed no significant genetic nor genotype-by-density interaction effects. Only the density effect was strongly driving the variation (Two-way ANOVA, p-value <0.0001). The same results were found in the high VPD season trial in Senegal. There, using a

Source of Variation	**Yield 2017 (India)**		**Yield 2018 (India)**	
Two-way ANOVA	Significance	Wald statistic	Significance	Wald statistic
Genotype	***	2.95	ns	1.33
Density	****	26.7	****	29.36
Genotype x Density	ns	0.97	ns	1.01

Source of Variation	**Yield 2019 (Senegal)**		**Yield 2020 (Senegal)**	
Two-way ANOVA	Significance	Wald statistic	Significance	Wald statistic
Genotype	****	7.8	****	4.02
Density	****	23.9	*	3.13
Genotype x Density	ns	1.3	ns	0.36

Table 2.1.1: Two way ANOVA table showing significance and Wald statistic for the yield obtained in two different density (HD, LD) in India in 2017 and 2018 and in the three different density (D1, D2, D3) in 2019 and 2020 in Senegal.

different genotype panel than in India, the HD treatment also increased WUE significantly. The average WUE for LD was 1.32 g biomass kg^{-1} water transpired and 2 g biomass kg^{-1} water transpired for the HD treatment (Fig 2.1.5C). A two-way ANOVA showed a slightly significant genotypic effect (p-value = 0.02), and a much stronger density effect (two-way ANOVA, p-value <0.0001), but no genotype-by-density interaction effect. Density more than genotype effect drove the WUE variation (Wald statistic values: $F_{density} = 50.9$, $F_{genotype} = 1.7$).

Soil water moisture is higher in the deepest part of the cores.

From the 2018 field trial in India, water content differences between high and low-density treatments were found for the cores sampled between 60 and 90cm. Soil moisture in this part of the profile was 19.57 % in HD, higher than the 10.78 % moisture found in the LD treatment (t-test, p-value= 0.02). The upper parts (0-30 and 30-60cm) showed not significantly difference between density treatments (Fig 2.1.6A). In the 2020 field trial in Senegal, the soil moisture measured in the first two profiles (i.e. 0-30 an 30-60cm) showed no significant differences between the three densities. In the deepest part (60-90cm), although differences were not significant, we observe the same tendency as in the trial in India of a higher soil moisture in the deepest part and for the highest density (D3) with 7.9% against 5.9% and 5.7% for D1 and D2 respectively (Fig 2.1.6B).

Discussion

The main results of the paper were: (i) that higher density increased biomass and/or grain yield in high VPD seasons; (ii) there was no or very limited genotype-by-density interaction in the response to density, indicating that no genotypic variation in the response to density; (iii) The positive response to density was limited to seasons with no light limitation (iv) Higher density decreased VPD in the canopy and increased WUE in all cultivars.

The response to higher sowing density in 2017 and 2018 field trials in India and in the 2019 field trial in Senegal was positive for a large majority of the genotypes tested. These trials were conducted during seasons characterized by high evaporative demand. Indeed the two trials in India were carried out during the hot and dry counter season and the 2019 trial in Senegal took place during a rainy season characterized by a long rain gap in the first 60 days with only two small rainfalls events (Fig 2.1.1). These results indicate that intensifying pearl millet productivity

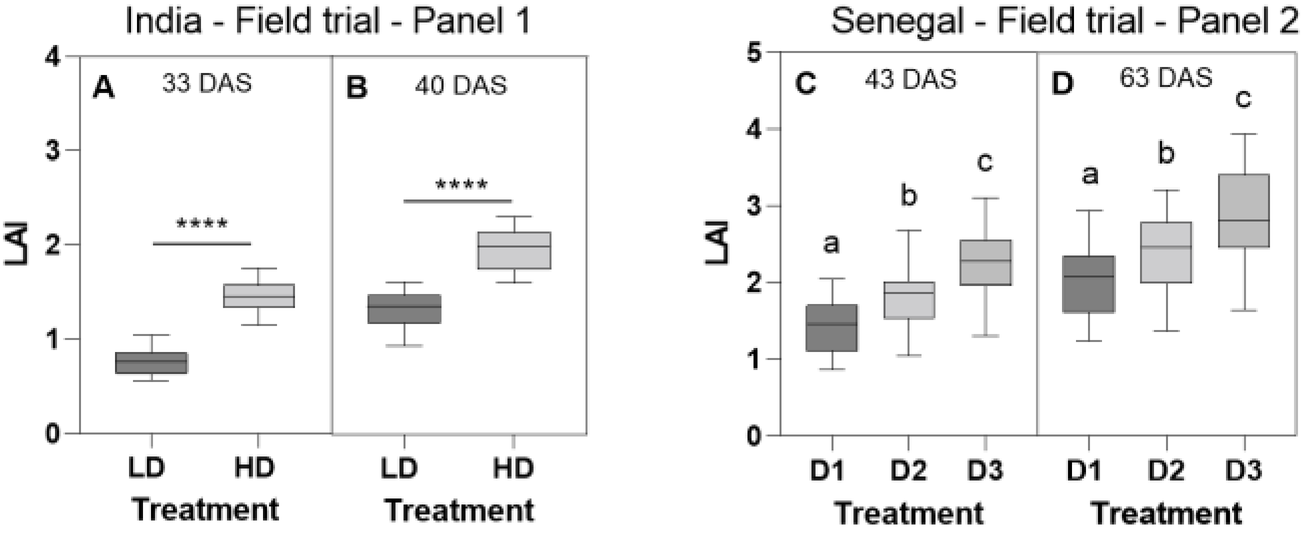

Figure 2.1.3: Leaf area index (LAI) measured at 33 DAS (A) and 40 DAS (B) in the 2018 field trial in India, and at 43 DAS (C) and 63 DAS (D) in the 2020 field trial in Senegal. Data are means and standard deviation of 20 genotypic means in India and 30 genotypic means in Senegal. Genotypic mean values were the average of 4 replicated plots in India and 3 replicated plots in Senegal, for each genotype-by-density combination.

should be possible using existing cultivars, and that this recommendation should target area with expected high evaporative demand. To our knowledge, this is the first report of a positive response to density in pearl millet. It is consistent with the positive response to density found earlier in maize (Mansfield & Mumm, 2014), or sunflower (Echarte et al., 2020), where the positive response to increased density was also associated with a high intercepted radiation.

Although there was a positive response to density, we showed no genotype-by-density interaction, indicating that there was no genotypic variation in the degree of response to density. These results indicated that, at least according to the response of the 50 cultivars tested in this work, there seemed to be no avenue to breed for density tolerance in pearl millet. This contrast with the earlier work in maize where they found genotypic variation in the response of hybrids to an increased density (Mansfield and Munns 2013). This also contrast with a recent work in sorghum where the authors found significant genotype-by-density interaction indicative of genotypic differences in the response to density (Pilloni et al, unpublished). The reasons for this lack of variation is unclear but could involve architecture traits related to light penetration in the canopy, as recently shown in maize (Perez et al., 2019). It could be that the high tillering ability of pearl millet and its usually narrow and droopy leave pattern may have limited the genotypic variation for light penetration, at least in the sets of genotypes that were considered. More work would be needed to search for genotypic variation in pearl millet for traits that have been found to influence the response to an increased density in other crops (Mansfield and Munns, 2013).

An environmental effect also seemed to alter the cultivar response to density, since the response to density in Senegal was positive in 2019 and negative in 2020. The 2020 season largely differed from 2019 in the number of days of rain that occurred during the trial, leading to more days with less light available for the crop in 2020. This was also supported by data collected from a meteorological station located 25 km south of the CNRA Bambey station (Roupsard et al., 2018) during 2019 and 2020 where the average radiation during the 2019 crop cycle was 334.3 $W.m^2.s^{-1}$ while the 2020 season was characterized by an average radiation of 304.2 $W.m^2.s^{-1}$. This relation between the number of rainy days and the radiation reaching the ground being supported by the literature (Az-Torres, 2017). This limitation may explain that plants grown in high density were experiencing an acute competition for light as this resource was less abundant due to the number of rainy days in 2020, and would explain the negative effect of higher densities in that season. Environmental importance in the choice of optimal density of planting in other crops being supported by literature (Guzman et al., 2019; Reddy et al., 2009).

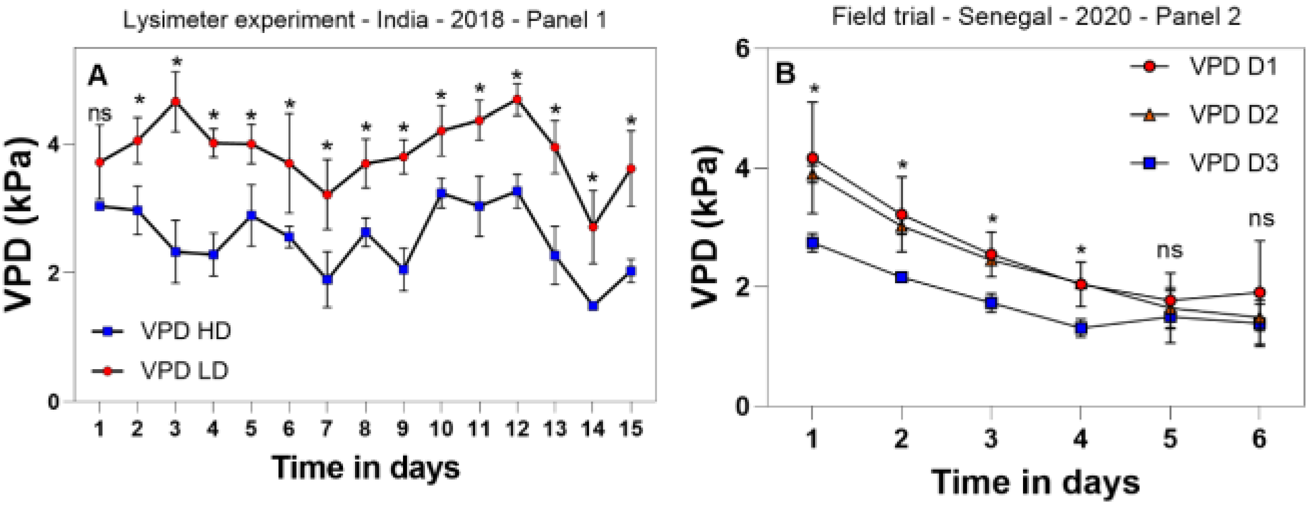

Figure 2.1.4: Vapor pressure deficit (VPD) measured on a 15 days period during vegetative stage in high and low density canopies in the lysimeter trial in India during the dry season (A) and in the 2020 field trial in the three different densities on a 6 days period before harvest in Senegal (B). Each data point is the average of data collected in three plots for each of the densities.

Together with those above, the results from this work tell us that an intensification of pearl millet production is possible in Senegal and in semi-arid regions of India, and that this recommendation should focus on areas where the evaporative demand is high and where there is no light limitation.

Our results from the lysimeter also showed that the small increase in water use due to higher density was proportionally lower than the benefit for the biomass. As a consequence, we showed a positive density effect on the water use efficiency in all the three trials. However, as in the field trials, no genotypes-by-density interactions were found, except for a slight interaction in the Senegal trial, indicating that no genotypes had any stronger WUE response to density than any other did. The lower VPD we measured within the high density canopy in the lysimeter trials likely explained the increase in WUE (Jauregui et al., 2018; Ryan et al., 2016) as there is a negative relation between the WUE and the VPD (Hatfield & Dold, 2019; T. R. Sinclair et al., 1984). The increase in LAI could explain this lowering of the VPD in the canopies (Gautier et al., 1999; Hu et al., 2008; Yang et al., 2014). This would imply that a substantial part of the incident light is able to penetrate inside the canopy and reaches lower level leaves. The fact there was no genotypic variation in the WUE response to density (no genotype-by-density interaction effect) suggest that light penetration would have been similar in all cultivars. We may speculate that, if the hypothesis is correct of a light penetration inside the canopy helps decreasing canopy VPD and increase WUE, genotypic variation in the response to sowing density in pearl millet could be found by searching cultivars with canopy architecture differences, using traits identified earlier in maize as a benchmark (Mansfield and Munns, 2013; Perez et al., 2019).

The VPD measurement also showed that differences in VPD occurred only above a certain air VPD threshold. Data from the field in 2020, suggests a VPD threshold around 2kPa below which the benefit of high density was lost. Therefore, we propose that in dry and hot condition with no light limitation, there is a comparative advantage for the crop to be planted under high density, which would then create a microclimate with lower VPD within the canopy that eventually contribute to increasing WUE. On the contrary, in season with lower light availability, leaves would be competing for light, letting little or no light reach the depth of the canopy where VPD is milder. These assumptions are comforted by the relation we found between the increases in WUE more positively related to the increase in biomass during the high VPD season in India and Senegal than the low VPD one in India. Indeed, the ratio of the WUE obtained in HD to the one obtained in LD plotted against the ratio of the biomass in the

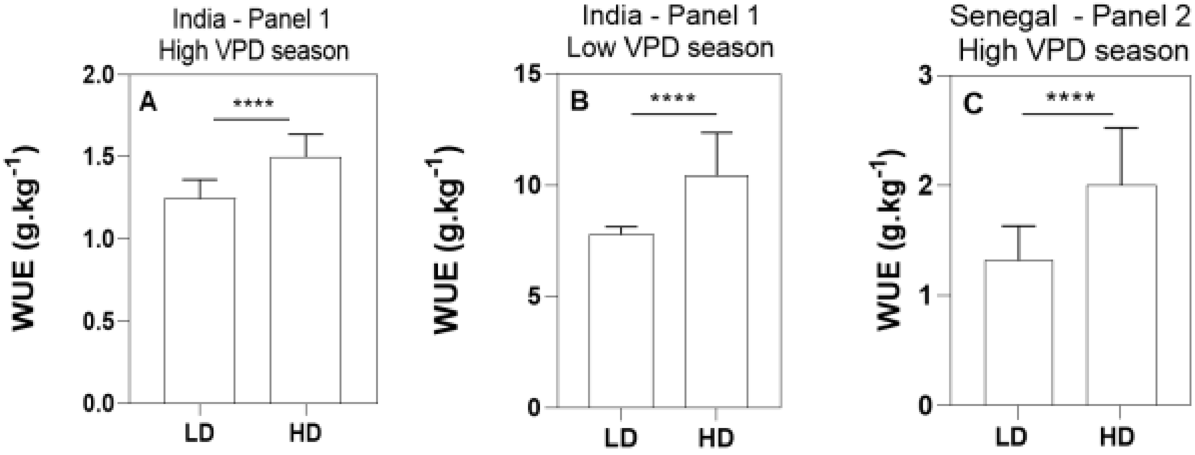

Figure 2.1.5: Water use efficiency (WUE) in grams of biomass per kilograms of water use of the 20 genotypes tested in the lysimeter platform in India in 2018 in both the dry (A) and rainy (B) seasons, and of the 30 genotypes tested in the Senegal lysimeter platform during the 2021 post rainy season (C). Data are means and standard deviation of 20 genotypic means in India (A, B) and 30 genotypic means in Senegal (C). Genotypic mean values were the average of 4 replicated plots in India and 3 replicated plots in Senegal, for each genotype-by-density combination

two density conditions (these ratios representing a change in both WUE and biomass variable due to density treatment) showed strongest correlation and significance in the high VPD season (r = 0.84, p-value < 0.0001 in India, r = 0.9, p-value < 0.0001 in Senegal) than in the low VPD one in India (r = 0.57, p-value < 0.01) (Figure 7). These relations are in lines with our interpretation that the higher WUE would also have more than compensated the higher water demand caused by the higher LAI. Other report also show an increase in WUE under higher sowing density (Echarte et al., 2019). However, no interpretation was made in this work of a possible VPD effect in these changes. This is also consistent with our soil cores measurements performed after harvest showing that the higher density treatments, both in Senegal and India, had not depleted deep soil water more than the low-density treatments. Rather, soil moisture was even higher in the 60-90cm layer under high density than under low density. Milder microclimate and the better WUE in higher plant stands could have explained part of these differences. While this higher soil moisture at depth that we found for the high-density treatments in India remains in part unexplained, they open the door for root system investigation in response to increased density.

Conclusion:

This work showed a positive effect of increased sowing density on the biomass and grain yield of pearl millet, in different location and genotypes. The benefit of higher density was maximum when the evaporative demand was high, suggesting an avenue to avoid atmospheric drought stresses, to the benefit of the water use efficiency of the crop and then of the yield. The strong environmental influence on the response of varieties already in use by farmers suggest the density have to be adapted according to areas and associated climate.

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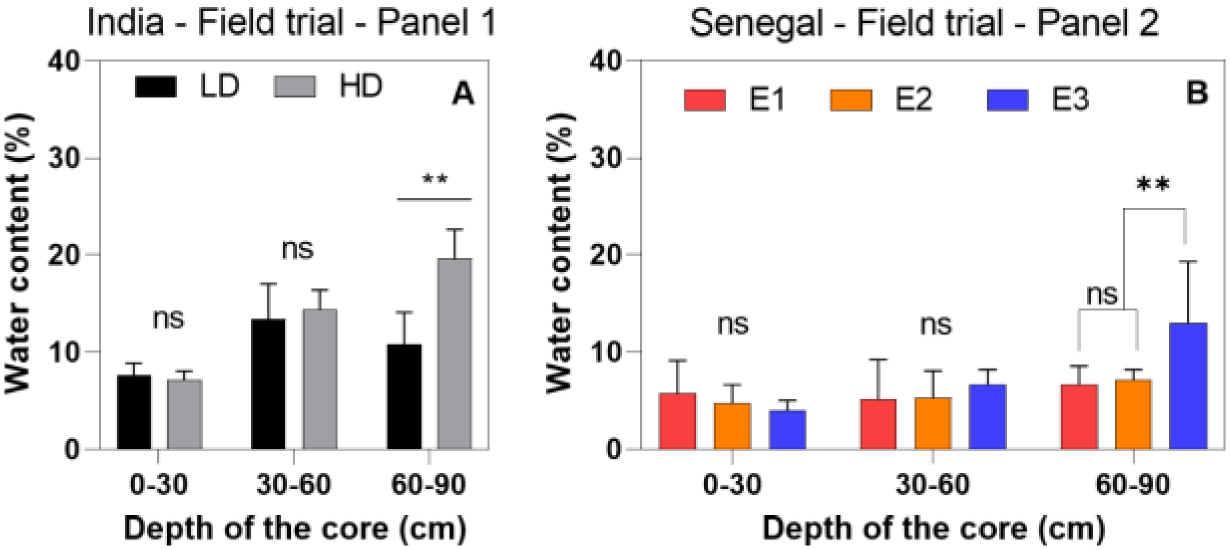

Figure 2.1.6: Percentage of soil moisture measured in three soil horizon profiles (0-30, 30-60 and 60-90cm) in the two density treatment tested in India (A) and the three different density tested in Senegal (B). Results were obtained through soil cores performed in the field immediately post-harvest. Means are average plus standard deviation of data collected in three plots for each of the density treatments.

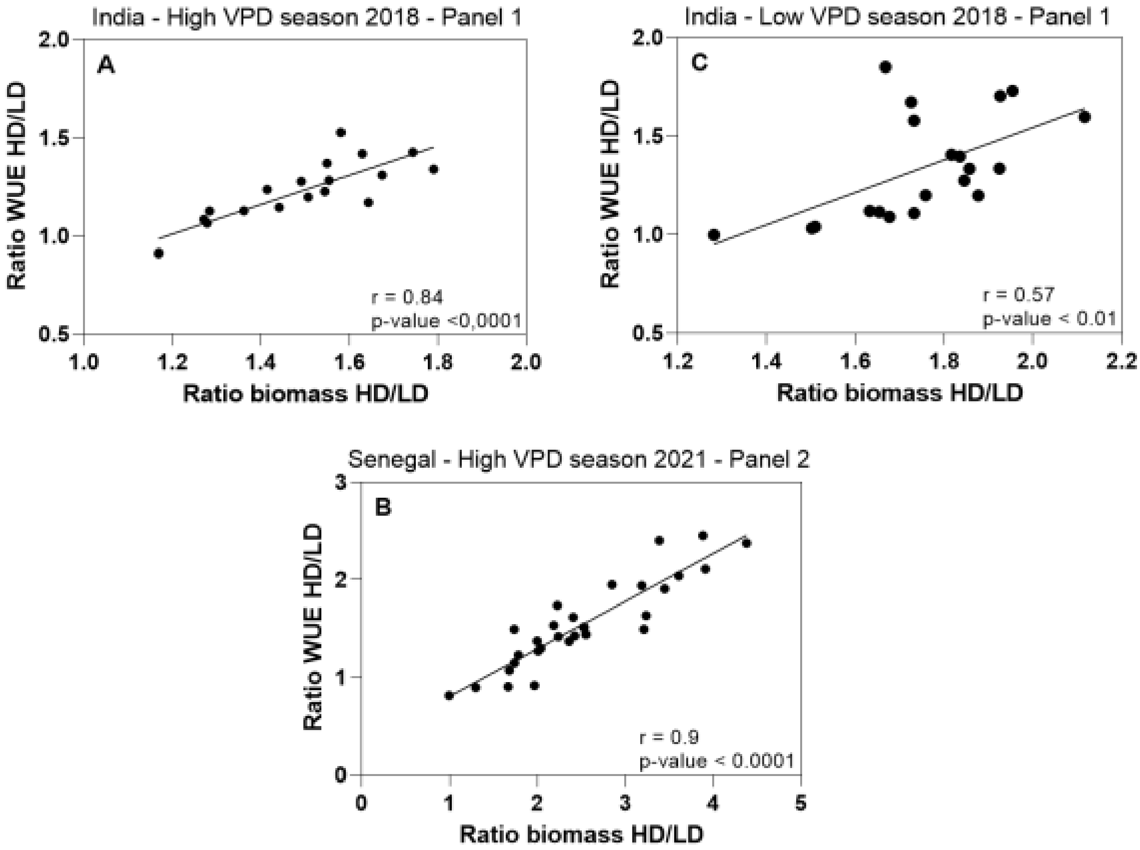

Figure 2.1.7: Correlation between the ratios of the WUE measured under high density to low density (WUE HD/WUE LD) as a function of the ratio of the biomass measured under high density to low density (Biomass HD/Biomass LD), using the data from the lysimeter trials carried out in India in 2018 and Senegal in 2021. Data shows positive and significant relation in both high VPD seasons in India ($r = 0.84$, p-value < 0.0001) (A) and Senegal ($r = 0.9$, p-value < 0.0001) (B) and also in the low VPD season in India ($r = 0.57$, p-value < 0.01) (C). Ratios were calculated from the genotypic means of WUE and biomass in each of the density treatments.

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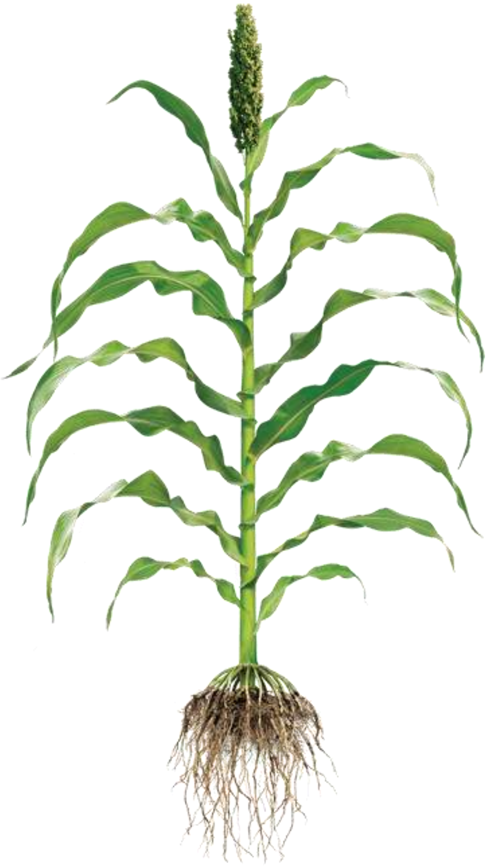
Chapter 2.2

Sorghum response to high sowing density varies genotypically and is related to an increase in water use efficiency.



Submitted in:

Field Crops Research
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Abstract

In semi-arid tropical areas, sorghum is traditionally sown at low plant densities but preliminary results suggest the possibility of a sustainable intensification by increasing the sowing density. However, an assessment of the extra water need associated with such intensification option is a pre-requisite in these drought prone areas. Doubling the conventional sowing density of commercial cultivars increased biomass and grain yields significantly and there was genotypic variability in the degree of response. These results were obtained applying the same amount of fertilizer and irrigation in both density treatments. No link was found with either the maintenance of the tillering capacity or with a differential increase in the leaf area index . Lysimeter experiments showed that high-density plantations had 62% higher biomass and only a 38% higher water use, resulting in a 17% higher water use efficiency (WUE). There was an appreciable genotypic variability in the degree of WUE increase. This work opens the door to intensification, in the short term by increasing the sowing density in dry land areas using cultivars that show a strong response to density. While the genotypic variation in the response to density was moderate, it was found in a very small panel of cultivars and calls for the search of additional variants towards the breeding high-density adapted sorghum cultivars.

Key Words: Sowing density, productivity, intensification, semi-arid tropics.

Introduction

Sorghum is a staple food in semi-arid regions of developing countries (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, an absolute necessity in the current demographic context of these regions (Godber & 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). However, higher densities (40-50 plant m^{-2}) can result in higher yield in fodder sorghum crops under temperate latitudes (Corleto et al., 1990). In Western large scale farming systems, extension services recommend a sowing density ranging from 26 to 35 plant m^{-2} according to the soil profile (Arvalis, 2020). Increasing sowing densities could therefore be a solution to increase sorghum production in tropical regions. Such benefit was indeed observed in dry areas (Andrade et al., 2002; Hatfield et al., 2001).

We have therefore explored to what extent an increase in the sowing density may also provide a comparative advantage in semi-arid regions, in spite of two risks associated with this technique. Firstly, evapotranspiration is usually high in these regions, and it increases with leaf area index, thereby potentially causing soil water depletion before then end of the crop season. Secondly, competition for light resource under high density potentially leads to a decreased tillering (Casal et al., 1986), although a large genetic variability was observed in the sensitivity of tiller number to density (Blanc et al., 2021). Hence, trade-offs need to be quantified between plant water use, leaf area, tiller number and biomass accumulation in response to increased plant density: we hypothesized that cultivars that, putatively, would have the most positive responses to plant density would be those able to optimize these tradeoffs, at least under certain environmental conditions. So far, no such work has been done on sorghum in dry land areas while benefits from adapted planting density was described in the literature for other species. This study consisted in several field and lysimetric trials with plants grown in different densities of sowing and using commercial hybrids commonly used by farmers in the semi-arid regions of India. In order to assess the risks abovementioned, the trials were conducted under equal fertilization and water regime in the two densities tested and under different evaporative demand conditions at the ICRISAT research station, Patancheru, India.

The objective of this study was therefore to investigate the consequences of an increased plant density on grain yield, biomass accumulation, total water used and water use efficiency in dry and rainy season conditions. In particular, we tested the range of genotypic variability in these

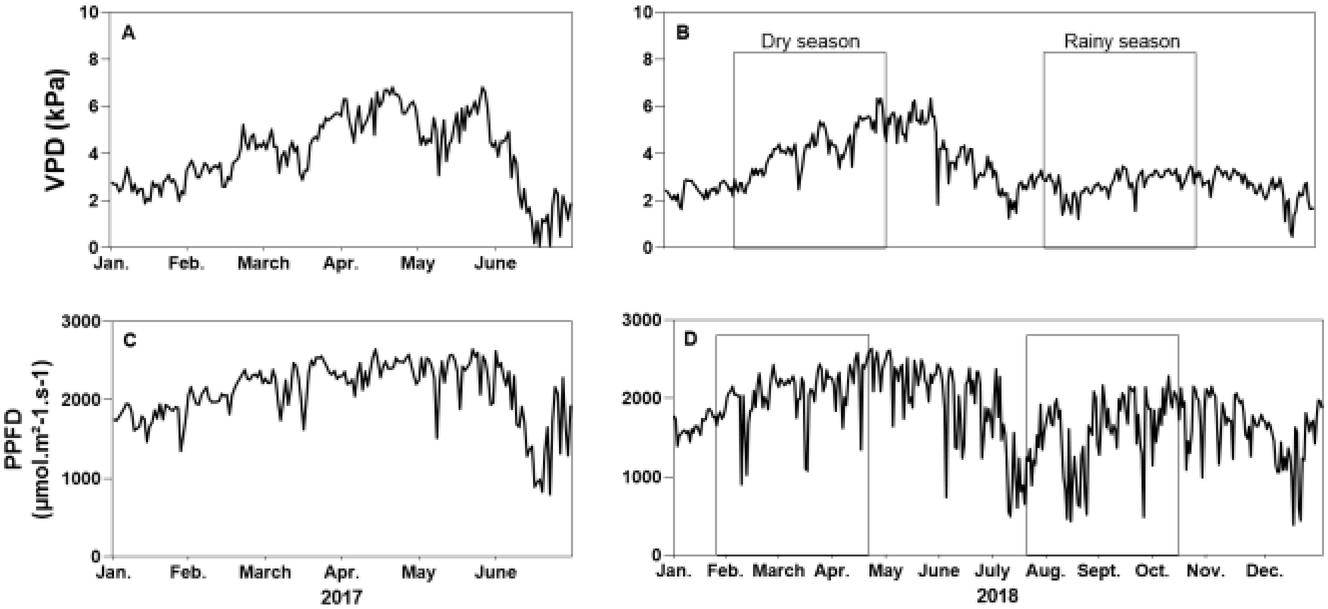


Figure 2.2.1: Daily vapor pressure deficit (VPD) and Photosynthetically active Photon Flux Density (PPFD) from ICRISAT meteorological station in the 2017 dry season (A, C) and the dry and rainy seasons of experiment in 2018 (B, D). Empty boxes correspond to the periods of lysimetric measurements in 2018.

responses and relationships between variables, using a small panel of sorghum cultivars. We also investigated the putative role of changes in the tillering ability and leaf area index in the degree of response of the crops in an increased sowing density.

Material and methods

The biological material used in this study consisted in a panel of 20 elite high-yielding sorghum genotypes from seed companies. 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). Two field experiments were carried out in 2017 and 2018, both during the dry season (February-May) characterized by a high evaporative demand and radiation condition. 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 (Figure 2.2.1). Lysimetric experiments were carried out on the same panel of genotypes to measure the effect of high density on soil evaporation, water use, and water use efficiency (WUE). The LysiField platform and design allowed a follow-up of the water use pattern over the growing period in both high and low density.

Genotypic response to density in the field

During the 2017 and 2018 dry seasons, field trials were conducted at the ICRISAT field facility (Hyderabad, India, 17°30 N; 78°16 E; altitude 549 m). The biological material used was a pool of twenty high yielding elite varieties and hybrids of sorghum coming mostly from the ICRISAT breeding program. Each of the twenty genotypes of sorghum was sown in a high density (HD) and low-density (LD) conditions. In HD condition, row-to-row distance was 30 cm, whereas in LD condition row-to-row distance was 60 cm. Plant-to-plant distance was 15 cm in both density treatments so that a density of sowing of 11 plants/m² and 22 plants/m² were reached in LD and HD treatment respectively. The field trials were fully irrigated and received 40mm of irrigation every week from sowing to maturity. These trials followed a fallow period during the previous rainy season.The trial was a randomized complete block design with density as the main factor and genotypes randomized within each block with three replications. A replicate consisted in a plot of 4 and 8 rows for the LD and HD treatments respectively, each row being 4 meters long. Seeds were manually sown (24th February 2017 and 22nd February

2018) at an amount exceeding the need for plant stand and, after emergence, the stand was thinned down to the right plant density. Just after sowing, the field was treated

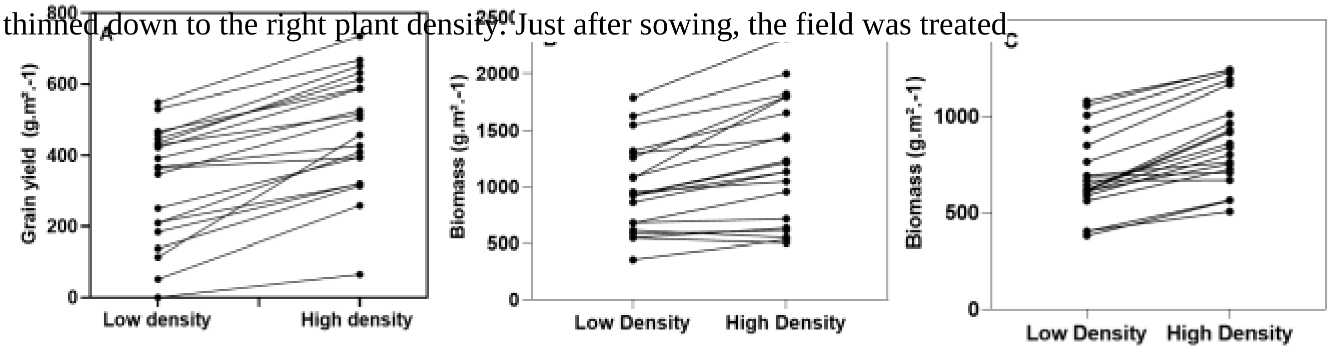


Figure 2.2.2: Grain yield (grams per m²) during the dry season field trial 2017 (A), and vegetative biomass accumulation (grams per m²) in 2017 (B) and the 2018 trial (C), in 20 genotypes grown under high and low density. Data points are genotypic means of the three replications in each treatment. Lines connecting dots shows the degree of response of the genotypes to the density treatment.

	Field trials				Lysimeter trials		
Two-way ANOVA	Biomass 2017	Grain 2017	Biomass 2018	Panicle no. Per m² 2017	LAI 2018	WUE Dry season 2018	WUE Rainy season 2018
Source of Variation							
Genotype	****	****	****	****	****	*	****
Density	****	****	****	****	****	****	****
Genotype x Density	***	ns	*	ns	ns	***	**
Heritability (H²)	0.7	0.22	0.25			0.67	0.78

Table 2.2.1*:* Analysis of variance of the different traits measured; biomass and grain yield, panicle number, water use efficiency (WUE), leaf area index (LAI) across the different trials (field and Lysifield) and year of experiment. Two-ways ANOVA shows strongly significant genotypic and density treatment effect. Genotype x Density interaction is significant for biomass and grain yield and WUE in the two seasons of experiment.

with herbicide (Atrazine, 1,5g/L, 1kg/ha). Before sowing, the field was fertilized with di-ammonium phosphate at a rate of 100kg/ha and top dressing with 100kg/ha urea occurred at four weeks after sowing in both high and low density treatment. A four meters wide border was set up around the field, and a border of two rows of plants between the different experimental conditions. In 2017, the experiment was conducted until harvest to measure vegetative and grain total dry biomass of the 20 lines in both HD and LD condition, and the number of panicle was counted at harvest as an estimate of the number of fertile tillers in the two treatments. In 2018, a storm forced us to harvest the entire field before maturity, at 79 days after sowing (DAS), and the total aboveground biomass was obtained after complete drying.

Leaf area index measurement in the field

In order to assess soil coverage in both high and low density canopies, the leaf area index (LAI) was measured in 2018 using a 1-meter long ceptometer (AccuPAR LP-80). Measures were taken at 40 days after emergence in both high and low density. The aim was to compare the genotypes' light interception profiles and infer their ability to protect the soil surface from solar radiation. In each plot, two measures from above the plants assessed the incident radiation on that particular day and moment of measurement. Then, four measures were taken from the ground, below the canopy, in different locations of the plot. For this step, the ceptometer was placed diagonally between two rows, in order to have a more representative measurement of the quantity of light on a square meter of the plot. The ceptometer measured the amount of photosynthetically active photon in $\mu mol.s^{-1}.m^{2\,-1}$ and converted the light quantity into leaf area index using the following formula: $LAI = \ln(\frac{I}{Io})/k$ where I is the incident light above the canopy Io, the light at ground level and k a crop extinction equal to 0.6 for sorghum crops.

Water budget response to density in lysimeter trials

In order to measure the effect of density on plant water use (WU) and on whole plant water use efficiency (WUE), two trials on a lysimetric platform were set up with the same 20 genotypes during the dry season (February to May) and the low VPD (August to October) 2018. The trials were performed at the LysiField facility of ICRISAT, equipped with a rain out shelter, at the ICRISAT experimental station. The twenty genotypes of sorghum were grown in lysimeters, consisting in tubes made of PVC and filled with alfisol collected from the ICRISAT farm. Each

Genotypes		Grain yield 2017 (g/m^2		Field stover yield 2017 (g/m^2)		Field stover yield 2018 (g/m^2)		WUE 2018 dry season (g-1.kg-1)		WUE 2018 rainy season (g-1.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	

***Table 2.2.2**:* Genotypic means of total aboveground biomass for both 2017 and 2018 field trials, leaf area index (LAI) and water use efficiency (WUE) in the two 2018 seasons of lysimetric experiment for the 20 genotypes studied. Grand mean, max, min, standard deviation, Wald statistic and probability for genotype (G), density (D) and interaction (GxD) of each traits are presented. Values are genotypic means of the three or four reps according to the trials.

tube was 1.2 m long and had a diameter of 20 cm. The tubes were placed in a pit over which a pulley system could easily move for weighing the lysimeters. Each tube had a steel collar at its top where an S-type load cell hanging from the pulley could be attached and tubes could be easily weighted by lifting them with a block chain pulley (see Vadez et al., 2011, 2014). Watering method was the same as described in the chapter 2.1. The twenty genotypes of sorghum were planted in four replicates on the experimental platform. A replicate consisted in four tubes. For the HD treatment, there was one plant in each of the four tubes (4 plants). For the LD treatment, only two tubes out of four were planted with one individual plant per planted tube, the two other tubes remaining empty (2 plants). The trial was also a randomized complete block design with density as the main factor and genotypes as a sub-factor randomized within each block with three replications. This way, the design created a LD and HD canopy with a density of 10 and 20 plants per m² respectively, similar to the field conditions. Fifteen days after sowing, water was added in the tubes to bring them to field capacity. The tubes were left to drain for about 24h to reach their field capacity. Tubes were then weighted weekly, which allowed to measure plant evapo-transpiration (ETr), which was expressed in L per replicate (i.e. L/4 tubes) and converted into mm. During the rainy season experiment, within a replication of four tubes, half of the tubes were covered with plastic beads in order to prevent evaporation, while the other half was not. In this way, transpiration and evapotranspiration could also be measured from the tubes with and without beads, giving the opportunity to measure potential soil evaporation savings.

Plants were harvested 79 days after sowing (DAS) and dried for one week in an oven at 60°C. The dry weight obtained allowed to measure the water use efficiency (WUE) and transpiration efficiency (TE) when beads was applied in the tubes for each replicate (*WUE/TE = Total biomass (g) / Total Water Use (kg)).*

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 (H²) was computed as in Falconer et al, 2005 with $H^2 = \frac{\sigma p^2}{\sigma g^2}$ where σp^2 is the phenotypic variance and σg^2 the genotypic variance plus residuals.

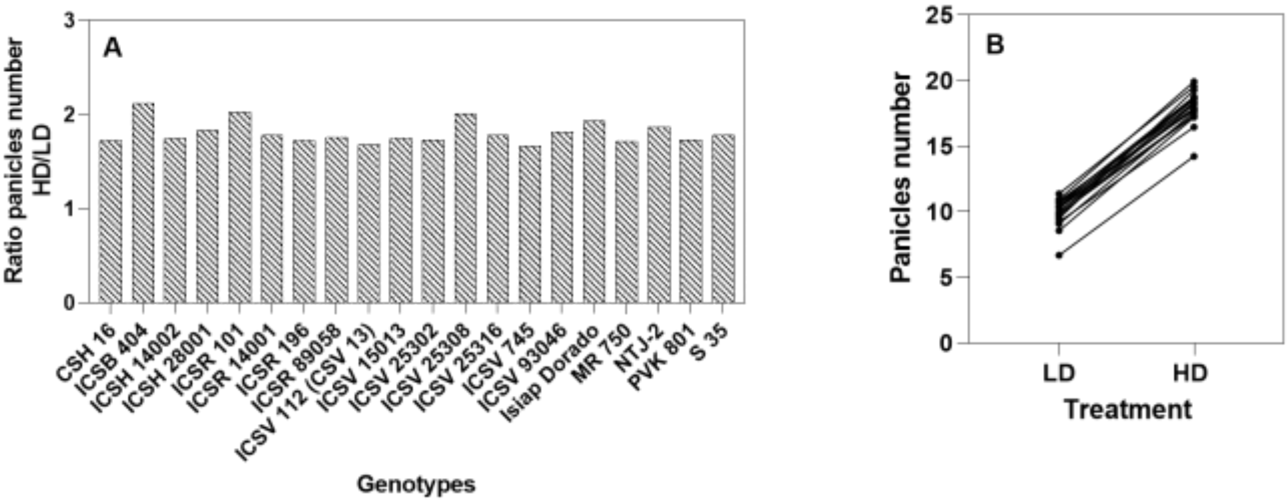


Figure 2.2.3: (A) Ratio of the panicles number counted in HD to LD treatments, (B) and panicle number in high and low density. Data points are genotypic means of the three replications in each treatment. Lines connecting dots shows the degree of response of the genotypes to the density treatment. Data are from the 2017 field trial.

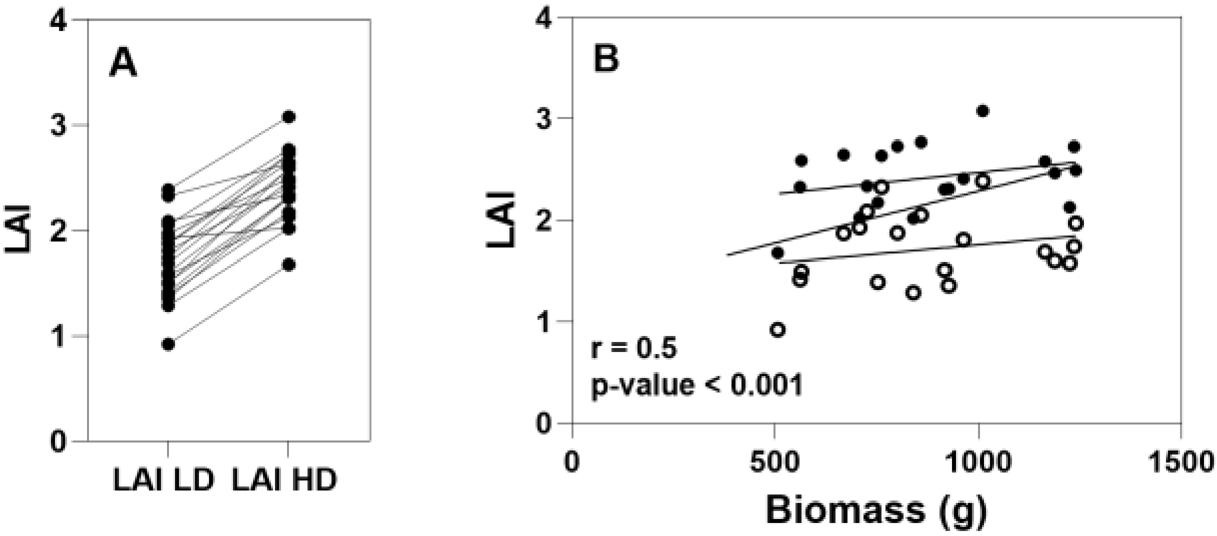


Figure 2.2.4: Total aboveground biomass of the 20 genotypes in high and low density (A) and relationship between biomass and the leaf area index (LAI) (i.e. HD, filled symbols and LD, empty symbols) (B). Significant and positive relation (r = 0,5, p-value < 0,001) was found. (B) Ratio of high density (HD) biomass to low density (LD) biomass plotted against the ratio of LAI measured in HD to LD (r =0.12, p-value=0.6). Dry season 2018 field trial.

Results

High density increased the biomass production in most genotypes, with no specific effects of tillering nor leaf area index

The 20 studied lines had higher yield and biomass accumulation under high than low density, for both the 2017 and 2018 experiments. In 2017, the increase in grain yield with plant density ranged from 8.4 % to 93 %, but this corresponded to an essentially constant absolute advantage for all studied genotypes, regardless of yield in low density (insignificant genotype by density interaction for the grain yield, Fig. 2.2.2A, Table 2.2.1 & 2.2.2). The aboveground biomass also positively responded to plant density in nearly all studied genotypes in 2017 and 2018 (Fig. 2.2.2 B and C), but the degree of response of genotypes differed and then showed a significant genotype-by-density interaction (Table 2.2.1). The heritability for biomass accumulation 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 1.65 to 2 for all except three genotypes, (Fig. 2.2.3 A) thereby suggesting a decrease in tillering at high density. However, the absolute increase in panicle number was similar for all genotypes (Fig. 2.2.3 B) and panicle number showed no genotype-by-density interaction, thereby indicating no genetic variability in the response of tillering to density treatment. Furthermore, panicle number had no effect on yield as the relation between the increase in panicle number and the increase in biomass from a density to another was not significant (p value 0.12, data non-shown).

Leaf area index (LAI) ranged from 0.92 to 2.39 in LD and from 1.67 to 3.08 in HD treatment (Suppl. Table 2.2.1). The increase in LAI with plant density was similar in almost all genotypes (Fig. 4 A), shown by the absence of a significant genotype-by-density interaction for LAI (Table 2.2.1 and 2.2.2). Higher LAI correlated with higher biomass (Fig. 2.2.4B). However, there was no specific effect once plant density was taken into account ($r = 0.3$, p-value > 0.18 and $r= 0.33$, p-value > 0.15 for HD and LD respectively).

WUE increased under high density and drove the genotypic biomass response

Water use was higher in high than in low sowing density: 607 ± 13 mm and 431 ± 5 mm in the HD and LD canopies respectively during the dry season (Fig 2.2.5A) and 166 ± 8 mm and 122 ± 3

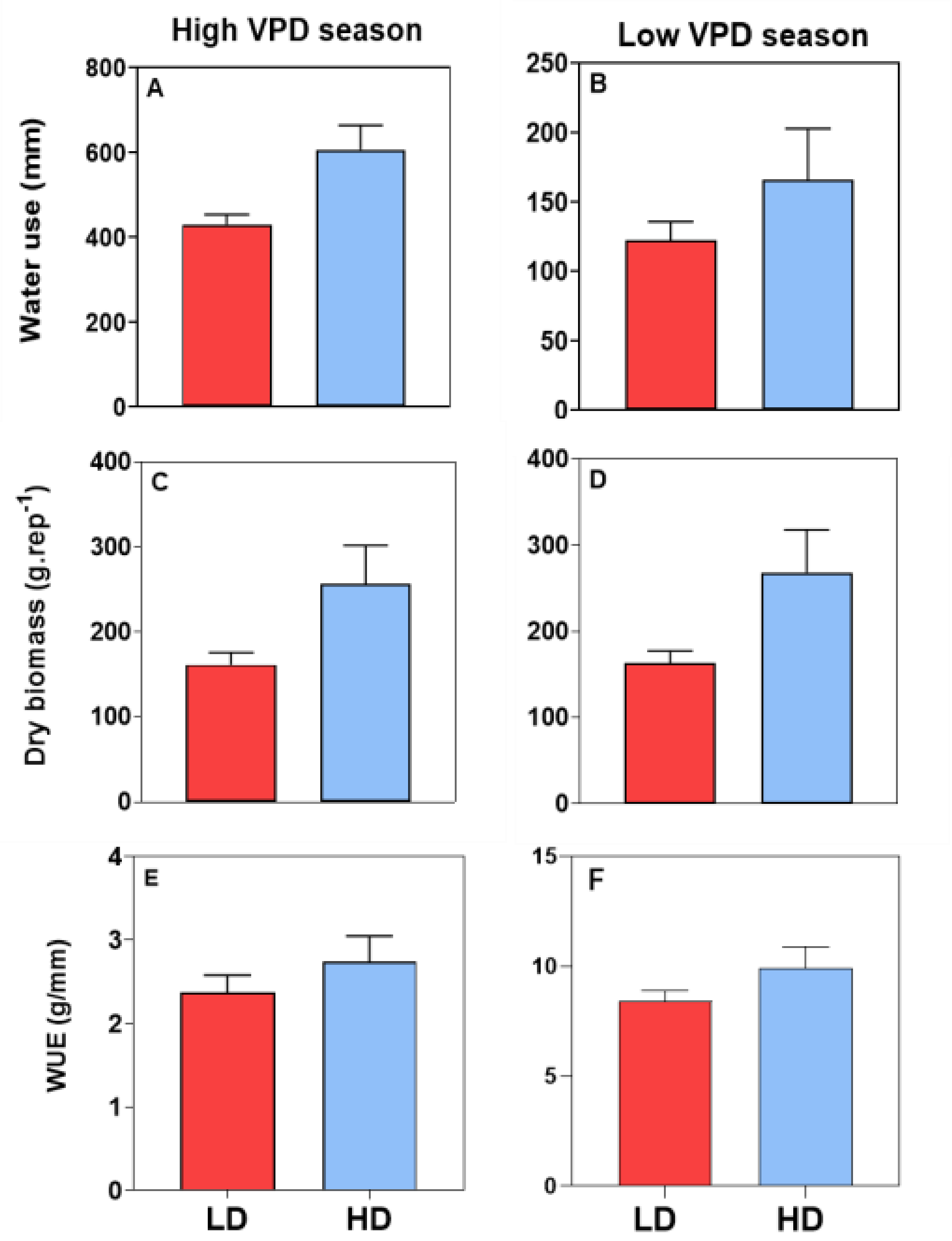


Figure 2.2.5: (A,B) biomass accumulation (grams of biomass per replicate) (C, D) total water use (E, F) water use efficiency (WUE, grams of biomass per kilograms of water used) under high (HD) and low (LD) density in dry and rainy season for the 20 genotypes grown on the lysimeter platform in 2018. Values are the genotypic means of the four replications.

mm for HD and LD treatment respectively in the rainy season (Fig. 2.2.5B, two-way ANOVA, P value <0.0001) as an average on the 4 replications of the 20 genotypes studied.

Biomass also significantly increased under high sowing density in both seasons (Fig. 2.2.5 C & D). This increase in biomass was higher than the increase in water use (65% vs 41% for the dry season and 59% vs 35% for the rainy season respectively). Therefore, WUE was higher in the HD than in the LD treatment ($p<0.0001$) in both the seasons (Fig.2.2. 5E&F), Table 2.2.2). Direct soil evaporation represented a small proportion of it, as it represented 42 mm of the LD water use from empty pots directly subjected to light, vs 431 and 609 mm for LD and HD canopies

respectively (Fig. 2.2.6 A). In addition, the treatment with beads on the top of the pot during the rainy season did not allow showing significant differences in the total water use compare to the pots without beads (Fig. 2.2.6 B). However this water saving allowed to increase slightly (but significantly, p-value <0,0001) the TE of the plants grown with beads on the top of the pots compare to the WUE measured in the pots without beads passing from WUE = 8.4 to TE = 9.2 g.kg in low density and WUE = 9.9 to TE = 11.25 g.kg in high density treatment. This increase in high density represents a 3% increase for TE compare to WUE (Fig. 2.2.7).

The increase in WUE was genotype dependent during both the rainy and dry seasons. Indeed, a significant genotype-by-density interaction on WUE was detected during both seasons (Table 2.2.2), similar in magnitude as the genotypic effect, indicating that the response of WUE to plant density differed between genotypes, and more so in the dry season. Confirming this, the ratios of WUE in the two treatment ranged from 0.96 to 1.39 in dry season and from 1.03 and 1.36 during the rainy season ($p< 0.0001$). Interestingly, the ratio of the biomass under HD to LD showed a strong and positive correlation (p value <0.0001, r = 0.91) against the ratio of WUE under HD and LD during the dry season (Fig. 2.2.8 A). In other words, the more WUE increased under high density, the more biomass did. Oppositely, during the rainy season, the reverse relation was found with a significant and negative regression between the increase in WUE and the increase in biomass. The significance of the relation was however less important than in the dry season (p value =0.02, r = - 0.48) (Fig 2.2.8 B).

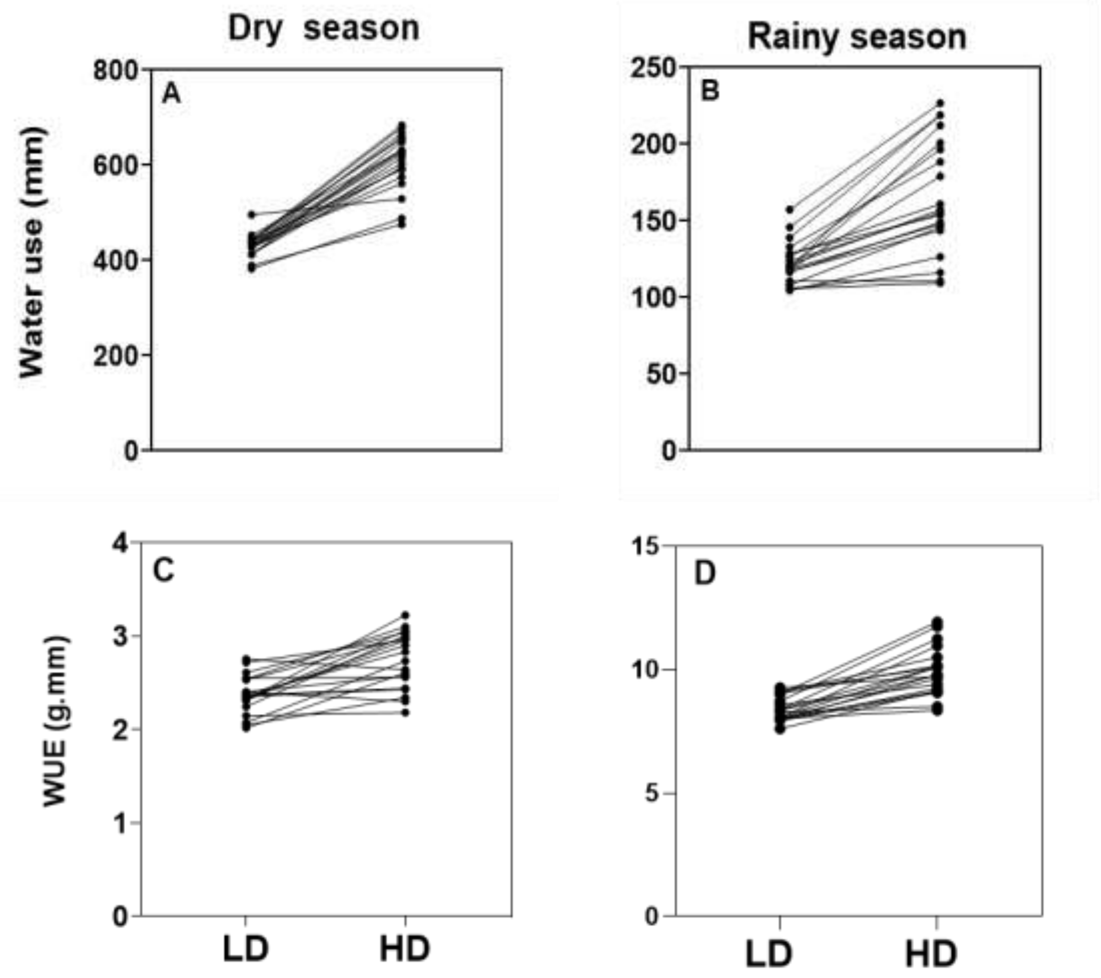


Figure 2.2.6: Water use in dry (A) and rainy (B) season and WUE in dry (C) and rainy (D) season for each of the 20 genotypes tested in India in both high (HD) and low (LD) density treatments. Lines connecting dots highlights the degree for these traits in the panel tested. Values are means of the four replications.

Discussion

Our results showed that grain yield and biomass increased for most genotypes when cultivated under higher sowing density. This opens 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 agronomic management as in the regular density (same fertilization, irrigation and phytosanitary products amount and application). This would be possible by just adapting the crop management, using existing varieties that showed the strongest response to density.

The biomass production, responded positively to an increase in density in a manner that differed across genotypes. So far, no breeding program has purposely taken the tolerance to higher density as a breeding target and the literature existing on the reaction to density for sorghum dealt with much lower densities than the one in the present paper (Berenguer & Faci, 2001; Tang et al., 2018). Others studies found no effect of density on biomass accumulation and/or on yield (Carmi et al., 2006). Our results then showed a positive response with a genotypic variation in the degree of response to an increased density. This propensity to increase the yield while increasing the density of planting has been shown in other species like maize and sunflower (Barbieri et al., 2012; Hernández et al., 2020). In high tillering cereals, some studies show that the ability to maintain tillering was necessary for good 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) it was therefore an aspect to take into account in order to estimate its importance in the response. In the current study, the response to density was not driven by tillering (Fig 2.2.3).

The experiments on the lysimeter platform were set up to quantify the water budgets and assess putative effects on the water budget and on the water use efficiency of the crop due to density treatments. The biomass response to higher density in the lysimeters also showed genotypic variation and confirmed the field results. The strength of these experiments was in showing that the accumulation of biomass was proportionally more important than the increase in water use, resulting in higher WUE in the HD treatment. This is consistent with recent results in maize showing that WUE was also increased under higher sowing densities (Hernández et al., 2020). However, in this maize study the densities were lower than in our trials, their higher densities corresponding to our low densities (10 plants/m²). Another recent study in sunflower also showed an increase in WUE under higher sowing density (grain and oil). There, the authors interpreted that the gain in WUE was attributed to the protection of the soil from solar radiation

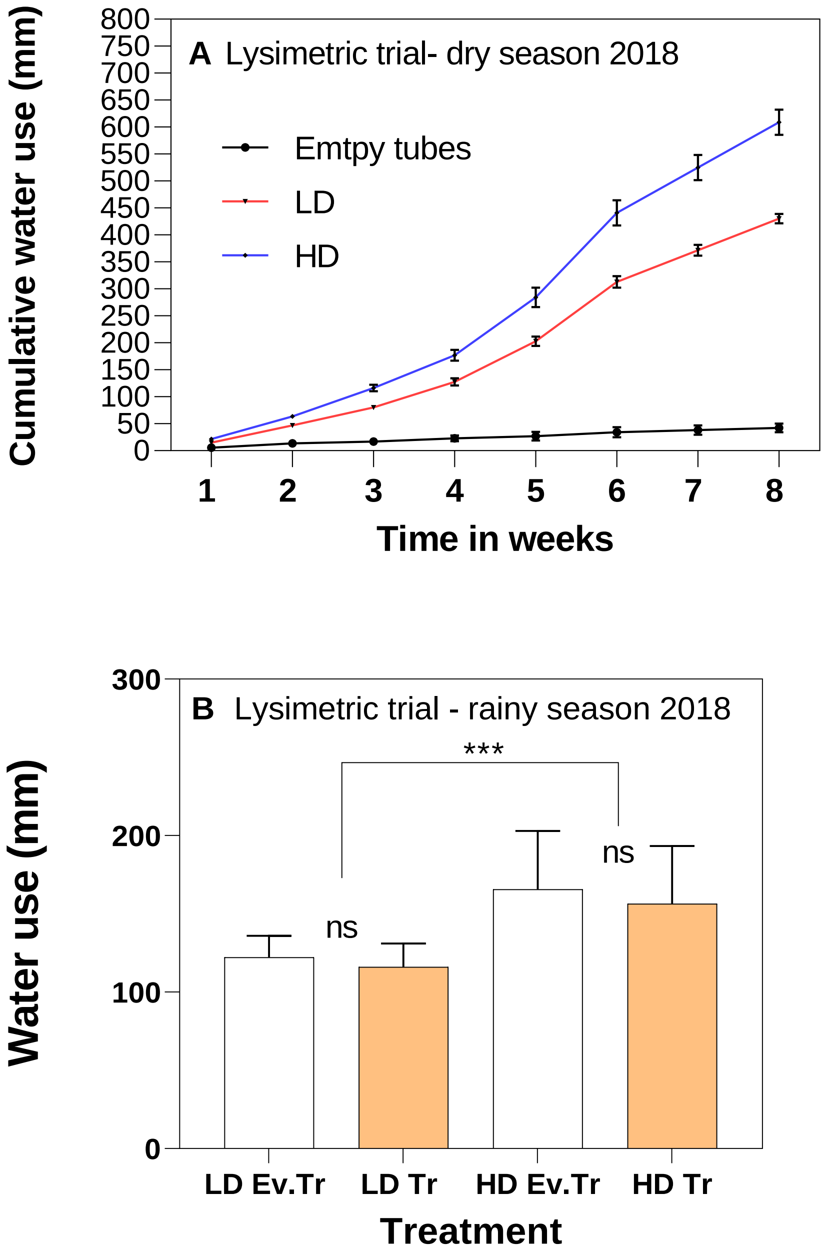


Figure 2.2.7: (A) Sum of the total water use of the High-density tubes (average of the 4 tubes of the replication), the low-density tubes (only the two tubes filled with plants) and the empty tubes from low density at harvest during the dry season trial. (B) Total water use of the crops in both high and low density comparing the treatment with (evapotranspiration, white bars) and without beads on the top of the pots (transpiration, orange bars), showing no significant differences in the total water use.

(Echarte et al., 2020). Here, we demonstrated that the part of water saved by soil coverage was small and contributed only 3% of the increase in TE compare to WUE.

In these recent work in maize and sunflower, the authors also made the hypothesis of a better light interception in sunflower to explain the increase in WUE (Echarte et al., 2020). However, they did not consider the possibility that a higher WUE could have occurred if the photosynthesis of that additional intercepted light had occurred under more favorable vapor pressure deficit (VPD). This would then agree with our recent results showing an increase in WUE in lines that also allowed light to penetrate deeper inside the crop canopy after canopy closure, where lower VPD than in the air were also recorded (Pilloni et al., 2022, under review). This could also explain the smaller density effect observed in the 2018 trial, which was stopped prematurely soon after canopy closure, and when the genotypic effects in light penetration would start being the strongest. These studies suggest physiological response involved in the genetic variability for increased WUE under high sowing density, suggesting a link between the increase in WUE and the lowered evaporative demand recorded in high density.

These results opens the possibility to increase the sowing density of existing varieties that most positively responded to an increased density. In the mid-term, the genotypic variation in the biomass response to density we found in this relatively small panel of cultivars 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.

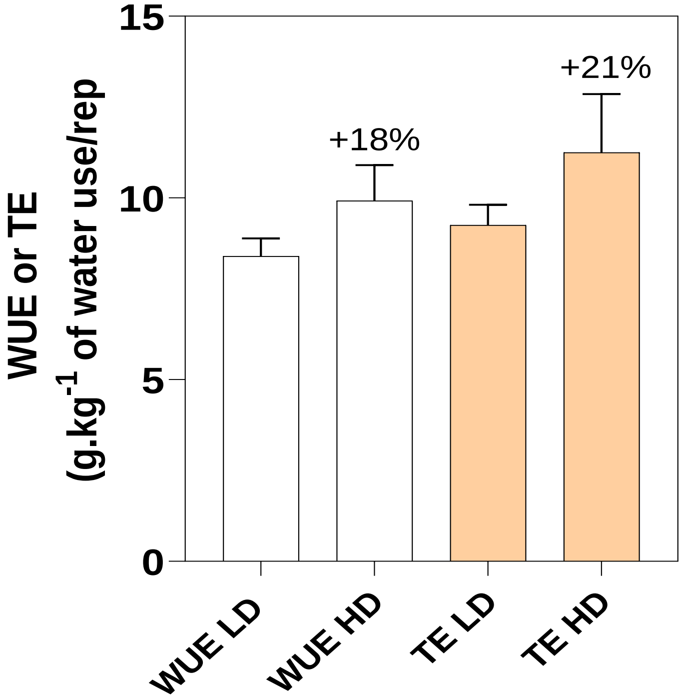
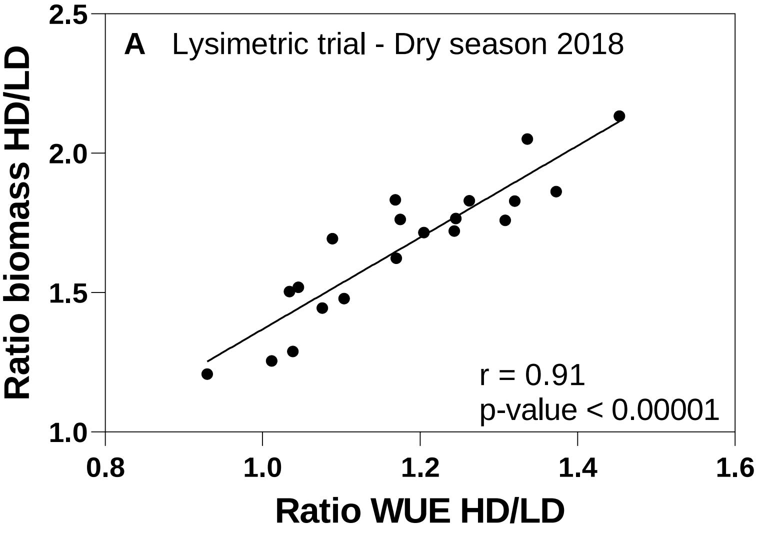


Figure 2.2.8: Water use efficiency (WUE) measured in the pots without bead at the top (white bars) and transpiration efficiency (TE) measured in the pots with beads at the top (orange bars) in both high and low density during the 2018 rainy season lysimetric trial.

Genotypes	Ratio WUE HD/LD rainy season	Ratio WUE HD/LD dry season
CSH 16	1.23	1.03
ICSB 404	1.32	1.01
ICSH 14002	1.16	1.19
ICSH 28001	1.19	1.09
ICSR 101	1.36	1.14
ICSR 14001	1.1	1.23
ICSR 196	1.25	1.29
ICSR 89058	1.34	0.96
ICSV 112 (CSV 13)	1.20	1.32
ICSV 15013	1.03	1.27
ICSV 25302	1.04	1.17
ICSV 25308	1.14	1.32
ICSV 25316	1.17	1.39
ICSV 745	1.15	1.34
ICSV 93046	1.09	1.22
Isiap Dorado	1.12	0.96
MR 750	1.35	1.07
NTJ-2	1.05	1.00
PVK 801	1.20	1.03
S 35	1.10	1.16
Mean	1.18	1.16
One sample t-test	****	****

Suppl. table 2.2.1: Change in water use efficiency (WUE) proxies by the ratio of the two densities values in the two seasons of experiment for the 20 genotypes studied.



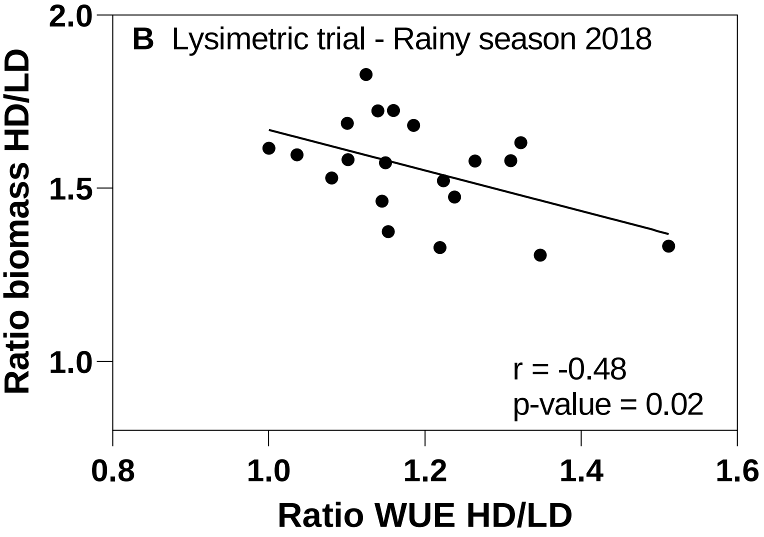


Figure 2.2.9: Simple linear regression of the ratio of the biomass accumulated under high density to low density (biomass HD/LD) and the ratio of the WUE under high density to low density (WUE HD/LD) during the summer season 2018 (r= 0.91, p-value <0.0001).

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Chapter 3.1

Transpiration and water use efficiency of sorghum canopies have a large genetic variability and are positively related under naturally high evaporative demand.

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Abstract

Indoor experiments with individual plants often show that transpiration rate is restricted under high vapor pressure deficit (VPD), resulting in a plateau of transpiration that increases water use efficiency (WUE) of some genotypes. We tested this hypothesis outdoors during dry or rainy seasons of India and Senegal, based on the response of the transpiration of canopy-grown sorghum plants to the reference evapotranspiration that takes both light and VPD into account. This response showed no plateau at high evaporative demand in 47 genotypes, but a large genetic variability was observed for the slope of the relationship over the whole range of evaporative demand. Unexpectedly, this slope was genetically correlated with WUE in two experiments with high evaporative demand: genotypes that most transpired had the highest WUE. Conversely, a negative correlation was observed under low evaporative demand. Genotypes with high WUE and response to evaporative demand were also those allowing maximum light penetration into the canopy. We suggest that this caused the observed high WUE of these genotypes because leaves within the canopy had sufficient light for photosynthesis whereas we observed a lower VPD in the canopy than in open air when leaf area index reached 2.5-3, thereby decreasing transpiration.

Key words: Transpiration, water use efficiency, genetic variability, vapor pressure deficit, light penetration

Introduction

Water use efficiency (WUE) tends to decrease with vapor pressure deficit (VPD) because transpiration, but not photosynthesis, increases with VPD (Condon et al., 2002, 2004; T. R. Sinclair et al., 1984). A mechanism used by plants to avoid such a decrease is stomatal closure at high VPD, which affects transpiration more than photosynthesis because of the non-linear relation between these two variables (Farquhar & Sharkey, 1982). Hence, restricting transpiration under high VPD theoretically increases WUE, especially during hottest hours of the day, provided that no confounding effects occur. Indeed, genotypes that most close stomata during the afternoon were reported to have highest WUE (Sinclair et al., 2005, Vadez et al., 2014). These analyses have triggered research on the genetic variability of the transpiration restriction under high VPD (Fletcher et al., 2008; Vadez et al., 2014). Genetic variation in the transpiration response to increasing VPD was observed in several species such as soybean (Fletcher et al., 2008), wheat (Schoppach & Sadok, 2012), pearl millet (Choudhary et al., 2020; Kholová et al., 2010), maize (Choudhary et al., 2020; M. Gholipoor et al., 2013), sorghum (Choudhary et al., 2020; Manoochehr Gholipoor et al., 2012) and rice (Affortit et al., 2022). A genetic variability for WUE was also described in sorghum (Donatelli et al., 1992, V. Vadez et al., 2011; Xin et al., 2009), and was interpreted as a consequence of the plant ability to restrict transpiration in response to evaporative demand, thereby generating a plateau of transpiration rate at high VPDs (Hatfield & Dold, 2019; Thomas R. Sinclair et al., 2005).

Intriguingly, plateaus of transpiration under increasingly high VPD were essentially observed in growth chamber experiments, with constant light at intensities usually lower than 500 µmol m^{-2} s^{-1}. Yet, light intensity outdoors is often much higher, e.g. in sahelian conditions with light intensity higher than 2000 µmol m^{-2} s^{-1} (Adeniji et al., 2020), and fluctuates in the course of the day. Results for transpiration under naturally occurring light and VPD did show a trend for a plateau (e.g. Fig.9B in Vadez et al., 2015), although not in all days. Smoothened transpiration profiles also showed a transpiration restriction in some wild chickpea cultivars (Kar et al., 2020), although not as clear as in the theoretical figure 1 of Sinclair et al., (2005). A specific difficulty occurs when examining the relationship between transpiration and evaporative demand in naturally fluctuating light conditions. Transpiration is indeed driven by light intensity and wind in addition to VPD, resulting in the reference evapotranspiration (ETref) as computed with the Penman-Monteith equation (Zotarelli et al., 2014). Because VPD and light intensity are often correlated during a day and between sites, several field studies considered VPD only as the driving variable of the evaporative demand (e.g. Schoppach et al., 2017). This assumption may be acceptable in regions in which no massive light variations occur between days and fields during the crop cycle e.g. during dry seasons in India (Kar et al., 2020), but less so in the opposite case. Hence, the genetic variability of the response of transpiration to the evaporative demand needs to be analyzed in natural conditions with light variations, in addition to variations of VPD. We developed a method for that and applied it to two panels of genotypes in three environmental scenarios, namely the dry and wet season in the drylands of India and a dry season in the Sahel of Senegal.

Another difficulty for translating the analyses of the genetic variability of transpiration rate from growth chamber to field is associated to the vertical variations of light and VPD and little studied (Geetika et al., 2019) . Plants in growth chambers have all leaves exposed to similar light and VPD (predominance of diffuse light, low competition for light and similar VPD at all altitudes in the growth chamber). This differs from plants grown in canopies, where the leaves inside the canopy are expected to be subjected to a lower VPD than the upper leaves because of canopy transpiration, and to a lower light because of self-shading. Provided they receive sufficient light, leaves inside the canopy, with partly closed stomata because of lower light and facing a milder VPD than the upper leaves, may have a higher WUE than upper leaves. The proportion of incident light that reaches the lower parts of the canopy, an essential feature under this hypothesis, has a clear genetic variability (Yin & Struik, 2015) related to plant architecture (Niinemets, 2010), plant density (Song et al., 2013) and the vertical distribution of leaf area (Perez et al., 2019).

We therefore aimed at evaluating to what extent the genetic variability of the transpiration response to evaporative demand and that of WUE, demonstrated in indoor experiments, is also appreciable under natural conditions for two panels of sorghum genotypes grown under high light and high or low VPD in Senegal and India. For that, we examined the response of each studied genotype to evaporative demand, calculated via a reference evapotranspiration (ET_{ref}) taking both VPD and light into account, rather than via VPD alone as in previous studies (Fletcher et al., 2008; Kholová et al., 2010). In order to better understand this genetic variability, we tested if the VPD inside the canopy appreciably differed from that in the air in canopies with different leaf area indices, which were obtained by setting up different plant density treatments, over several days and at different times of the day. We finally explored to what extent WUE differences were linked to light penetration inside the canopy.

Material and method

Genetic material, experimental design and growth conditions

A panel of 20 elite hybrids of sorghum provided by seed companies was used in experiments in India (panel A), and a panel of 27 lines from the germplasm collection of ICRISAT was used in all other experiments (panel B). The field experiments in Senegal involved the whole panel B, while sub samples of nine and two lines of the latter panel were analyzed in the indoor lysimetric platform and greenhouse experiments, respectively (see below). A first set of experiments was carried out in the field lysimetric platform (LysiField) of the ICRISAT station (Hyderabad, India, 17°30 N; 78°16 E; elevation 549 m) during the dry (February to May) and rainy (August to October) seasons of 2018, which largely differed for VPD (4.7 vs 2.8 kPa in average) and light intensity (2217 vs 1545 µmol m^{-2} s^{-1}), (Fig. 1). The platform consisted of large pits in which PVC tubes (20 cm diameter,1.20 m length) were arranged side by side and filled with alfisol. A S-type load cell system set up and a block-chain pulley allowed the tubes to be lifted and weighed every 7th-10th day. Direct soil evaporation was prevented via beads located on the soil. Each replicate consisted of a set of 4 tubes arranged in square, all tubes carrying the same genotype. Four replicates were used in both experiments, organized as a complete randomized block design with density as the main factor and genotypes randomized. In the high density treatments, each tube carried one plant, vs one tube out of two in the low density treatments leading to plant densities of 20 and 10 plants m^{-2}. A second experiment took place in the field lysimetric facility of the ISRA/CNRA station (Bambey, Senegal, 14° 41 N; 16° 27 W, elevation 20 m) during the 2021 dry season (March to June 2021) characterized by high VPD and light (4.9 kPa and 2068 µmol m^{-2} s^{-1} on average) (Fig 3.1.1). The platform had the same characteristic as the Hyderabad platform with small differences: tubes were 25 cm in diameter and 1.50 m long, they were filled with a sandy soil and soil evaporation was prevented via gravels located on the soil. The experimental design was the same as above, except that high and low plant densities were 16 and 8 plants m^{-2}, respectively. Two field experiments were carried out near the lysimeter experiments in India and Senegal, with panels A and B, respectively, with two plant densities. The field experiment in Senegal was carried out at the CNRA Bambey station. Microplots (2 m wide, 4 m long) were sown with one genotype each, with three reps per genotype. The low density plots harbored 11 plant m^{-2} consisting in 4 plant rows, 15 cm between plants within a row, and an inter row spacing of 60 cm. High density (HD) plots harbored 22 plants m^{-2} with an inter row spacing of 30 cm and the

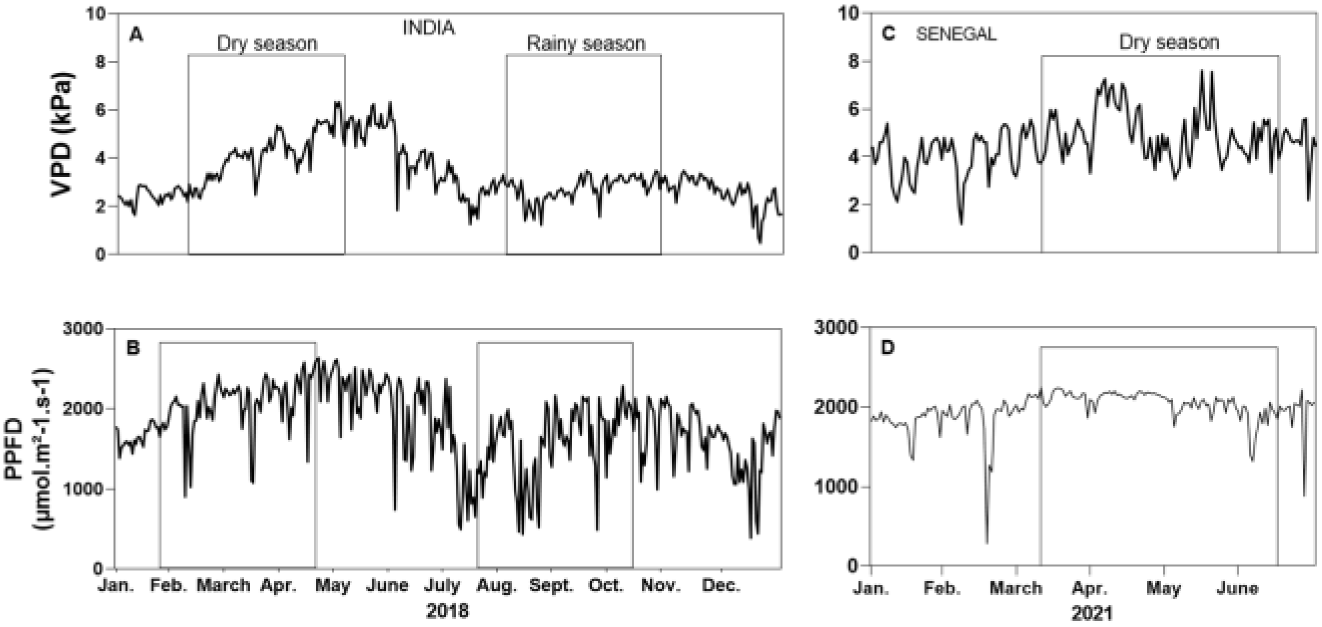

Figure 3.1.1*:* Daily vapor pressure deficit (VPD) and Photosynthetically active Photon Flux Density (PPFD) in experiments in India (ICRISAT meteorological station in 2018, A and B) and Senegal (CNRA Bambey station for the VPD, (C) and Niakhar meteorological station for PPFD in 2021, (D)). Empty boxes correspond to the periods of lysimetric measurements.

same plant distribution otherwise. Before sowing, the field was fertilized with di-ammonium phosphate at a rate of 100kg/ha and top dressing with 100kg/ha urea occurred at four weeks after sowing in both high and low density treatment. The field experiments in India was carried out at the ICRISAT station close to the lysimeter facility. The experimental design was the same as in the Senegal experiment, with the same two plant densities. Before sowing, the field was fertilized with di-ammonium phosphate at a rate of 100kg/ha and top dressing with 100kg/ha urea four weeks after sowing. Daily temperature, hygrometry and solar radiation data were collected on site at the ICRISAT and CNRA Bambey meteorological stations for experiments in India and Senegal, respectively, except for the radiation data in Senegal that was recorded in anther meteorological station located 25 km south from the field.

An experiment was performed in the indoor IRD automated lysimetric platform of Montpellier, France in April-May 2020-21. Light conditions and VPD fluctuated in the greenhouse, with VPD values ranging from 1 kPa at sunrise to 3.5 kPa at 2 pm. 16 L pots were filled with a mix of loamy clay agricultural soil and sand, each containing four plants and organized in mini canopies of 20 plants m^{-2}. A replication consisted of nine pots set up on contiguous 2m long and 0.8m wide tables. Three replicates of each genotypes were randomly distributed on the platform. Each pot was a closed mini lysimeter system that avoided any drainage loss during irrigation. Plastic beads covered the soil surface (4-5 cm layer) to avoid water loss due to direct soil evaporation. Pots were weighed every week and immediately irrigated after weighing to precisely compensate the amount of water transpired by plants of the considered pot. Five weeks after emergence, the mini lysimeters were transferred to an automated lysimetric platform recording the weight of each pot every 30 minutes for a 4-day period.

Finally, an experiment was carried out in the greenhouse experiment at Montpellier in April 2020 with a daytime VPD of 3 kPa maintained over the studied period. Plants were grown in four liters squared pots filled with horticultural potting soil. Pots were placed on 1m^2 tables. The high and low density treatments consisted in 1 m^2 tables with 24 or 12 pots. Each treatment was replicated three times for each genotype in the glasshouse.

Evapotranspiration response to the evaporative demand in a lysimeter setup

Evapotranspiration was measured with lysimeters over several days with natural fluctuations of light and VPD. To maximize the range of evapotranspiration, we also varied leaf area index (LAI) by setting up two plant density treatments. Hence, this protocol profoundly differs from that most often used for establishing response curves to VPD, which involves measuring individual plant transpiration over time scale of 30-60 minute per VPD increment in a growth chamber.

Evapotranspiration data, initially measured in kg of water loss per replication (4 tubes), was first converted into mm m^{-2} day^{-1}, by dividing raw values by the reciprocal of plant density in each replicate and by the time between two weighing dates. In India, lysimeters were weighed nine and eight times during the rainy and dry seasons, respectively, resulting in 30 evapotranspiration values for each genotype across the two plant densities. In Senegal, lysimeters were weighed 11 times, giving 20 evapotranspiration values for each genotype. In both cases, measurements covered a period of 7-10 days.

The evaporative demand (ET_{ref}) during the same periods of time was calculated in two ways. The first method was based on the simulation of evapotranspiration of a reference genotypes by using the APSIM crop simulation model parametrized with a sorghum genotype having a phenology and leaf area similar to those of hybrids of panel A, based on Priestley-Taylor equation (Priesltley and Taylor, 1972). The transpiration of this reference genotype, simulated with unlimited water supply, is akin to an environmental variable, and was considered as a reference evapotranspiration. The second method involved the Penman-Monteith equation corrected by a crop coefficient (Kc) (Zotarelli et al., 2014) which takes into account the change with time of LAI, ranging from 0.4 to 0.85 according to plant stages (Piccinni et al., 2009). For both methods, we used as inputs the daily temperature, hygrometry, wind and solar radiation data collected on site at the ICRISAT and CNRA Bambey meteorological stations (Solar radiation data was collected at the Niakhar meteorological station located 25km south of the CNRA statin). Daily ET_{ref} values obtained with either method were averaged over the respective measurement periods and plotted against measured evapotranspiration values for the same perios. Only the second method was used for the Senegal experiment because no closely related genotype was parameterized in APSIM. ET_{ref} data from both methods were compared in the experiment in India. Leaf area index (LAI) was measured 33 and 40 days after sowing in the field directly adjacent to the lysimetric platform by using a 1-meter long ceptometer (AccuPAR LP-80). Four measurement were taken in each replication for the 20 genotypes in both high and low density treatments.

Transpiration response to the evaporative demand in the glasshouse

In the indoor lysimetric facility of Montpellier, a data analysis pipeline (adapted from Kar et al., 2020) allowed estimation of the time course of transpiration over 24 h. The slope of the time course of transpiration was estimated during the three hours preceding the maximum transpiration, corresponding to the maximum VPD window on a particular day. Because the time course of VPD was similar between days, this slope was an indirect way for comparing genotypes for their transpiration response to evaporative demand.

The slope of the time course of transpiration was estimated during the three hours preceding maximum evapotranspiration, corresponding to the maximum VPD window on a particular day. Because the time course of VPD was similar between days, this slope was an indirect way for comparing genotypes for their transpiration response to the evaporative demand. After 4 days on the load cells, the aboveground biomass of these 6-weeks old plants was harvested, dried in an oven during 72h at 60°C, and used to compute WUE.

Genotypic variation for water use efficiency in canopy-grown plants

At 13 and 14 weeks after sowing in dry and rainy VPD seasons respectively in India, and at 14 weeks in Senegal, the total above ground biomass was harvested from the lysimeters, dried in an oven during 72h at 60°C and weighted. It was used to compute the water use efficiency (WUE, g of biomass accumulated per kg of water loss through evapotranspiration).

VPD assessment within canopies

Temperature and relative humidity sensors were positioned in the low and high density plots of the field experiment in Bambey in 2021 (TinyTag ultra 2, TGU-4500, Gemini Datalogger Ltd, Chichester, UK). A daily average of temperature and hygrometry was recorded at the Bambey meteorological station. Air and canopy VPD were calculated based on relative humidity (RH) and air temperature values measured every 30 minutes, either 2 meters above the canopy for air VPD (i.e. from the meteorological station located 50m from the field) or within the canopy at mid height of the plants. Every week, the position of the sensors was adjusted according the height of the plants to be continuously placed at mid height of the canopies. Recording of data started at four weeks after emergence.

Similar temperature and relative humidity measurements were performed in the greenhouse facilities of CIRAD with two genotypes from panel B in April 2020. The sensors were placed at 15 cm above the soil surface (i.e. few centimeters above the pot brim). Recording of data started at fifteen days in the greenhouse. At 16 and 22 days after sowing, leaf area index (LAI) was measured in both low and high-density canopies using a light sensor (Spectrol LI-80, Li-Cor).

In both experiments, the sensors were covered with open polystyrene boxes to avoid direct solar radiation. Temperature and relative humidity were recorded every 30 min during a 15 days period in the glasshouse and during 6 weeks in the field trial allowing to calculate VPD.

Measurement of LAI and light penetration in the canopy in Senegal

In the field trial at Senegal, leaf area index (LAI) was measured using PPFD measurement performed in both low and high-density canopies using a light sensor (LI-190R-BNC-2 Quantum Sensor) placed above the canopy, and a sensor bar (LI-191R-BNC-2 Line Quantum Sensor) placed at the ground level. LAI was calculated indirectly as $LAI = \ln(\frac{I}{Io})/k$ where Io is the incident light above the canopy, I is the light at ground level, and k is a crop extinction equal to 0.6 for sorghum. The Photosynthetic Photon Flux Density (PPFD) was also measured above the canopy and at two heights in the canopy on days 45 and 60 after sowing. The measurements were done with a light sensor (LI-190R-BNC-2 Quantum Sensor) placed above the canopy, and two sensor bars (LI-191R-BNC-2 Line Quantum Sensor) fixed perpendicularly on a pole placed vertically in the plots and adjusted respectively at mid-canopy level and at ground level. Mid canopy height was assessed for each genotypes thanks to graduations marks on a vertical pole. All sensors were connected to a data logger (LI-1500 Light Sensor Logger), and data were recorded between 12:30 to 1:30 pm. Each data point was the mean of four replicates in each plot. At 44 days after sowing, a light measurement was also done at ground level in a plot of high and low density at four time-point during this particular day, at 7am, 10am, 2pm and 5pm.

Statistical analysis

Statistical analysis presented in this study were performed using GraphPad Prism version 9.2.0 for Windows, (GraphPad Software, San Diego, California USA, http://www.graphpad.com) Slopes and time courses of transpiration were smoothed and analyzed using R software version 4.1.2. Broad sense heritability of the slopes was computed as in Falconer et al, 2005 with $H^2 = \frac{\sigma\, g^2}{\sigma g^2 + residuals}$ where $\sigma\, p^2$ is the genotypic variance.

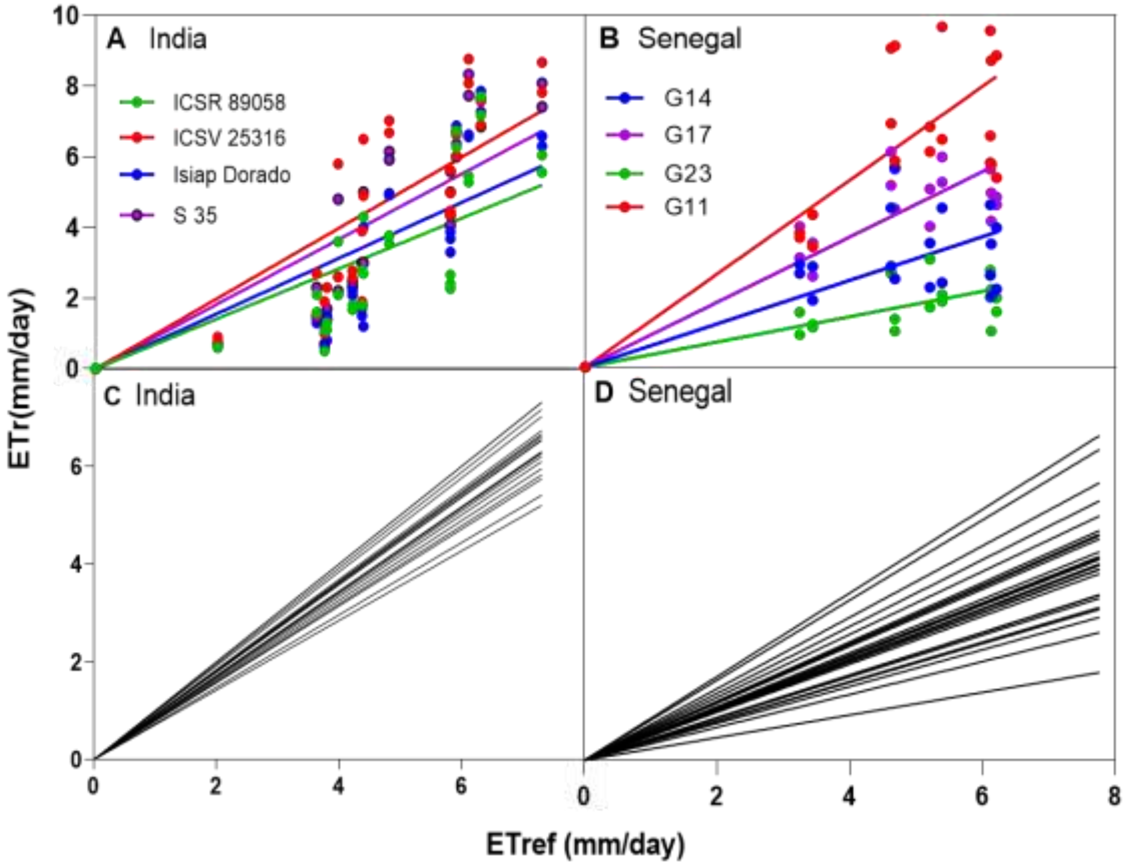

Figure 3.1.2: Measured evapotranspiration (ETr) plotted against the reference evapotranspiration (ETref), for 20 genotypes in the lysimeter platform in India (A, C) and 27 genotypes in the lysimeter platform in Senegal (B, D). A and B: point cloud regressions for 4 contrasting genotypes, C and D regression lines for all studied genotypes (one line per genotype). Data are means of 4 replications per genotype and treatment (P-values < 0.0001).

Results

The response of evapotranspiration to the evaporative demand was genotype-dependent

We examined the response of each studied genotype to the reference evapotranspiration (ET_{ref}), which takes both VPD and light into account. Indeed, light intensity underwent large fluctuations in our experiments with time of day, between days and between seasons (Fig. 3.1.1), so regression lines presented in Fig. 3.1.2 correspond to evaporative demands varying with both VPD and light. Point clouds correspond to coupled values, for one genotype, of measured evapotranspiration and ET_{ref}, calculated for periods of 7 to 10 days during either the dry or rainy seasons in India, or during the dry season in Senegal, at two plant densities.

In the dataset collected in India, linear regressions were significant for all tested genotypes (p-values <0.0001; Suppl. table 3.1.1). We tested the linearity of relationships by considering a quadratic term in regressions, which was significantly positive for all hybrids (values of ET slightly higher than those expected under a linear assumption, Suppl. table 3.1.1, Suppl. Fig 3.1.1). Hence, we never observed a tendency towards a plateau of evapotranspiration at high evaporative demands. Notably, all regressions with VPD alone were also significant, and none of them showed a tendency to plateau (non-significant quadratic term in 25% of cases, positive term otherwise). In order to facilitate genetic analyses, and in view of the small contribution of positive quadratic terms, we only considered the linear relationships in the following analyses. The slopes of linear regressions significantly differed between genotypes (Fig. 2, p-value = 0.03), with a high heritability ($H^2 = 0.56$, Fig. 3.1.2 A&C). Hence, the studied genotypes differed in their ability to transpire at a given evaporative demand with up to three-fold differences, e.g. from 2.3. to 6.28 mm d^{-1} at an ET_{ref} of 5.80 mm d^{-1}. Importantly, the slopes of relationships for each genotype were independent of leaf area for both the first (r = 0.2, p-value=0.42) and the second (r= 0.03, p-value=0.86) measurement in the adjacent field, so the differences between genotypes were probably not related to leaf area, and linked to stomatal behaviour (Suppl. Fig 3.1.2). The same set of results was observed whether ET_{ref} was calculated either via the Penman-Monteith equation corrected for leaf area, or with the APSIM model, with a very high correlation between calculated slopes (Suppl. Fig 3.1.3 A).

The dataset collected in Senegal provided consistent results, with regressions between evapotranspiration and ET_{ref} significant for all studied genotypes (Fig. 3.1.2 B&D, p-values <0.0001; Suppl. table 3.1.2). Quadratic terms were non-significant in 56% of cases and slightly

Genotypes Panel A	Slopes (ETref)	R^2 (ETref)	Slopes p-values	x^2 term	X^2 term p-value	Genotypes Panel A	Slopes (VPD)	R^2 (VPD)	p-values	x^2 term	X^2 term p-value
CSH 16	0,86	0,65	<0,0001	0,09	0,021	*CSH 16*	1,20	0,62	<0,0001	0,08	0,150
ICSB 404	0,74	0,65	<0,0001	0,14	0,005	*ICSB 404*	1,04	0,68	<0,0001	0,23	0,000
ICSH 14002	0,91	0,75	<0,0001	0,13	0,408	*ICSH 14002*	1,27	0,74	<0,0001	0,15	0,079
ICSH 28001	0,96	0,75	<0,0001	0,13	0,322	*ICSH 28001*	1,34	0,73	<0,0001	0,12	0,074
ICSR 101	0,80	0,73	<0,0001	0,11	0,067	*ICSR 101*	1,12	0,74	<0,0001	0,14	0,004
ICSR 14001	0,85	0,75	<0,0001	0,15	0,096	*ICSR 14001*	1,19	0,73	<0,0001	0,17	0,003
ICSR 196	0,86	0,73	<0,0001	0,15	0,305	*ICSR 196*	1,21	0,74	<0,0001	0,18	0,001
ICSR 89058	0,71	0,61	<0,0001	0,12	0,010	*ICSR 89058*	1,00	0,64	<0,0001	0,20	0,000
ICSV 112	0,84	0,76	<0,0001	0,17	0,236	*ICSV 112*	1,17	0,75	<0,0001	0,21	0,001
ICSV 15013	0,91	0,70	<0,0001	0,15	0,282	*ICSV 15013*	1,27	0,67	<0,0001	0,14	0,036
ICSV 25302	0,98	0,73	<0,0001	0,12	0,455	*ICSV 25302*	1,36	0,67	<0,0001	0,10	0,349
ICSV 25308	1,00	0,69	<0,0001	0,10	0,371	*ICSV 25308*	1,39	0,62	<0,0001	0,05	0,452
ICSV 25316	1,00	0,71	<0,0001	0,10	0,354	*ICSV 25316*	1,39	0,65	<0,0001	0,06	0,330
ICSV 745	0,90	0,74	<0,0001	0,18	0,236	*ICSV 745*	1,26	0,76	<0,0001	0,22	<0,0001
ICSV 93046	0,86	0,69	<0,0001	0,16	0,145	*ICSV 93046*	1,20	0,65	<0,0001	0,18	0,043
Isiap Dorado	0,79	0,71	<0,0001	0,16	0,005	*Isiap Dorado*	1,11	0,77	<0,0001	0,24	<0,0001
MR 750	0,82	0,73	<0,0001	0,18	0,002	*MR 750*	1,15	0,79	<0,0001	0,26	<0,0001
NTJ-2	0,91	0,70	<0,0001	0,13	0,368	*NTJ-2*	1,26	0,67	<0,0001	0,12	0,230
PVK 801	0,89	0,74	<0,0001	0,15	0,053	*PVK 801*	1,26	0,77	<0,0001	0,20	0,000
S 35	0,92	0,75	<0,0001	0,15	0,319	*S 35*	1,29	0,74	<0,0001	0,14	0,017
p-values	0,03					p-values	0,0003				
H^2	0, 56					H^2	0,66				

***Suppl. table 3.1.1**:* Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for all genotypes from the panel A tested in India. Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software) or VPD (Calculated with T° et RH data from the adjacent meteorological station).

positive otherwise (Suppl. table 3.1.2), so following analyses only considered the linear regressions as in panel A. The genetic variability of slopes was even larger than in experiments in India (p value = 0.02), with slopes ranging from 0.26 to 0.96, and a heritability of slopes of $H^2 = 0.41$. For example, transpiration rate ranged from 1.03 to 9.14 mm d^{-1} at an ET_{ref} of 5.83 mm d^{-1} (Fig. 3.1.2 B & D). The low range of VPD in this dataset resulted in the fact that regressions of ET with VPD alone were non-significant in most cases. Hence, regressions with ET_{ref} provided more insights than those with VPD alone in this case. Consistent results were again observed in the greenhouse, where the slopes of the transpiration response to the evaporative demand differed between genotypes (p-value < 0.01, $H^2 = 0.39$) (data not shown).

A large genotypic variability for water use efficiency

WUE showed a high genotypic variability in panel A during both the dry and rainy seasons in India (2.2 to 3.2 and 8.4 to 11.9 g kg^{-1}, respectively, p-value = 0.01 and < 0.0001), with a high heritability calculated across seasons ($H^2 =0.64$) (Fig. 3.1.3 A and 3B). A significant genotype x season interaction was observed, suggesting that the decrease in WUE with ET_{ref} depended on other traits that differ between genotypes (p-value = 0.0004) (Table 3.1.1). Similar results were observed in Senegal with panel B, with a high genetic variability for WUE (1.4 to 3.9 $g.kg^{-1}$, p-value < 0.01) and a high heritability ($H^2 = 0.65$, Fig. 3.1.3 C).

The genetic correlation between WUE and evapotranspiration was positive under high evaporative demand, and negative under low evaporative demand

We then examined the genetic link between WUE and the response of transpiration rate to evaporative demand. In experiments performed in dry seasons of India or Senegal (Fig. 3.1.4 A and B), WUE correlated positively and significantly with the slope of the regressions presented in Fig. 3.1.2 for the same genotypes. Counter intuitively, the genotypes that most transpired at a given ET_{ref} had the highest WUE, with a high correlation between WUE and the slope of response curves in India (r = 0.79, P value <0.0001,) (Fig 3.1.4 A) and lower but significant in Senegal (r = 0.54, p-value <0.01) (Fig 3.1.4 B). The same pattern was observed in the greenhouse under high VPD, where the slope of the regression between transpiration and time at high transpiration hour, was positively correlated with WUE (r = 0.78, p-value < 0.05) (Suppl. Fig.3.1.4). Conversely, this relationship was reversed and strongly negative in the rainy season of India (r = -0.71, p-value <0.0001, Fig 3.1.4 C). That is, the genotypes that most transpired in response to ET_{ref} had the lowest WUE in this case. This result held whether ET_{ref} was calculated via either the APSIM model or the Penman Monteith equation corrected for leaf area (Suppl. Fig 3.1.3 B and C).

Genotypes Panel B	Slopes (ETref)	R^2 (ETref)	Slopes p-values	x^2 term	X^2 term p-value
IS 11119	0,85	0,61	<0,0001	0,15	0,010
CE-145-66	0,91	0,35	<0,0001	0,10	0,072
IS 3507	1,07	0,61	<0,0001	0,18	0,023
IS 33209	0,73	0,50	<0,0001	0,11	0,017
ICSV 745	0,63	0,59	<0,0001	0,09	0,186
IS 14539	0,50	0,25	<0,0001	0,04	0,512
IS 33173	0,74	0,32	<0,0001	0,10	0,079
IS 13452	0,68	0,57	<0,0001	0,10	0,018
FAURO	0,74	0,66	<0,0001	0,11	0,079
IS 19016	0,64	0,27	<0,0001	0,10	0,166
IS 16396	0,75	0,43	<0,0001	0,11	0,014
IS 7958	0,64	0,37	<0,0001	0,16	0,005
IS 14556	0,50	0,65	<0,0001	0,04	0,346
IS 15443	0,64	0,31	<0,0001	0,07	0,274
IS 14963	0,67	0,42	<0,0001	0,08	0,075
IS 13845	0,29	0,39	<0,0001	0,02	0,319
IS 2678	0,54	0,51	<0,0001	0,06	0,030
IS 25207	0,53	0,47	<0,0001	0,06	0,266
E36-1	0,54	0,21	<0,0001	0,07	0,320
IS 31202	0,80	0,75	<0,0001	0,12	0,037
IS 15702	0,47	0,58	<0,0001	0,07	0,252
IS 33423	0,42	0,50	<0,0001	0,06	0,271
IS 33261	0,66	0,47	<0,0001	0,09	0,070
IS 16125	0,66	0,39	<0,0001	0,12	0,068
IS 2986	1,02	0,44	<0,0001	0,11	0,027
IS 10876	0,61	0,63	<0,0001	0,10	0,070
PAYENNE	0,63	0,71	<0,0001	0,10	0,014
p-values	0,02				
H^2	0,41				

***Suppl. table 3.1.2**:* Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms in all genotypes from the panel B. Regressions are ETr values from the lysimeters as a function of ETref calculated via the Penman-Monteith equation corrected with a crop constant (Kc).

The intra canopy VPD was lower than air VPD during the day

The measured VPD was lower within the canopy than in the air, to a greater extent in high than in low plant density. In high-density canopies, this difference became significant from day 42 onwards (Fig. 3.1.5A), when LAI was approximately 2.9 (measured on day 45). In low-density canopies, it became significant 14 days later (day 56) when LAI was also close to 3 (3.2 on day 53) (Fig. 3.1.5 A). Differences in VPD were maximum in the morning and early afternoon (average difference of 0.97 kPa, p-value = 0.05 over this period, Fig 5B). Differences in VPD between canopy and air were also observed in the greenhouse experiment during a 15-days period, with a mean difference in VPD of 0.63 kPa (0.55 to 0.93 kPa, p-value = 0.001, Suppl. Fig 3.1.5). Overall, VPD within the canopy was lower than that sensed by upper leaves in direct relation to open air, and more so in dense canopies. This was likely due to the intra canopy transpiration because differences nullified during the night and increased with LAI.

Is the positive relation between WUE and transpiration linked to the vertical distribution of VPD and light penetration in the canopy?

We measured light penetration in the canopy to better understand the positive genetic correlation between WUE and high transpiration under high VPD. In the experiment in India, light measurement on day 44 showed that light was appreciable within the canopy, to a larger extent for canopies with low than high density, at 10am (488 vs 165 µmol m^{-2} s^{-1} respectively) and 2pm (958 vs 479 µmol m^{-2} s^{-1} respectively) (Fig 3.1.5 C). In the Senegal experiment, genotypes that most let the light penetrate at mid-canopy level were those that had highest WUE at 60 DAS, when LAI was 3.9 (r = 0.44, p-value = 0.009) (Fig. 3.1.6 B). At this phenological stage, the genotypic correlation was observed with light intensity at mid-canopy level and not at ground level because studied canopies intercepted nearly all the incident light. At an earlier stage (45 DAS, LAI = 2.9), the genotypic correlation was observed with light at ground level, reached by light at this stage (r = 0.56, p-value = 0.04) (Fig. 3.1.6A). Hence, genotypes that most transpired at a given evaporative demand were also those for which more light reached leaves inside the canopy, potentially increasing the photosynthesis of these leaves. Because the intra-canopy VPD was lower than air VPD (Fig. 3.1.5A&B), the response of transpiration rate to ET_{ref} had a higher effect on biomass than on transpiration, thereby increasing WUE (Suppl. fig. 3.1.6 A). This was not observed in the rainy season (Suppl. fig. 3.1.6B), probably explaining the observed negative genotypic effect of transpiration on WUE. Hence, we raise the possibility that genetic correlations between transpiration and WUE depend on

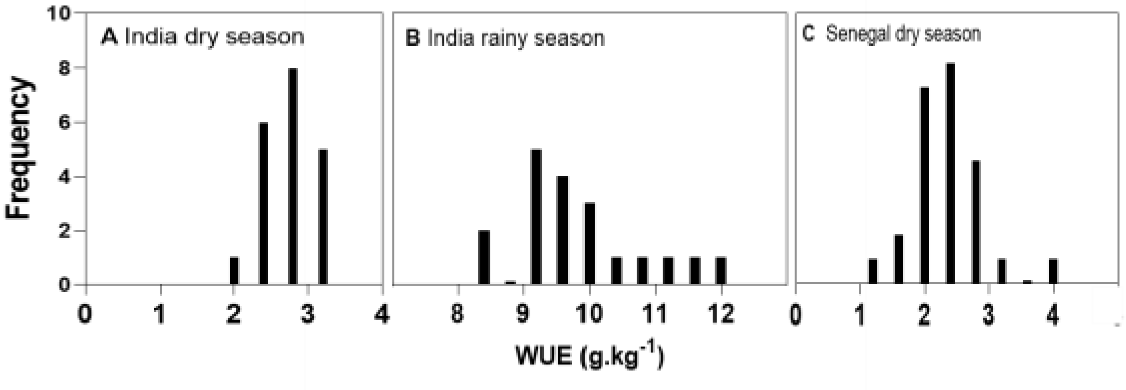

Figure 3.1.3: Frequency distribution of the water use efficiency (WUE) values measured on the lysimeter platforms for the 20 genotypes from panel A India during the 2018 dry (A) and rainy seasons (B), and for the 27 genotypes from panel B in Senegal during the 2021 dry season (C).

Source of Variation	Dry season 2018	Rainy season 2018	Dry season 2021
Location	India	India	Senegal
Genotypes panel	A	A	B
Two-way ANOVA			
Genotype	*	****	*
Genotype x Season	***		NA

Table 3.1.1: ANOVA table showing the genotypic variability for the water use efficiency (WUE) in the dry and rainy season experiment in India and the dry season experiment in Senegal. *, p-value <0.05, **, p-value <0.01, *** p-value <0.001, ****, p-value <0.0001.

plant architecture and the vertical distribution of VPD, with a positive relationship due to light penetration under the high light and VPD observed during dry seasons, and a negative relationship under lower light and VPD observed during the studied rainy season.

Discussion

A large genetic variability of the response of transpiration to evaporative demand in natural conditions

This work extends to natural conditions, with high light and VPD, the observation of a genetic variability of the response of transpiration to evaporative demand, of particular importance for dry tropical areas. Here, we considered evaporative demand via its two components, light intensity and VPD, and observed significant regressions between the resulting ETref and evapotranspiration of each of the 47 tested genotypes. Because slopes significantly differed between genotypes in both panels A and B, we can conclude that a significant genetic variability exists for the ability of a genotype to transpire at a given evaporative demand. This variation was not related to differences in leaf area. Given the high heritability of slopes, this genetic variability may be used for designing genotypes for either high transpiration rate (suited to favourable environmental scenarios) or lower transpiration rate (suited to drought-prone areas). Notably, the accessions of panel A had a lower genetic variability and heritability for slopes than the elite hybrids of panel B, thereby suggesting that the response of transpiration to evaporative demand may have faced a selection pressure. Accessions from panel A came from the ICRISAT breeding program and were mostly bred for the rainy season, which could have explained the lower variation for a trait addressing drought-prone situations.

It was considered by Sinclair and other authors that the differential responses to evaporative demand is both characterized by stomatal closure which occurs at differently high VPD for each genotype (VPD breakpoints) and by differences in the slopes before and after the breakpoint, thereby resulting in a genetic variability of responses (e.g. Fletcher et al., 2008; Kholova et al., 2010). Here, we did not observe such a non-linear response, as quadratic terms of regressions, all positive when significant, indicated a concave relationship rather than a plateau. Indeed several studies carried in the field showed responses that did not always present a non-linear response, even when the evaporative demand was expressed via VPD only (Tharanya *et al.*, 2018; Devi and Reddy, 2018). In the same way, when a confusion of effect between temperature and VPD was avoided, only a small proportion of wheat genotypes presented a plateau under high evaporative demand (Tamang et al., 2022). Importantly, some of the genotypes that did

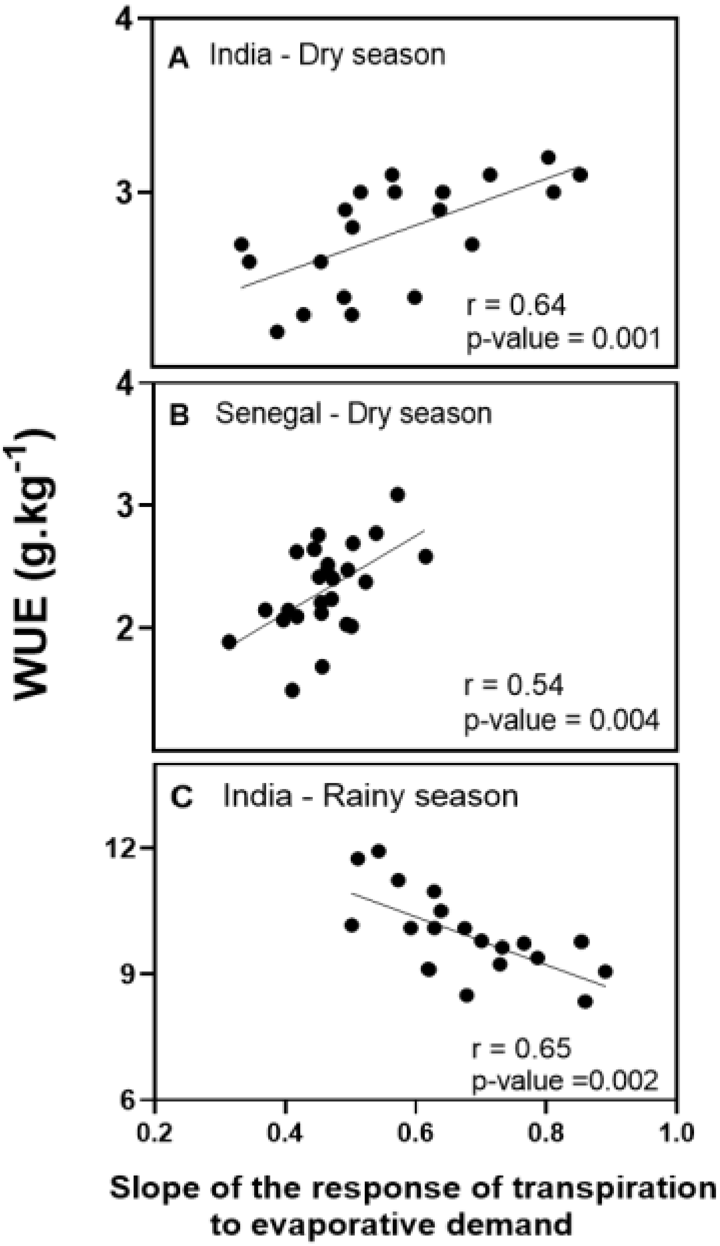

Figure 3.1.4: Water use efficiency (WUE) plotted against the slopes of the evapotranspiration response to ETref (see figure 2). (A) 20 genotypes of panel A in the dry season, India (r = 0.64, p-value = 0.0001). (B) 27 genotypes of panel B in the dry season, Senegal (r= 0.54, p-value = 0.004). (C) 20 genotypes of the panel A in the wet season, India (r = - 0.65, p-value < 0.01). Data are means of 4 replications per genotype and treatment (P-values < 0.0001).

not present a plateau in our study, did show a marked plateau at high VPD in a study in growth chamber (Karthika et al., 2019). Hence, a different representation emerges from studies carried out in high evaporative demand carried out outdoor compared with those in growth chamber. Because no plateau for evaporative demand was observed, the cause of the genetic variability of the evapotranspiration was not stomatal closure at high VPD, but rather a difference in transpiration rate over the whole range of evaporative demand. Indeed, an appreciable genetic variability for stomatal conductance was observed in maize, even at relatively low evaporative demands, with high heritability and consistent QTLs (Alvarez Prado et al 2019, Welcker et al 2022). We therefore suggest that genetic differences in transpiration rate were due to intrinsic differences in stomatal conductance, rather than to an adaptive process of stomatal closure at high VPD.

Why different shapes of the response of transpiration to evaporative demand were observed between results in growth chamber and in our field study? We raise the possibility that this is due to the difference in light intensities between the two conditions. Under relatively low light and high air movement, as in a growth chamber, the decrease in stomatal conductance with high VPD directly translates into a reduction in transpiration rate. In conditions with high light and lower wind, uncoupling occurs between stomatal conductance and transpiration for most species (Jarvis & Mcnaughton, 1986), so leaf temperature increases with stomatal closure, thereby increasing leaf-to-air vapour pressure difference, largely decreasing the effect of stomatal closure on transpiration rate (Chaves et al., 2016). It was unexpected that a good relationship between transpiration and air VPD was still observed in our experiments in India, as it did in former studies (Kar et al., 2020; Kholova et al., 2010). This was probably due to the correlation between light intensity and VPD, observed in our study, but probably not in other climatic conditions, for example in regions where the wind brings either dry air from continental areas, or wet air from the sea, depending on its direction, with an unchanged light intensity (Ben Haj Salah & Tardieu, 1996). Indeed, this correlation was not observed in the Senegal experiment.

A genetic variability of WUE related to the response of evapotranspiration to evaporative demand

The results presented here suggest that lower VPD within the canopy may increase the WUE of leaves transpiring within the canopy, during seasons facing high VPD, because WUE is inversely related to VPD (Condon et al., 2002; T. R. Sinclair et al., 1984). We propose

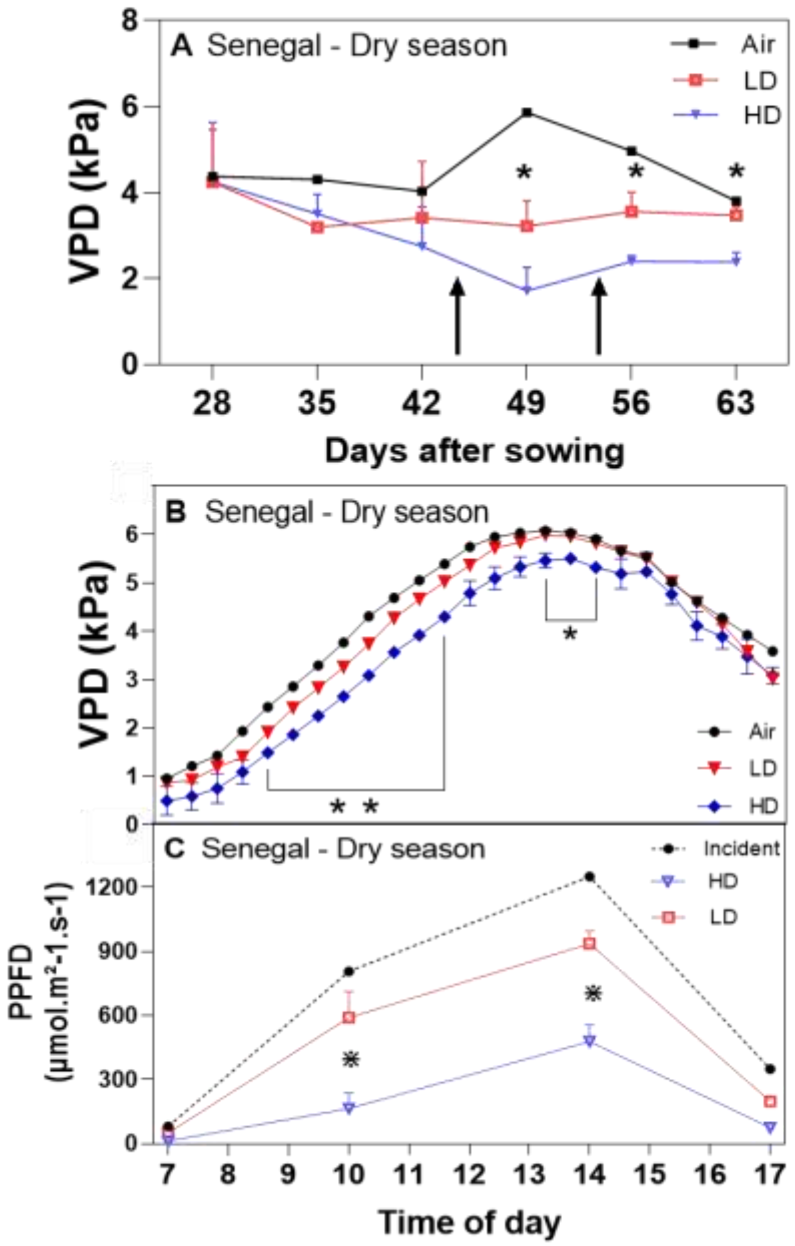

***Figure 3.1.5**:* (A) Vapor pressure deficit (VPD) measured in the air and within canopies with high and low densities (12 and 24 plants/m^2 respectively, HD and LD), as a function of time after sowing in the dry season field trial in Senegal. Black arrows in (A) represent dates at which LAI was measured (45 and 53 days after sowing), and stars indicates significant differences between HD and LD (paired t-test, p-value <0.001). (B) Daily time course of VPD during the 4th week of the same field experiment from 7am to 7 pm, where stars indicate significant differences between HD and LD (paired t-test, p-value <0.001). (C) Photosynthetically active Photon Flux Density (PPFD) at 4 time points across the same day in the two densities. Stars indicates significant differences between HD and LD (paired t-test, p-value <0.0001). Each data point is the average of sensor data collected in three plots for each of the densities.

that observed differences in WUE among genotypes were, in part, a consequence of the proportion of transpiration contributed by leaves within the canopy. The large and heritable genotypic variation response of evapotranspiration to ET_{ref} supports this hypothesis. Our interpretation is that a stronger evapotranspiration response would come from more leaves actively participating in plant transpiration, i.e. by involving leaves inside the canopy. Genotypes with highest WUE in high VPD seasons were those for which more light was available inside the canopy. Architectural features could be involved in the variability of response, with genotypes that allow the lower canopy levels to participate in transpiration, benefitting from lower VPD values, allowing for an increase in WUE over the long term. Indeed plant architecture strongly influences variables such as light interception (Duursma et al., 2012; Falster & Westoby, 2003; Iii et al., 2015) and radiation use efficiency (RUE) (George-jaeggli et al., 2013; Perez et al., 2019; Truong et al., 2015). Other studies have also shown a better efficiency of water use in varieties allowing a better distribution of the light resource in other species (Falster & Westoby, 2003; Lee & Tollenaar, 2007).

Why WUE was highest in genotypes with high response to transpiration in dry seasons and lowest in wet season?

WUE was either positively or negatively genetically correlated to the evapotranspiration response to the evaporative demand, depending on the prevailing light and VPD during the season. This contrasts with earlier results showing that it is related to transpiration restriction under high VPD (Thomas R. Sinclair et al., 2005; Vincent Vadez et al., 2014). We propose that differences in WUE may be related to differences in the distribution of the light resource inside the canopy, not taken into account in earlier studies that only considered VPD for variation of transpiration. During the dry season with high VPD and light, highly responsive genotypes for transpiration also allowed light to penetrate deeper in the canopy, letting lower level leaves to transpire and photosynthesize. This caused proportionally more transpiration to occur for intra-canopy than for top leaves in genotypes with high transpiration. Intra-canopy leaves benefited from the low VPD and they could maintain photosynthesis better than genotypes with lower light penetration, and at a lower water cost than genotypes photosynthesizing mostly from the top leaves. Accordingly, the increase in biomass was proportionally higher than that of the evapotranspiration in the high VPD season (Suppl fig. 3.1.3A), suggesting a positive trade-off of biomass for water.

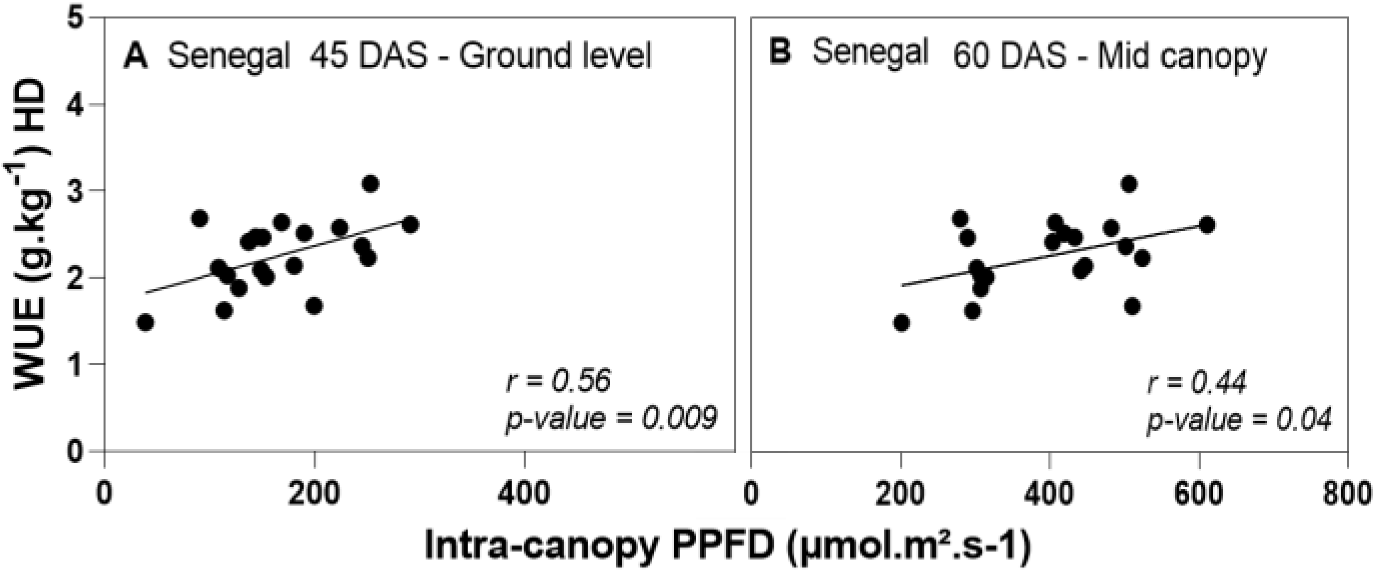

Figure 3.1.6: Water use efficiency (WUE) measured in lysimeters plotted against the amount of light measured at two altitude in the canopy. Senegal dry season, at (A) 45 DAS (ground level) and (B) 60 DAS (mid-canopy level). Data are the mean of four replications for WUE and three replications for light measurements.

On the contrary, during the rainy season with less incident light and VPD, a higher slope of the ETr response to ETref correlated to a lower WUE. Our interpretation is that light resource was insufficient to penetrate deeply inside the canopy, so both transpiration and light interception essentially involved the top leaves in the canopy, i.e. those exposed more to air VPD of the environment, VPD value that were still above 2kPa during that season. Hence, we propose here that differences in canopy architecture allowed variations in the light available inside the canopy, which drove transpiration under high evaporative demand, and increased WUE because of a higher proportion of transpiration benefitting from milder VPD conditions allowed by dense canopies.

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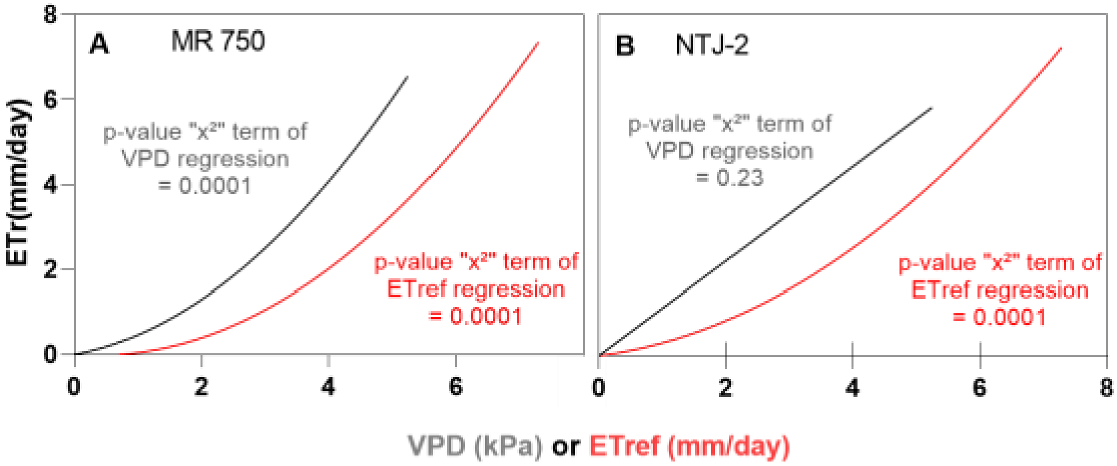
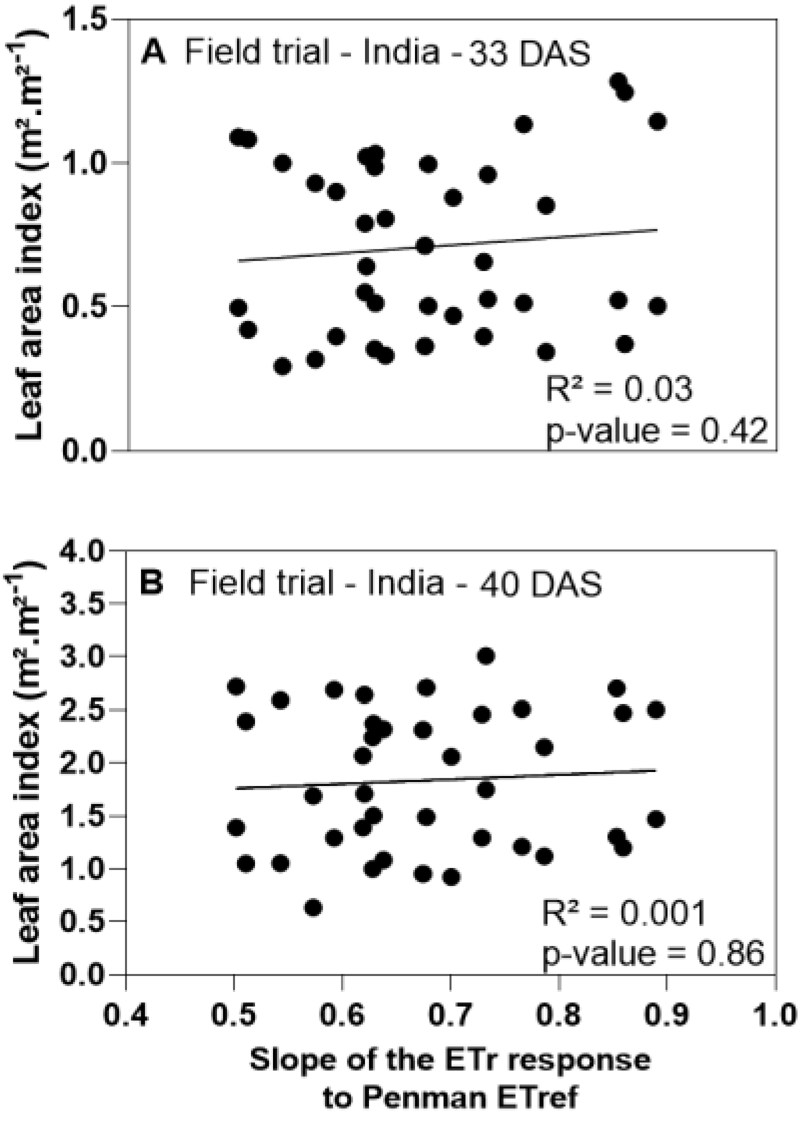
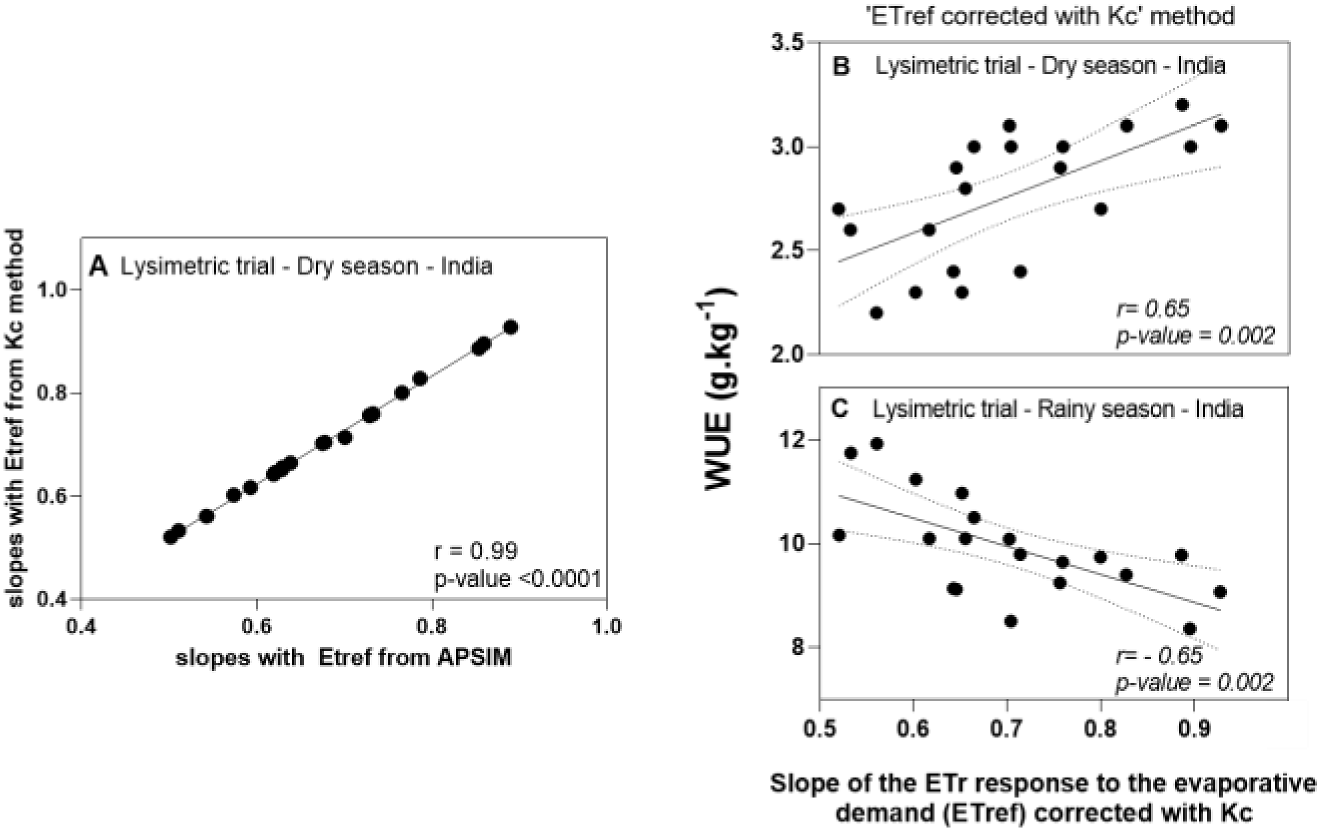
Supplementary figures:



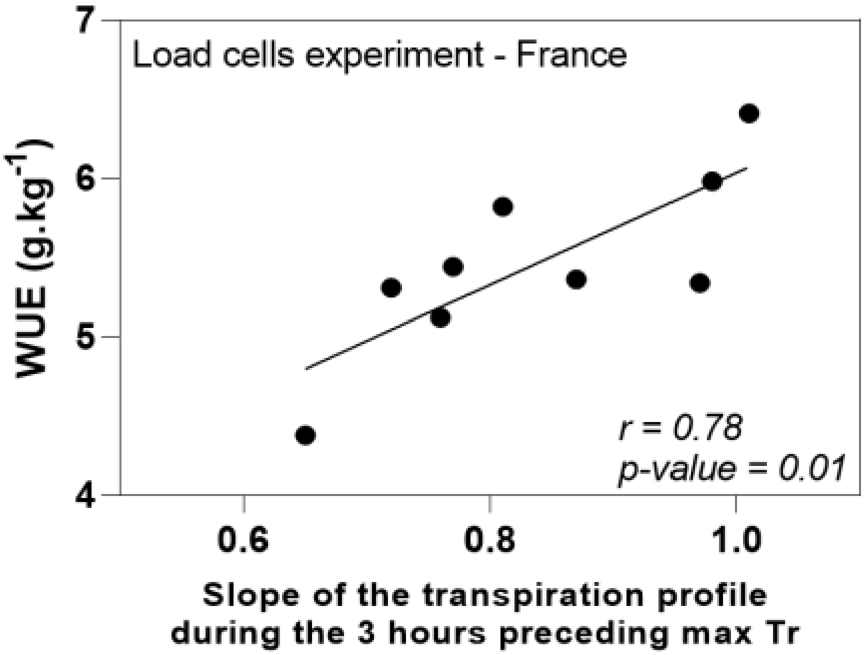
Suppl. Fig 3.1.1*:* Example of the polynomial regressions using both VPD (grey lines) and ETref (red lines) in x-axis for two genotypes from the panel A. Genotypes MR750 showed significant quadratic term with both variables with a concave relationship (A). Genotype NTJ-2 showed non-significant quadratic term with VPD and significant one with concave relationship with ET ref.



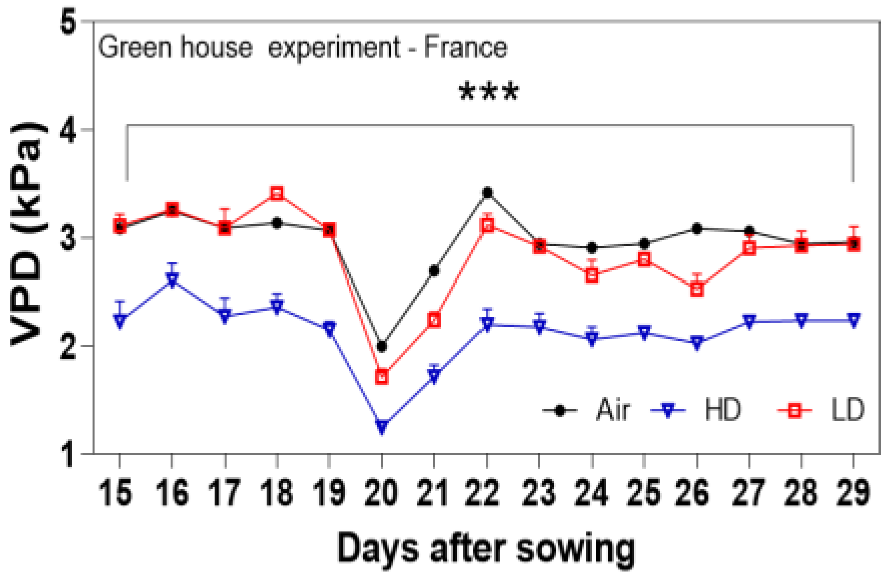
Suppl. Fig 3.1.2: Leaf area index (LAI) measured 33 days (A) and 40 (B) days after sowing in the 2018 dry season field experiment as a function of the slope of the transpiration response to evaporative demand (ETref) from the adjacent lysimetric experiment. (India, Panel A).



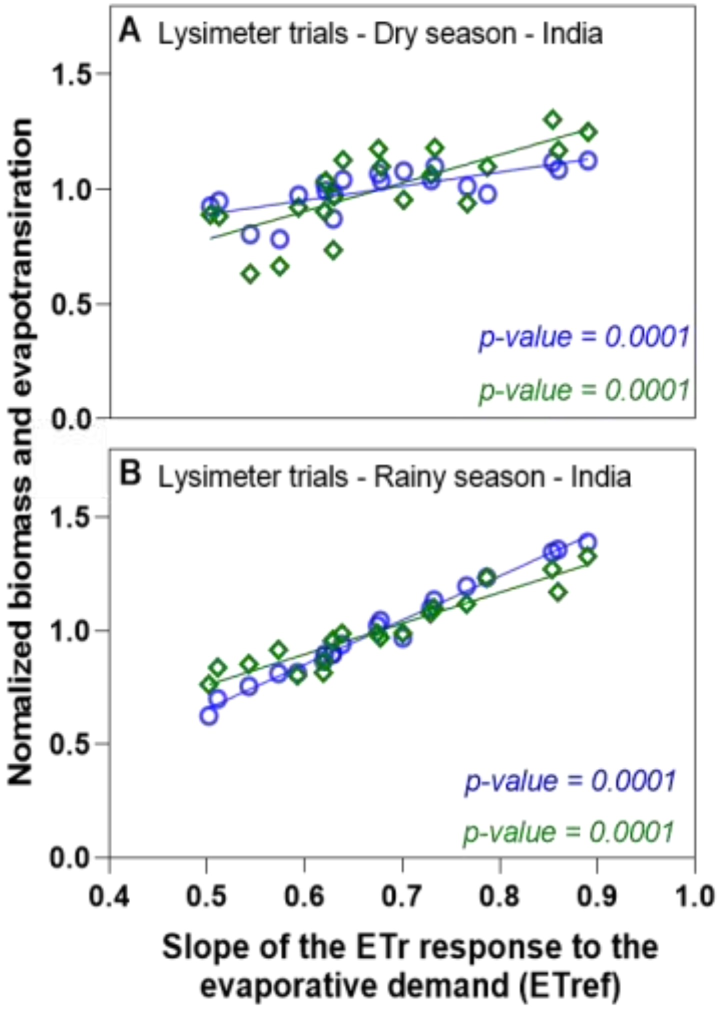
***Suppl. Fig 3.1.3**:* Linear regression of the slopes values generated with Kc method as a function of the values of the slopes generated with APSIM for the dry season experiment in India (r= 0.99) (A). Water use efficiency (WUE) as a function of the slope generated by the regression of the measured evapotranspiration against the ETref calculated with the Penman-Monteith corrected with a crop constant (Kc) for the 20 genotypes of the panel A in the dry (B) and rainy (C) season in India.



Suppl. Fig 3.1.4: Water use efficiency (WUE) plotted against the slope of the time course of transpiration rate during the 3 hours preceding the maximum transpiration in the indoor lysimeter experiment (Montpellier, France, 9 genotypes from panel B).



Suppl. Fig3.1.5: Vapor pressure deficit (VPD) measured in air and within canopies with high and low densities (12 and 24 plants/m² respectively, HD and LD), as a function of time after sowing in the glasshouse experiment (Montpellier, France, 2 genotypes from panel B). Stars indicates significant differences between air and HD VPD.



***Suppl. Fig 3.1.6**:* Normalized biomass (green) and evapotranspiration (blue) plotted against the slopes generated by the regression of measured evapotranspiration and ET_{ref} during the dry (A) and wet (B) seasons in India.

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Chapter 3.2

Response of canopies to evaporative demand differs among species: the case of pearl millet.

Unpublished

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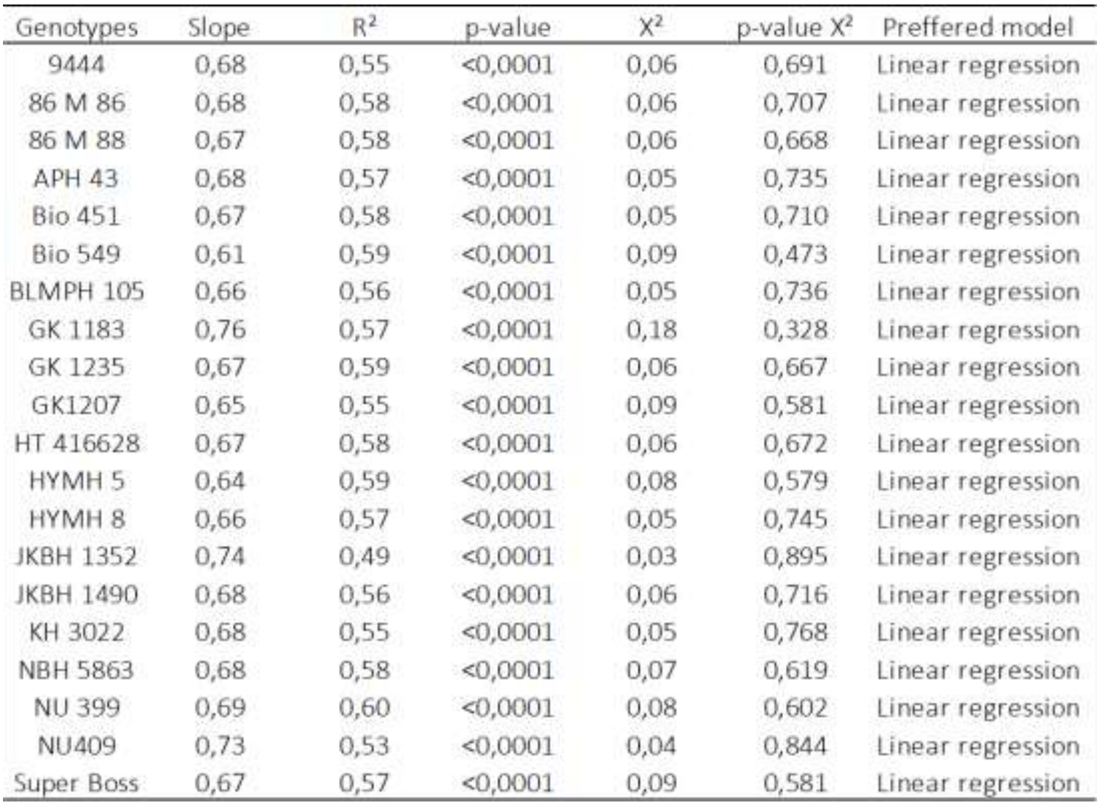
Abstract

This section aimed at testing the evapotranspiration response of dense pearl millet canopies to the evaporative demand, as it was undertaken in sorghum in the previous chapter (3.1). The objectives were to assess putative genotypic variation in this response. As in the previous chapter, the evaporative demand was measured with the Penman-Monteith ETref , that takes into account solar radiation, temperature, relative humidity and wind), rather than the more classical approach of using VPD alone. Lysimetric trials were carried out in India and Senegal in seasons contrasting for the evaporative demand. In contrast to sorghum where we found large genotypic variation, there was no or very limited genotypic variation in the degree of transpiration response to the evaporative demand in pearl millet. In addition, again contrary to sorghum, the slope of this response was not related with water use efficiency (WUE) regardless of the seasons or locations. These results are in lines with our findings of an absence of genotypic variation for the biomass accumulation and WUE response to density (i.e. no genotype x density interaction). Differences in the canopy architecture of pearl millet compared to sorghum may explain these differences. Different mechanisms of transpiration regulation in this specie may also be responsible for the intrinsic genotypic variation for WUE.

Introduction

Background

The second chapter of this manuscript (See chapter 2.1) showed that the pearl millet biomass and/or yield was higher in the high density treatment, but this effect was only observed in seasons with more light and higher evaporative demand. In addition, when positive, the high-density effect benefited all genotypes similarly, so that there was no genotypic variation in the response to higher density in pearl millet, shown by a non-significant genotype-by-density interaction effect for biomass and grain yield. Water use efficiency (WUE) followed a similar pattern: It increased under higher density, more so in high evaporative demand seasons, and also showed no genotypic variation in the response. In both species, we interpreted that the WUE increased because of the lower intra canopy VPD of the higher density treatment, since WUE and VPD are reversely correlated variables (Sinclair et al., 2017). The second chapter (See chapter 2.2), focused on sorghum, showed that there was a genotypic variability in the response to density. Indeed, we reported significant genotype-by-density interactions for the biomass and WUE increase in response to density (See chapter 2.2), indicating that genotypes responded differently to the increase in sowing density. We also showed a strong and positive relation between the increase in WUE and the increase in biomass and, in contrast to pearl millet, a strong genotype x density effect for WUE. Following this, chapter 3 reported a strong genetic variability and high heritability in the degree of the transpiration response to the evaporative demand of sorghum genotypes. The relationship between the slopes of this response and WUE was highly and positively correlated in high evaporative demand seasons but not in low evaporative demand seasons. Thirdly, the increase in WUE under high density was strongly and positively related to the increase in biomass, which we interpret as potentially related to the strong genotype x density interaction that we have highlighted in sorghum (for WUE but also for biomass response to density). These results prompted us to undertake the same comparison with pearl millet using the same method. To this end, this work aimed to assess the degree of genotypic variation of the transpiration response to the evaporative demand of pearl millet genotypes, and to assess a putative link with the genotypic variation for WUE.



Genotypes	Slope	R^2	p-value	X^2	p-value X^2	Preffered model
9444	0,68	0,55	<0,0001	0,06	0,691	Linear regression
86 M 86	0,68	0,58	<0,0001	0,06	0,707	Linear regression
86 M 88	0,67	0,58	<0,0001	0,06	0,668	Linear regression
APH 43	0,68	0,57	<0,0001	0,05	0,735	Linear regression
Bio 451	0,67	0,58	<0,0001	0,05	0,710	Linear regression
Bio 549	0,61	0,59	<0,0001	0,09	0,473	Linear regression
BLMPH 105	0,66	0,56	<0,0001	0,05	0,736	Linear regression
GK 1183	0,76	0,57	<0,0001	0,18	0,328	Linear regression
GK 1235	0,67	0,59	<0,0001	0,06	0,667	Linear regression
GK1207	0,65	0,55	<0,0001	0,09	0,581	Linear regression
HT 416628	0,67	0,58	<0,0001	0,06	0,672	Linear regression
HYMH 5	0,64	0,59	<0,0001	0,08	0,579	Linear regression
HYMH 8	0,66	0,57	<0,0001	0,05	0,745	Linear regression
JKBH 1352	0,74	0,49	<0,0001	0,03	0,895	Linear regression
JKBH 1490	0,68	0,56	<0,0001	0,06	0,716	Linear regression
KH 3022	0,68	0,55	<0,0001	0,05	0,768	Linear regression
NBH 5863	0,68	0,58	<0,0001	0,07	0,619	Linear regression
NU 399	0,69	0,60	<0,0001	0,08	0,602	Linear regression
NU409	0,73	0,53	<0,0001	0,04	0,844	Linear regression
Super Boss	0,67	0,57	<0,0001	0,09	0,581	Linear regression

***Table 3.2.1**:* Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for the 20 genotypes tested in India. Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software)

Hypothesis

Parting from the differences in the genotype x density interaction effects for biomass and WUE reported between the two crops (significant for sorghum, non-significant for pearl millet), and on the significant and positive relationship between WUE and the slope of the transpiration response to the evaporative demand in sorghum, we hypothesized that the response of the evapotranspiration to the evaporative demand may be different in pearl millet compare to sorghum crops.

The goal of the following work was then twofold: i) test whether we can find genetic variability in the degree of response of the evapotranspiration to the evaporative demand in two panels of 20 and 30 pearl millet varieties and ii) relate this putative variability in the response to WUE and biomass under high density canopies condition.

Material and method

Evapotranspiration response to evaporative demand

From the different lysimeter trials carried out using the pearl millet material and under different evaporative demand conditions and locations described in the first chapter, the same approach as for sorghum in the third chapter was used. We computed averaged weekly values of evapotranspiration (ETr) of each genotype in both high and low density and in both the dry and rainy season of experiments carried out in India and the dry season in Senegal to generate a regression, plotting the ETr values against the evapotranspiration reference (ETref) corresponding to the same time window (usually 7-10 days). This evaporation potential was calculated using APSIM modelling software in the two trials in India or with an ET ref calculated via the Penman-Monteith equation (Zotarelli et al., 2014) and corrected with a crop constant (Kc) taking into account the leaf area of pearl millet crops according to the development stage for the trial in Senegal.

Measurement of the water use efficiency

Data from the three lysimetric trials carried out in India and Senegal in different seasons and described in the first chapter were used (See chapter 2.1). The two trials in India were done in a dry and a rainy season while only a dry season trial was carried out in Senegal. In

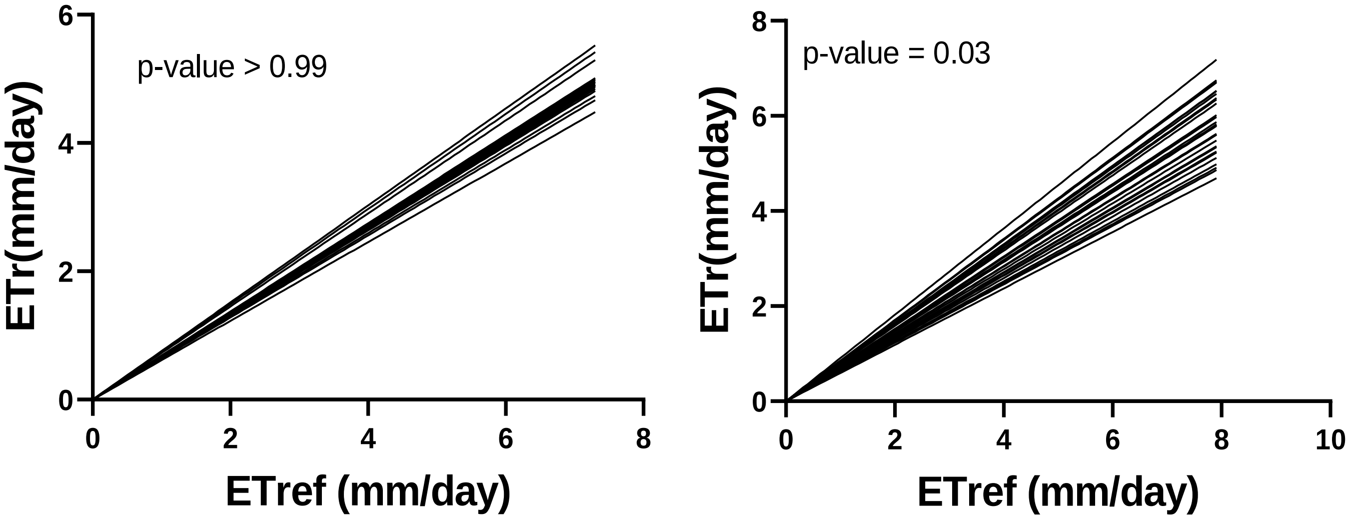


Figure 3.2.1: Measured evapotranspiration (ETr) plotted against the reference evapotranspiration (ETref), for 20 genotypes in the lysimeter platform in India (A) and 30 genotypes in the lysimeter platform in Senegal (B). regression lines for all studied genotypes (one line per genotype). Data are means of four and three replications per genotype and treatment in India and Senegal respectively (P-values < 0.0001).

both high and low density canopies, the total above ground biomass harvested and the total water use measured in the lysimeters trials allowed to calculate WUE in g of biomass per kilogram of total water use. Each value was the genotypic mean of the four replications disposed on the platform in each of the density treatments.

Leaf area assessment in the field trial

In India, the field trial from the 2018 dry season described in the chapter 1 (See chapter 2.1) was set up in order to assess the genotype response to density. This trial was also used to measure the leaf area index (LAI) in both densities of sowing and in each genotype of the panel of elites varieties. Measurement were done at two different date (33 and 40 days after sowing). The genotypic means values of LAI in the different date and density condition were plotted against the slope of the transpiration response to evaporative demand in order to ensure that the degree of response was not linked to differences in leaf area.

Results

Transpiration response to evaporative demand was not genotypic

In India, the regressions of the evapotranspiration values (ETr) against the corresponding evapotranspiration reference (ETref) showed significant regression (p-value = 0.0001) in all the genotypes with R^2 ranging from 0.49 to 0.60 (Table 3.2.1). Second order polynomial regressions were also calculated to possibly reveal a potential plateau in the transpiration response with increasing values of ETref. None of the genotypes had significant quadratic terms (p-values ranging from 0.473 to 0.885) (Table 3.2.1). We therefore consider the most fitted model was a linear regression for all of the genotypes, as it was the case in sorghum (See chapter 3.1). Most importantly, the slopes of the linear regressions were not significantly different among the 20 genotypes (p-value = 0.99) (Fig. 3.2.1).

From the dry season trial in Senegal, significant regression between the ETr and the ETref values (calculated via the Penman-Monteith equation corrected with Kc) were found in 28 of 30 genotypes tested (p-value ranging from 0.01 to 0.39) but with low values of R^2, ranging from 0.04 to 0.29 (Table 3.2.2). This was likely related to the limited range of evaporative demand during that season. Here also no significant quadratic term was found in any of the regressions.

Genotypes	Slope	R^2	p-value	X^2	p-value X^2	Preffered model
CHAKTI	0,94	0,09	0,043	-0,05	0,634	Linear regression
GB 8735	0,95	0,07	0,061	-0,07	0,453	Linear regression
ICMB 177002	1,19	0,13	0,012	-0,05	0,642	Linear regression
ICMH 177111	0,99	0,18	0,003	-0,05	0,668	Linear regression
ICMHIS 14006	1,10	0,17	0,003	-0,06	0,551	Linear regression
ICMHIS 14007	1,20	0,18	0,003	-0,01	0,934	Linear regression
ICMP 177001	0,86	0,09	0,039	-0,06	0,604	Linear regression
ICMP 177002	0,83	0,17	0,003	-0,04	0,655	Linear regression
ICMP 187092	1,07	0,05	0,119	-0,10	0,432	Linear regression
ICMP 187093	0,98	0,09	0,036	-0,08	0,463	Linear regression
ICMR 08888	0,90	0,24	0,000	-0,02	0,823	Linear regression
ICMV 147141	1,01	0,18	0,003	-0,03	0,780	Linear regression
ICMV 147142	1,13	0,09	0,044	-0,05	0,679	Linear regression
ICMV 147143	1,23	0,25	0,000	-0,03	0,752	Linear regression
ICMV 147144	1,27	0,16	0,006	-0,05	0,540	Linear regression
ICMV 167001	0,93	0,10	0,029	-0,06	0,587	Linear regression
ICMV 167002	0,96	0,15	0,007	-0,06	0,560	Linear regression
ICMV 167003	1,16	0,14	0,010	-0,08	0,446	Linear regression
ICMV 167004	1,02	0,25	0,000	-0,04	0,659	Linear regression
ICMV 167005	0,83	0,12	0,014	-0,07	0,496	Linear regression
ICMV 167006	1,02	0,11	0,019	-0,08	0,538	Linear regression
ICMV 167012	0,99	0,26	0,000	0,01	0,898	Linear regression
ICMVIS 89305	1,14	0,17	0,003	-0,06	0,633	Linear regression
ICMVIS 92222	1,02	0,15	0,007	-0,07	0,583	Linear regression
ICMVIS 94206	0,97	0,29	<0,0001	0,04	0,625	Linear regression
ICMVIS 99001	1,17	0,19	0,002	-0,04	0,667	Linear regression
LCICMV-4	0,87	0,26	0,000	-0,04	0,684	Linear regression
PE08043	1,06	0,13	0,011	0,00	0,971	Linear regression
SL 423	0,91	0,10	0,027	-0,05	0,592	Linear regression
SOSAT-C88	0,86	0,11	0,020	-0,07	0,486	Linear regression

***Table 3.2.2**:* Values of slopes, R^2, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for the 30 genotypes tested in Senegal . Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software)

Therefore, a linear regression model was also considered the best fit for all genotypes in this trial (Table 3.2.2). Differences between slopes values were slightly significant in this case (p-value = 0.03).

No link between transpiration response and leaf area

No significant relationship was found between the transpiration response of the genotypes to ETref in the lysimeters (i.e. the slopes of the regressions) and the leaf area index measured in the adjacent field in none of the density conditions nor date of measurement in the Indian trials (Fig. 3.2.2)

Water use efficiency was not related to the slope of the response of the evapotranspiration to the evaporative demand

We then evaluated the putative link between the slopes of the regressions between the evapotranspiration and the evaporative demand (Table 3.2.1 and 3.2.2) and the WUE of the genotypes in the three experiments. We tested separately the dry and rainy seasons in India and the dry season in Senegal. To this end, we generated linear regressions of the genotypic mean value of the WUE as a function of the slope of the response of the transpiration to evaporative demand of the 20 and 30 genotypes from India and Senegal respectively. We found no significant relationship between the two variables in all three trials with p-values of the regression of 0.99 and 0.38 in the dry and rainy season of India respectively and 0.72 in the dry season of Senegal (Fig. 3.2.3)

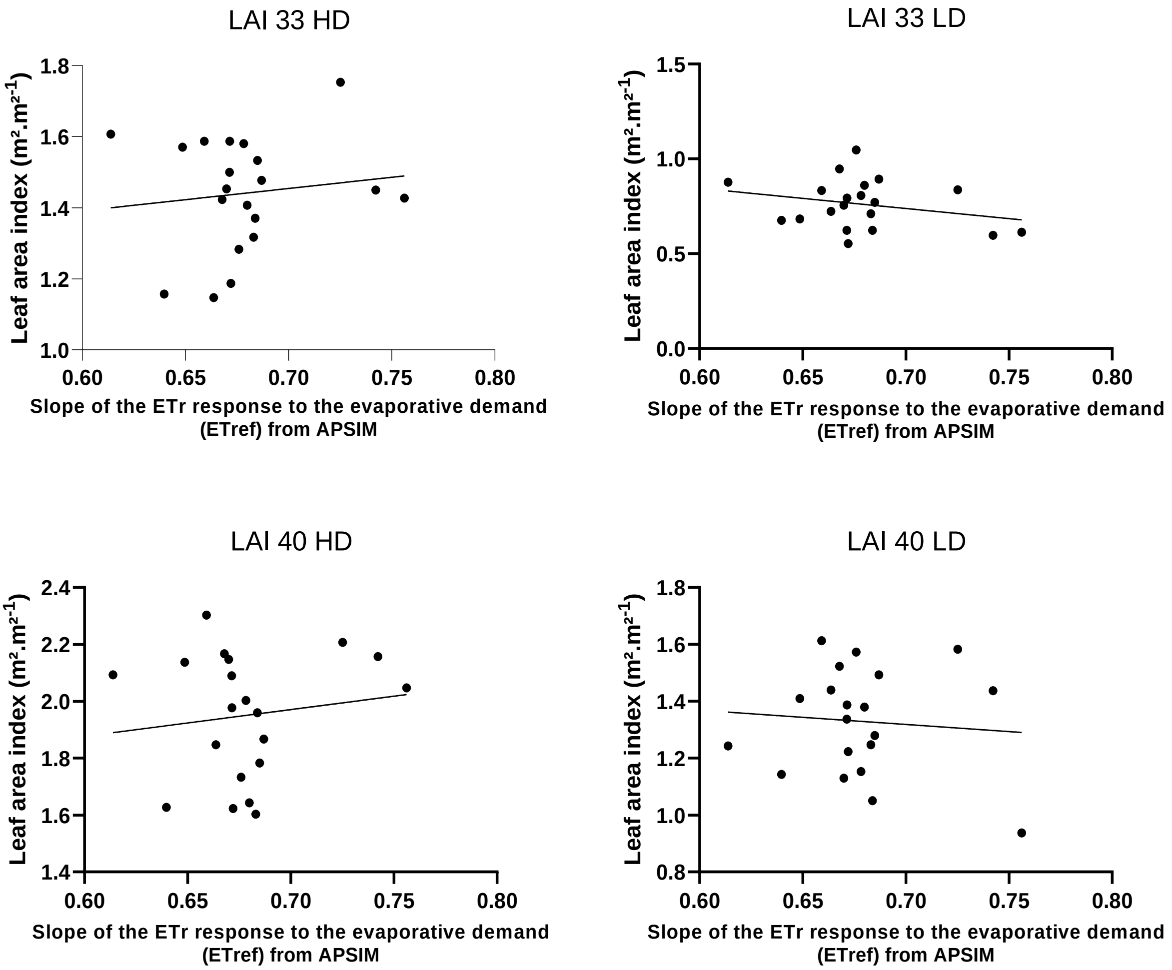


Figure 3.2.2: Leaf area index (LAI) measured 33 days (A) and 40 (B) days after sowing in both high and low density in the 2018 dry season field experiment as a function of the slope of the transpiration response to evaporative demand (ETref) from the adjacent lysimetric experiment. (India).

Discussion and conclusion

The response of the evapotranspiration to the evaporative demand, measured in two panels of 20 and 30 genotypes, showed either no or very limited variation. In addition, there was no significant relationship between the slope of the transpiration response to the evaporative demand and WUE. These results were opposite to those in sorghum.

Such results are in line with the previous results generated on pearl millet in this study. Indeed, in section 2.1 we reported no genotypic variation in the biomass and WUE response of pearl millet to high density, and we showed that the positive crop response to high density occurred only in climatic conditions with no rainfalls and high radiations. These results reinforce the strength of our findings in sorghum where we showed a strong positive link between the increase in biomass accumulation and the increase in WUE in high-density conditions, and between WUE and the degree of the transpiration response to evaporative demand during dry seasons may explain the genotypic variability in the tolerance to high-density for sorghum, which was not the case in pearl millet. The different traits measured in the different location and seasons are synthetized in the table 3 with the degree of response and/or interaction between genotype and density effect. Interestingly, these differences between the two species suggests that other mechanisms could explain the intrinsic genetic variability for the WUE measured in pearl millet within density treatment that we found in this study. Indeed, even if there was no genotype x density interaction for WUE interaction, still there is large genotypic differences for WUE in both high and low density WUE taken separately that we were not able to explain as in sorghum.

We may interpret that the light distribution along the vertical axis does not vary among pearl millet genotypes tested in this work. However, VPD was lower also inside the dense canopies of pearl millet and this would have explained the increase in WUE. Therefore, it is reasonable to think that the lower VPD in high-density canopies would have allowed a significant increase in WUE in all genotypes equally (no genotype x density interaction), but the lack of variability among them for light distribution would have disallowed to discriminate genotypes for the transpiration response to ETref. The very different architecture of sorghum and pearl millet could be the reason of such differences. Indeed, while sorghum is low tillering, has wider leaves and long internodes, pearl millet tends to be bushier, and has more tillers and leaves. This could have reduced the variability for light transmission through the canopy, as well as the response to the evaporative demand, and *in fine* the variability in the response of WUE and biomass

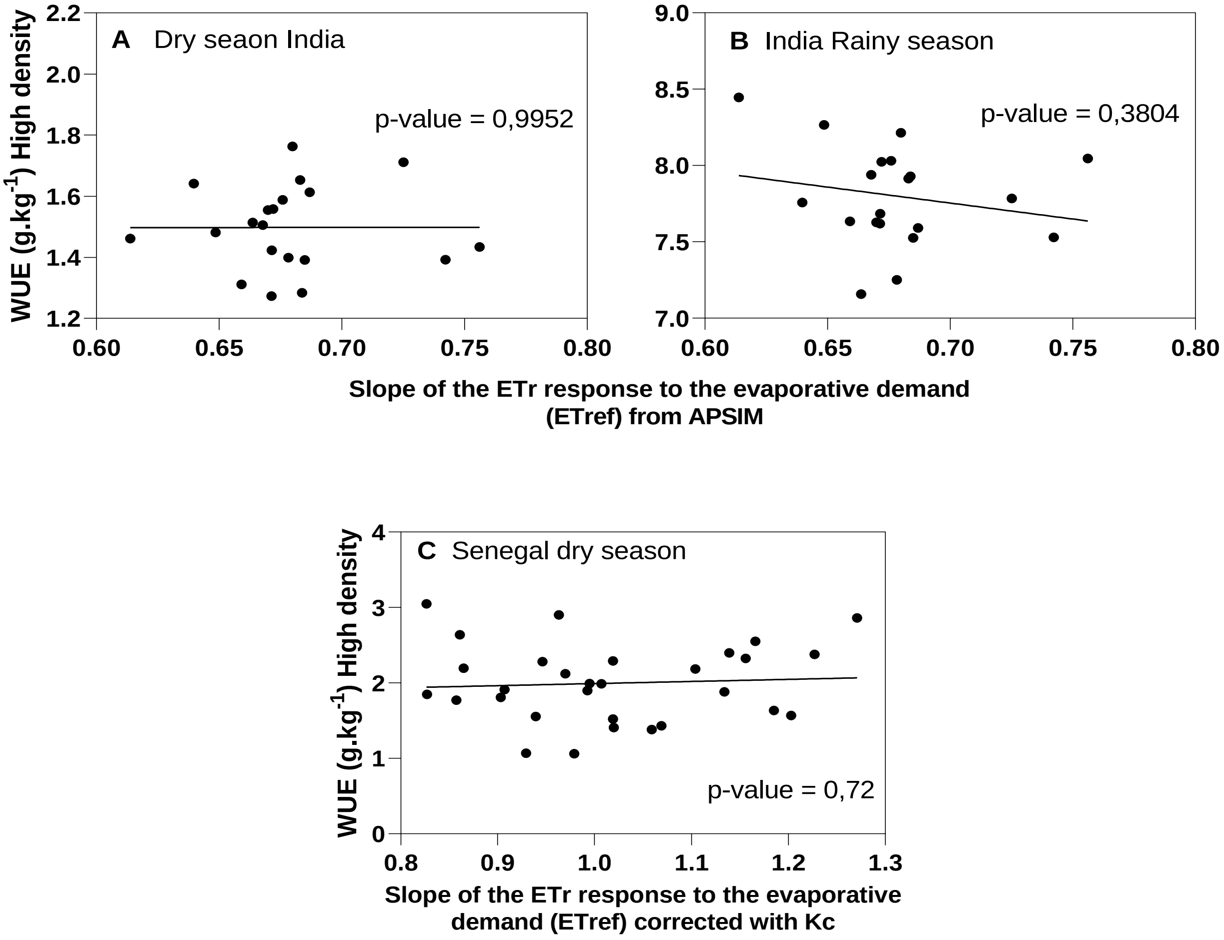


***Figure 3.2.3**:* Water use efficiency (WUE) plotted against the slopes of the evapotranspiration response to ETref (see figure 1). (A) 20 genotypes of genotypes tested in the dry season, India. (B) 20 genotypes in the wet season, India. (C) 30 genotypes in the dry season, Senegal. Data are means of four replications per genotype and treatment.

accumulation to a higher density. These are assumptions because an assessment of light distribution within pearl millet canopies was not done and should be undertaken in a large panel of pearl millet varieties in order to test above-mentioned hypothesis. Pearl millet shoot architecture is very little studied and the few papers on dealing with radiation diffusion in the canopies cannot be extrapolated to the present case due to the low density of planting in regions (Begue et al., 1991) as it can be for sorghum (Gitz Iii et al., 2015).

In this study, and similar to sorghum, there was no plateau in the regressions of the transpiration response to the evaporative demand. This is contrary to earlier studies carried out in grow chambers (Kholová et al., 2016; Sinclair et al., 2017). This highlight the differences we can find in the response of the plants to evaporative demand considering canopies experiencing large range of light and VPD conditions leading to an uncoupling between stomatal conductance and transpiration in pearl millet as well, in opposition to at what is observed in single plants, cultivated in growth chamber with limited variation in climatic conditions and low and stable light intensity. Moreover, the absence of sign of transpiration restriction also suggest that a substantial part of the transpiration occurred in the deep part of the canopies and is decoupling from air-canopy interface. The major difference with sorghum would therefore be the absence of significant variation in the contribution of this lower leaves transpiration in the global transpiration of the plants.

Specie	***Sorghum***	***Pearl millet***
Response to density treatment (D Effect) for yield and biomass	++	++ in dry season + in wet season
Genotypic response (GxD interaction effect) for yield	-	-
Genotypic response (GxD interaction effect) for biomass	+	-
Response to density treatment (D Effect) for WUE	+++	++
Genotypic response (GxD interaction effect) for WUE	++	-
Genotypic variation in the ETr response to ETref	+++	-
Relationship between slope of ETr response to ETref and WUE	++ in dry season ++ in wet season	-- --
Relationship between light penetration depth and WUE	++ in dry season ?? in wet season	?? ??
Relationship between biomass increase and WUE increase	++ in dry season - In wet season	??

Table 3.2.3: Synthesis of the differences between sorghum and pearl millet crops to density regarding the traits measured in the different experiment and seasons.

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General discussion and conclusion

The increase in plant density can increase yield in many crops species. The present work highlighted that this was also possible in sorghum and pearl millet crops in semi-arid regions. Indeed, field trials showed positive response of biomass and grain yield in the different locations and varieties tested with a strong density effect on both variable. We also showed a positive effect of the high-density treatment on the whole-plant water use efficiency (WUE) in both crops in contrasting environments. The increase in WUE due to high-density treatment varied with genotype in sorghum, but not in pearl millet. The monitoring of plant transpiration in the lysimetric trials allowed to develop a method assessing the evapotranspiration response to the evaporative demand using long time intervals (7-10 days), from which large genotypic variation were found in sorghum, but not in pearl millet. These results shed new light on the possibility to breed for density tolerance and WUE in sorghum.

The following sections recall the main hypotheses that were drawn up in the introduction and discuss how much the findings from the results validate or not these hypotheses. Further research needed to build on the results of this thesis work are also discussed.

Sorghum and pearl millet yield response to density

The agronomical work done on pearl millet showed a positive effect of increased sowing density for both biomass and grain yield. Consistent and positive reaction to increased density were found in the different studied locations and in almost all tested genotypes except during the 2020 rainy season that had a high number of rainy days and low average solar radiation during the crop cycle. The benefit of high density was highest in season with high radiation and vapor pressure deficit, revealing that increasing density could be a way to cope with these harsh environmental conditions. The strong influence of climatic conditions on yield response to high density of varieties that are already in use by farmers suggests that sowing density might be adapted to given areas and associated climate. Regarding sorghum, it appeared that, unlike pearl millet, a genotype x density interaction was found in biomass and WUE response to increased density. This genotypic variation in biomass and WUE response was observed in all material, seasons and locations. This opens an avenue to breed to density tolerance in sorghum crops, and the next step would be to screen large panel of genotypes for density tolerance in the field but also for the different physiological traits related to the tolerance in this thesis.

Soil coverage hypothesis

A fast soil coverage from higher density stand is expected to save water used by soil evaporation, and is an hypothesis to explain higher response to increased density (T. Winkel, 1992) and quickly intercept the maximum amount of light as it has been recently described in the literature (P. Barbieri et al., 2012; Echarte et al., 2020; Hernández et al., 2020). The lysimetric analyses on sorghum and pearl millet grown under two plant densities have shown that this soil protection effect, although existing, was limited. Results showed indeed that the part of water loss due to soil evaporation in low-density treatment was 10% of the total water use (averaged on 20 genotypes). WUE in the same experiment was increased by more than 20% under high-density condition (average for 20 genotypes), and up to 60% for some of the most responsive varieties of sorghum. Moreover, WUE increased only 3% by protecting the soil from evaporation. In field experiments with pearl millet, we also showed that the combination of soil protection from solar radiation and the increased WUE preserved soil water content from a larger depletion in high density compare to low density. It was surprising to find more water in the deepest part of the soil profiles under the higher density stand in both India and Senegal. The reason for that may involve differences in the radiative balance between the two density treatment. In particular, soil evaporation from the field in the low-density stands may have been higher than those estimated in the lysimeters.

WUE benefit from high-density: a VPD effect.

In the different lysimetric trials carried out in the scope of this work, we showed that the high-density treatment increased significantly the total water use of the crops but, proportionally, the increase in the accumulation of biomass was higher, leading to an increase in water use efficiency (WUE). According to (Sinclair et al., 2017) WUE is a variable reversely related to the VPD the plants are exposed to. At the leaf level, the following equation is proposed by Condon et al., 2002 to calculate transpiration efficiency (TE): $TE = 0.6. Ca.\frac{1-\frac{Ci}{Ca}}{(Wi-Wa)}$ where $\frac{1-\frac{Ci}{Ca}}{(Wi-Wa)}$ is the ratio of CO_2 assimilation rate to transpiration rate at the stomata. If much research took place to improve photosystems and decrease the $\frac{Ci}{Ca}$ ratio, the $Wi - Wa$ term of the equation has rather been considered a mere environmental factor that can be simply impacted by a change in the climate surrounding the plants. A lower evaporative demand (i.e the higher is the relative humidity in this case) would lower the Wi-Wa term so that TE would be higher. Such decrease of VPD conditions within the high-density canopies was highlighted in this work. We showed that the differences between in-canopy VPD and air-VPD was strongest during highest air-VPD conditions during the day and nullified below a certain threshold of ambient VPD (2kPa in pearl millet field trials). Under high VPD conditions (i.e above 2kPa), often encountered during the crop cycle, a density effect would then occur by creating a lower within-canopy VPD, allowing to decrease the W_i-W_a term of the equation of Condon et al., 2002, and to increase TE (or WUE in the case of field crops). However, this would only be possible if transpiration occured within the canopy, where those lower VPD conditions were measured. Different light measurement performed in sorghum and pearl millet crops revealed that light were still avalable in the canopies, even at advanced stages, and could ensure photosynthetic activity.

Measuring the transpiration response to ETref instead of VPD only

In order to validate that the genotypic response of transpiration depended on more variable than sole VPD but also on light conditions in the canopies, we needed to integrate other components of the transpiration drivers, for instance including solar radiation. Recent literature related to the transpiration restriction trait has only focused on VPD being the factor triggering the restriction. However, plant evapotranspiration is also driven by light or wind. Therefore, we developed a method to measure the transpiration response to the evaporative demand in the broad sense, taking the reference evapotranspiration (ETref) calculated in crops with the Penman-Monteith equation. The equation allow estimating a potential evapotranspiration of crops for any climatic parameters, which are mainly temperature, relative humidity, wind and solar radiation. Solar radiation plays a large role in the equation and its use was an interesting approach to compare the reaction of the crops to natural and varying climatic conditions, particularly in the case of multi-seasonal lysimetric trials, largely differing for solar radiation conditions, but less so for VPD, as it was the case in this study.

Thus, the transpiration response was measured against a wider range of evaporative demand conditions than VPD alone (2mm to 7mm/day), as the two seasons (dry and rainy) in India could be combined. In Senegal also, the range of ETref values was wider (effect of light and wind added to the variation) than VPD alone in the course of the crop cycle, despite having only one season. The regressions of ETref versus ETr followed linear models in all experiments, and did not show any sign of a plateau in response to high evaporative demand values. This showed that none of the genotypes tested restricted transpiration as shown earlier in experiments considering VPD only. However, some genotypes of the tested panel had previously been reported to show transpiration restriction in the form of a breakpoint or plateau in experiments conducted in a growth chamber with isolated plants and not grown as a canopy, and using only VPD as the driver of transpiration (Karthika et al., 2019). In these experiments, plants were separated from each other, with good air circulation among them so that all leaves must have been exposed to the same air VPD, and light was constant.

Explaining the differences in ETr response to ETref

Then, a large variation in the slopes of the transpiration response to ETref was found, and these slopes were not related to the LAI. These slopes were also positively related to WUE in the high evaporative demand seasons, not in low evaporative demand seasons. We also found that WUE was also positively related with more light availability deep inside the canopy, at times when in-canopy VPD was also lower than air VPD. Our interpretation of these results is that genotypes capable of allowing the most light into the canopy were able to maintain transpiration, and therefore photosynthetic activity, at canopy levels where we measured lower VPD conditions than at the air-canopy interface. Transpiration from leaves inside the canopy then helped sustained a steeper slope of the ETref/ETr regression and increased WUE because this part of the plant transpiration took place under lower VPD. On the contrary, genotypes that did not allow light to penetrate inside the canopy, had most of the transpiration coming from the top of the canopy that is exposed to high values of air VPD.

Photosynthesis and light: a vertical gradient to explore in sorghum?

As above-mentioned, the increase in WUE due to high-density treatment showed large genotypic variation in sorghum, although not in pearl millet. The degree of response of WUE was also strongly linked to the degree of response of the biomass, particularly in high evaporative demand season. Such seasons are also characterized by saturating light conditions that can go deeper through the canopies.

It is described that the Rubisco enzyme activity decreases with depth in plant canopies. This is commonly determined through the hypothesis that the limiting rate of carbon uptake (*V*max) vary with canopy depth as the photosynthetically active radiation decreases (PAR; e.g, Sellers et al., 1992). With such an assumption, canopy properties, such as *V*max and potential electron transport rate (*J*max) of the whole plant could be related to the corresponding measurable properties of the leaves at the canopy top only, lower leaves not contributing significantly to whole plant photosynthetic activity. But in a context of saturating light conditions as in the dry season lysimeter trial presented in this work, an appropriate architecture would allow a denser canopy letting some light in the lower part of the canopy in order to ensure photosynthesis and then vary the vertical distribution of Rubisco enzyme activity. This could be measured and the Rubisco enzyme activity deep inside the canopy would become a proxy of the light conditions of these leaves. Next research should then focus on an assessment of both transpiration of specific layers of canopies on the vertical axis and photosynthetic activity of these layers. Stomatal conductance (Farquhar & Sharkey, 1982; Henry et al., 2019) and photometric assessment of Rubisco activity (Sharkey et al., 1991) could be undertaken in field-like conditions grown plants, such as lysimetric design presented in this study and reveal putative genotypic variations for lowest part of the canopies photosynthetic activity.

New tools: phenotyping for density tolerance?

The genotypic variability in the response of sorghum cultivars to an increased density opens the opportunity to breed for density tolerance. Part of the positive response was related to an increase in WUE, itself explained by a deeper light penetration that allowed more of the photosynthesis to happen under the milder VPD conditions inside the canopy. Therefore, next research needs to target in priority the genetic variability for architectural traits, especially those involved in a deeper light distribution within the canopies. Today, 3D images analysis has become a chance to decipher plants traits with a resolution never reached. Image based phenotyping is now a common approach (Bao et al., 2019; Deery et al., 2014; Perez et al., 2016, 2019). Remote sensing is also available to generate 3D images from which traits of interest can be extracted. A phenotyping platform combining automated lysimetry and 3D imaging scanners (Phenospex, 2017) is available at IRD research center and research is currently in progress to compare contrasting genotypes in their architectural characteristics. An approach will be about formalizing architectural features measured through 3D images analysis, and relating them to physiological measurements described in the previous section.

G x E x M: When Management affects the Environment and then shift the target for Genetics, the case of sorghum and density.

Drought, whether due to low soil water reserves or hot and dry air, is a major constraint to crop production. In many regions, particularly in the semi-arid tropics (SAT), the usually high temperature and low relative humidity lead to a high evaporative demand that can be seen as an atmospheric drought, potentially deleterious for the crop accomplishment. If the evaporative demand is higher than the water supply needed the plant is living a period of water stress. Breeding effort are directed towards varieties that are increasingly resistant to these climatic events. This search for resistance to abiotic stresses and stable yield production should put the management at the center of the thought regarding the improvement of crops in drought-affected areas. The present work highlighted the need to better integrate the management in the adaptation of crops to extreme weather events and so the breeding effort that need to be associated with (i.e. the genetic targets to be addressed). Today, one strategy is to focus programs on the search for cultivars able to adapt to environmental constraints, considering that genetics will allow maintaining yield under such and such limiting conditions, water deficit for example. In addition, work is done on the possibility to avoid the stress spatially and temporally. For example through an optimized sowing date to avoid a long rainfall gap (Hajjarpoor et al., 2021) or selecting root traits allowing escaping from a future and predicted period of stress during the crop cycle, between sowing and 3 leaves stage for example (Hajjarpoor et al., 2022). Regarding sorghum, this work propose that being tolerant to higher plant stand would bring the possibility to conserve genetic progress already made on yield as we showed that common varieties already largely improved and considered as high yielding responded favorably to high density. To fit with high density tolerance, the traits linked with the yield performance under increased density have to be altruistic (not selfish, Weiner et al., 2010) allowing at best the sharing of the resource between individuals while allowing a group effect offering the implementation of more favorable conditions to the benefit of WUE and *in fine* the biomass and grain yield. Traits related to light distribution variability would then become the new target for breeding for density tolerance. Reversely, increasing density could be a way for crops to be more resilient to face high VPD in SAT climates. To this end, the material that would benefit most would be that which accommodates density and distributes light better. Such varietal improvement should therefore do this in a staggered way combining genetic and crop management.

Soil fertility was not an issue: root system is to explore

Soil fertility was not considered as a factor in this study. However, it is frequently questioned to what extend an increase in plant density would require a substantial amount of extra fertilization. In our case, the same treatments were applied in both density stands and soil fertility did not appear to be a limitation to the accomplishment of the crops cycles. Crop simulation ran in APSIM predicted a very limited decrease in biomass (2%) and grain yield (5%) in high density compared to low density with the same fertilization conditions (data non-show). In any case, the same fertilization in the two density treatments allowed a positive grain and biomass yield response under higher sowing density in both species. It is in agreement with P. A. Barbieri et al., (2008) who even indicated a greater yield response to reduced row spacing in low nitrogen field treatment. According to Sharratt & McWilliams, (2005), this may due to a better rearrangement of the root distribution with a bigger proportion of root length exploring deeper the inter row space. We may speculate that a denser and deeper rooting system would allow a better recovery of nutrient, especially highly soluble nitrates, and more work would be needed to quantify this benefit.

Our results regarding the yield in response to density in sorghum and pearl millet, which are largely grown in poor soils (Breman et al., 2001), is prompting us to investigate root systems plasticity in the tolerance to density. For example, it was shown in maize that the root angle was steeper under a nitrogen deficit, changing the pattern of root prospection in the soil (Trachsel et al., 2013). Therefore, variations in root traits such as the crown root angle could explain a part of the high-density tolerance and the yield performance in high-density condition. A preliminary work has been started in this sense in order to make a screen of the variability and potential effect on the density of the variation in root angle.

Models can be improved

With the first intention to generate ETref values including crop leaf area development, we ran several simulation with crop models. The chapter 3 evidenced a lack of accuracy of the model to integrate the density and output correctly a trustable prediction. In short, the models gave similar values of ETref for both high and low density canopies. In addition, grain and biomass yield predicted by models and above mentioned differs from what we measure in the field on a large majority of the genotypes tested. These results suggests an avenue to improve the prediction of the model by a better crop parametrization in the field and also by adding parameters related to variation in density response in the functions. Typically, a power equation is used in crop models to define the allometric relationship coefficients between plant leaf area and main-stem leaf number (Soltani, Robertson, Mohammad-Nejad, et al., 2006). Their relationship to plant density can also be found using different plant density data. To do this, the data for each plant density can be fitted with a power equation, and the relationship between the resulting coefficients and plant density can then be found (Hajjarpoor et al., 2022). Parameters that are used in the model estimation of biomass production are the leaf area index (LAI) and extinction coefficient (KPAR). Plant and canopy characteristics are combined into a single composite property, KPAR, which can be obtained experimentally from PAR interception and crop LAI measurements (Soltani, Robertson, Rahemi-Karizaki, et al., 2006), however, in this case, different layers of the canopy are not considered. Such improvement could better predict the

Density and pearl millet, an unintended target for farmers?

The response of pearl millet to an increased sowing density showed non-significant genotype effect, but the density treatment was highly significant, and particularly in seasons with high evaporative demand and saturating light conditions. Therefore, while breeding density tolerant pearl millet genotypes seems out of scope, at least from the results of the panels that were tested, it remains beneficial to increase the sowing density of existing cultivars, if environmental conditions that promote a positive response exist. Regions with low rainfall, high evaporative demand and high light intensity would be the preferred zone to increase the density. This was rather counter intuitive because such environments would be thought to be limited by water. Literature research regarding farmer's decision on varieties in drylands of India where evaporative demand and drought stresses occurs (Van Oosterom et al., 1996) highlighted a preference for high-tillering locals genotypes (Kelley et al., 1996; Van Oosterom et al., 1996). On the contrary, farmers from wetter regions where drought episodes have less probability to occurs preferred using varieties with less tillers and larger panicles (Van Oosterom et al., 1996; Vom Brocke et al., 2003). These choices are driven by the fact that high tillering ideotypes would allow tillers to produce grain if the main tiller failed because of a drought event. By doing these choices, and increasing the number of tillers and leaves composing the canopies, the farmers also plays unintentionally on the microclimate surrounding the plants according to the climatic conditions of the regions they are growing the crop. In other words, in areas offering saturating light and high evaporative demand the choice of cultivar with a large numbers of tillers would increase the density of the canopies as would a density treatment do. These choices in varieties are known to be related to the probability of the crop to face favorable conditions or not (Catherine Ragasa, Antony Chapoto, 1997) which is in line with the Density x Environment interaction we found in this study.

Higher density in pearl millet, where is the target?

According to the results highlighted in this work, next steps are to test the pearl millet response to density in farmers' field. The higher response found in the driest seasons and conversely the absence of response during wet and rainy seasons led to plan trials in different areas contrasting for the evaporative demand conditions. Sahelian regions differ for rainfalls with a direct impact on the solar radiation and evaporative demand conditions that the crops may face. In the groundnut basin of Senegal where pearl millet is largely grown, a rainfall gradient on the northwest southeast axis exists, with a range of 300 to 850 mm. Such differences may alter the degree of response of the crops to high sowing density and needs to be addressed. Moreover, the highly significant density treatment effect found in this work indicates that the varieties already in use by farmers would respond favorably (according to environmental conditions). Social and cultural values of the varieties is important in small holders farming system from these Sahelian regions, and it is now well referenced that maintaining agro-biodiversity is a key in the resilience of developing countries farmers (Coomes et al., 2015; Pautasso et al., 2013; Westengen et al., 2019). Being able to assess where an increase in density may be beneficial for crop yield, testing farmer's seeds already in use on a gradient of climatic conditions, changing only the density in the crop management, seems to be a promising way to quickly increase crop productivity. Such a project is currently being implemented on the ground in Senegal (Pilloni et al., 2022).

Conclusion

This thesis, at the interface between ecophysiology and agronomy, shows the interconnection between these two disciplines and the close link between the effect of crop management and the physiological response of plants to environmental conditions. It highlights the possibility of significantly affecting positively many traits of agronomic interest by varying only one parameter in crop management, in this case sowing density. The yield results, put in the climatic context of the semi-arid regions, seem to indicate the direct possible application of the increase of the sowing density in areas most likely to encounter conditions of high evaporative demand. It appeared that material responding favorably to this change in density was already in the hands of the farmers who depend on these food crops. Social-cultural and technical aspects are now the levers to be used to make these results effective in the field. A genetic door has also been opened by this work with the possibility of breeding for density tolerance, which could be a target for future breeding programs. The understanding of the parameters affecting the response of the crops to these density changes should also make it possible to adjust the output of the crop models in order to test different density scenarios in contrasted climatic areas conditions, mainly for VPD and solar radiation. This work calls for new experiments to decipher with a higher resolution the physiological mechanisms involve in the response of plants to the increase in sowing density. Several tools, involving several domains and technologies, including physiology, 3D imaging, genetic and agronomical study might work together in these future researches.

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Abstract

In the semi-arid tropics, sorghum and pearl millet or key source of income and calories. Sustainable intensification is therefore needed to ensure food security. These two cereals are largely grown in smallholder farming system and cultivated at low density, opening an avenue to increase yield through this agronomic management. Through field and lysimetric trials carried out in India and Senegal this work showed the possibility to increase significantly, the biomass and grain yield in both species, with the same irrigation regime and fertilization. We highlighted a lowering of the vapour pressure deficit (VPD) in the canopies of high density, resulting in an increase in water use efficiency of the crops. While both crops responded positively to increased density, there were also large specie differences in the genotypic variation of the response to density, namely a strong genotype x density interaction in sorghum for biomass and WUE, but none in pearl millet. The genotypic variation in the degree of WUE response found in sorghum and its link with biomass accumulation led to investigate putative differences in the transpiration response of the crops to the evaporative demand. We tested this hypothesis outdoors with canopy-grown sorghum plants in field and lysimeter experiments. The response of the evapotranspiration was measured against the evaporative demand. This response was linear and, with WUE, showed large genetic variation. WUE was surprisingly higher in genotypes with the highest transpiration response to the evaporative demand (Penman-Monteith). These genotypes were also those that allowed maximum light penetration into the canopy. This work opens the door to intensification, in the short term by increasing sowing density in drylands using sorghum and pearl millet cultivars that show a strong response to density, and in the medium term by selecting sorghum cultivars adapted to high density.

Key Words: Sowing density, sorghum, pearl millet, intensification, semi-arid tropics.

Résumé

Dans les régions tropicales semi-arides, le sorgho et le mil sont des sources essentielles de revenus et de calories. Une intensification durable est donc nécessaire pour assurer la sécurité alimentaire. Ces deux céréales sont en grande partie cultivées dans des systèmes de petites exploitations et cultivées à faible densité, ce qui ouvre la voie à une augmentation du rendement grâce à cette gestion agronomique. A travers des essais au champ et des essais lysimétriques réalisés en Inde et au Sénégal, ce travail a montré la possibilité d'augmenter significativement la biomasse et le rendement en grain des deux espèces, avec le même régime d'irrigation et la même fertilisation. Nous avons mis en évidence une diminution du déficit de pression de vapeur (VPD) dans les canopées de haute densité, résultant en une augmentation de l'efficacité d'utilisation de l'eau des cultures. Alors que les deux cultures ont répondu positivement à l'augmentation de la densité, il y avait également de grandes différences entre les espèces dans la variation génotypique de la réponse à la densité, à savoir une forte interaction génotype x densité dans le sorgho pour la biomasse et la WUE, mais aucune dans le millet perlé. La variation génotypique dans le degré de réponse pour WUE trouvée chez le sorgho et son lien avec l'accumulation de biomasse ont conduit à étudier les différences putatives dans la réponse de transpiration des cultures à la demande evaporative. Nous avons testé cette hypothèse en plein air avec des plants de sorgho cultivés en canopée dans des expériences en champ et en lysimètre. La réponse de l'évapotranspiration a été mesurée en fonction de la demande evaporative. Cette réponse était linéaire et, avec le WUE, a montré une grande variation génétique. Le WUE était étonnamment plus élevé dans les génotypes avec la réponse de transpiration la plus élevée à la demande evaporative (Penman-Monteith). Ces génotypes étaient également ceux qui permettaient une pénétration maximale de la lumière dans la canopée. Ce travail ouvre la porte à l'intensification, à court terme en augmentant la densité de semis dans les zones sèches en utilisant des cultivars de sorgho et de millet perlé qui montrent une forte réponse à la densité, et à moyen terme en sélectionnant des cultivars de sorgho adaptés à une densité élevée.

Mots clés : Densité de semis, sorgho, mil, intensification, tropiques semi-arides.