



# Heterogeneity effects of plant density and fertilizer application on cowpea grain yield in soil types with different physicochemical characteristics

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## ABSTRACT

**Context or problem:** Although high-density cropping and fertilizer application are plausible techniques to improve crop production, there are difficulties in the growth environment that may undermine the positive effects.

**Objective or research question:** This study focused on soil types as a major environmental factor of the heterogeneity effects of plant density and fertilizer on grain yield. The objectives were to clarify the responses of grain yield to high plant density and fertilization under soil types with different physicochemical characteristics.

**Methods:** Three cowpea genotypes (KVx61-1, KVx421-2J, and Dja) were grown in three dominant soil types in the Sudan Savanna: Ferric Lixisols (LXfr), Petric Plinthisols (PTpt), and Pisoplinthic Petric Plinthosols (PTpt.px). The experiment was conducted two consecutive years in 2018 and 2019. The effect of plant density (31,250 and 62,500 plants ha<sup>-1</sup>) and fertilizer application (non-fertilizer and fertilizer application at N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 14:23:14 kg ha<sup>-1</sup>) on grain yields were evaluated. The fertilizer was applied with two different methods: basal dose and split dose.

**Results:** A larger yield increase by high plant density and fertilization was observed for PTpt and PTpt.px than for LXfr. Split dose of fertilizer drastically increased yield when combined with high plant density. However, fertilizer leaching was occurred for split dose applied during the peak of rainy season, which was larger in PTpt and PTpt.px because of higher topsoil permeability. The differences in grain yield among the cowpea varieties were attributed to the shoot growth and flowering date that were differently affected by plant density and fertilization. **Conclusions:** Excessive moisture stress depressing shoot growth and delaying flowering was a cause of the heterogeneity effects of high plant density and fertilization on grain yield. The stress was more severe in soil of lower topsoil permeability. In contrast, soil types with higher topsoil permeability caused late-season drought for the plants with delayed flowering. Split dose of fertilizer was a strong option for yield increase but the risk of fertilizer leaching should be considered. Genotypic differences in the yield in response to plant density and fertilizer application were presumably influenced by the ability to adapt to excessive soil moisture and drought conditions. **Implications or significance:** Plant density and fertilizer application should be optimized according to the soil type of the target region. Its optimization would help to achieve food security and better economic income of small households of the region.

## 1. Introduction

Grain yield of cowpea (*Vigna unguiculata* (L.) Walp) in West Africa has increased more than 2 times in the last 55 years; however, the 5-year average yield (0.57 t ha<sup>-1</sup>) is still lower than the potential yield (2–3 t ha<sup>-1</sup>) (Food and Agriculture Organization of the United Nations (FAO), 2022). In this region, cowpea cultivation has been conducted using traditional methods that strongly rely on natural environments, such as soil fertility and precipitation. Sandy soils in West Africa derived from

old basement rocks though repeated cycles of weathering or sand deposit from the Sahara Desert have low base reserve and soil organic matter level (Funakawa and Kosaki, 2017). Furthermore, soil erosion decreases soil nutrient and productivity (Stoorvogel and Smaling, 1990; Lal, 1995). Unstable precipitation causes variation in annual yield that accounts for 12 % of average yield, which is twice than that in United States (Food and Agriculture Organization of the United Nations (FAO), 2022). Precipitation in Sudano-Sahelian countries in West Africa has been predicted to decrease in the future, leading to an 18 % decrease in

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crop production (Roudier et al., 2011). Iizumi and Wagai (2019) reported that the effects of drought on crop production are accelerated by soil degradation. The environment surrounding cowpea production in West Africa is worsening but the importance of cowpea production will not change or become more apparent because farmers cultivate cowpea not only for consumption but also for selling to compensate for the scarcity of the main cereal crops (Shiratori et al., 2020). Therefore, traditional cultivation needs to be improved to alleviate the negative effects of the environment and increase cowpea yield.

High-density cropping and fertilizer application are simple techniques for small households to improve and stabilize grain yield. The effectiveness of these techniques has been confirmed in many studies performed in several environments (Kamara et al., 2018; Kwapata and Hall, 1990; Ishikawa et al., 2022). High-density cropping increases plant biomass and pod numbers per unit area (Kamara et al., 2018). As poor shoot growth is a primary yield-limiting factor under low fertility soil conditions (Iseki et al., 2021), the increase in shoot biomass directly improves pod number and grain yield. Kamara et al. (2018) reported that the grain yield increased by 78 % when the plant density was increased from 133,333 to 400,000 plants ha<sup>-1</sup>. Moreover, fertilizer application is also a suitable alternative for increasing yield by accelerating shoot growth and grain filling; however, its implementation cost is much higher than that of high-density cropping. In the case of legumes, nitrogen fertilizer application is known to decrease symbiotic nitrogen fixation (Elowad and Hall, 1987); however, it still has a large positive effect on grain yield, especially under conditions of low soil fertility (Chen et al., 1992). Because nitrogen fixation is influenced by phosphorus availability (Chen et al., 2011), phosphorus fertilization is effective in improving the yield.

Although high-density cropping is a plausible technique to improve traditional cowpea cultivation, there are difficulties in the growth environment that may undermine the positive effects. For example, high plant density promotes root water competition under dry soil conditions, thereby reducing grain yield (Kamara et al., 2014). Moreover, increasing plant density under dry soil conditions reduces flower production and increases flower abortion (Lemma et al., 2009). In contrast, Craufurd and Wheeler (1999) reported no relationship between seed yield and plant density under drought conditions. This inconsistency might be due to environmental differences, such as drought intensity and soil fertility. Plant responses to high plant density are also influenced by genotypic differences in shoot biomass, plant type (erect or prostrate), flowering type (determinate or indeterminate), and maturity (Egli, 1988; Kwapata and Hall, 1990).

Compared to the effect of plant density, fertilization has more stable effect on cowpea yield; however, the effect of fertilizers on plant growth and grain yield is still not well understood. Nitrogen application at the early flowering stage increased the number of branches and pod yield of cowpea, whereas that at sowing was less effective (Elowad and Hall, 1987). Ishikawa et al. (2022) reported that the cowpea grain yield was the highest when NPK compound fertilizer was applied at the early growth stage (four weeks after sowing); however, the effect differed by plant density and variety. In other cases, NPK application to cowpea just after sowing increased the net economic return by more than 30 % in different ecological zones (Abdul Rahman et al., 2018). Such complexity in the effects of fertilization might also be due to differences in soil water availability and soil fertility. Farmers hesitate to apply fertilizers because of these unpredictable factors as the implementation cost might not be recovered by the yield.

Based on the results of the above studies, soil water availability and fertility are major factors controlling the effects of high plant density and fertilizer application. In the Sudan Savanna, two dominant soil types, Lixisols and Plinthosols, with different chemical and physical properties, are widely distributed on a continental scale (Dewitte et al., 2013). At the local scale, the soil types are classified as Ferric Lixisols (LXfr), Petric Plinthosols (PTpt), and Pisoplinthic Petric Plinthosols (PTpt.px) according to the latest soil classification system (IUSS

Working Group WRB, 2015), and their geographical distributions are closely related to the position in the rolling hills (Ikazaki et al., 2018a). In our previous study, annual cowpea yield variation differed depending on these soil types (Iseki et al., 2021), indicating that yield responses to high plant density and fertilizer application might also be different for different soil types; however, this interaction has not yet been revealed.

In this study, the yield responses of cowpea genotypes to high plant density and fertilizer application were analyzed in the three dominant soils of the Sudan Savanna. The objectives of this study were to clarify the responses of shoot growth and grain yield to high plant density and fertilization under the three soil types. Then, these responses were compared among the cowpea genotypes to determine plant traits for better yields. The adaptability of high plant density and fertilizer application in each soil type was discussed to improve traditional cowpea cultivation and obtain a better and more stable yield.

## 2. Materials and methods

### 2.1. Experimental fields

Three experimental fields with different soil types (LXfr, PTpt, and PTpt.px) were selected in the Institute of Environment and Agricultural Research (INERA) Saria station located in the Central Plateau of Burkina Faso (12°16' N, 2°09' W, 300 m.a.s.l.), where rarely undulated landscape is expanded. The soil properties in each field are shown in Table 1 (after Ikazaki et al., 2018a). LXfr is observed at the lower to toe slope and has relatively higher fertility level but lower soil permeability in the topsoil caused by soil crusts. PTpt.px is observed around the upper slope and has relatively lower fertility level but higher soil permeability in the topsoil caused by macropores. PTpt is observed in between LXfr and PTpt.px and has soil properties intermediate between them. The major difference between PTpt.px, PTpt, and LXfr is the thickness of the soils overlying the petroplinthic horizon (Iron hardpan): about 25, 50, and 100 cm for PTpt.px, PTpt, and LXfr, respectively. Each field was located on a gentle slope (approximately 1 % gradient) starting from the river bottom, separated by 400–600 m (Supplemental Fig. S1). The long periods of water erosion would have transported soils from upper slope to lower slope and caused this difference.

### 2.2. Meteorological and soil conditions

Meteorological conditions were recorded at 10-min intervals with an automatic weather station that consisted of a temperature/relative humidity sensor (HygroVUE™5, Campbell Scientific), rain gauge (TE525MM-L, Campbell Scientific), and albedo meter (CHF-SRA01, Hukseflux, Delft, Netherlands). The meteorological conditions were considered identical for the three fields with different soil types because the fields were located near each other. Because the fields were close to each other, the meteorological conditions were assumed to be identical. Therefore, the results of the trials can be compared directly without considering differences in meteorological conditions. In 2018 and 2019, the average solar radiation (21.9 and 21.6 MJ day<sup>-1</sup>, respectively) and air temperature (26.2 and 26.3 °C, respectively) were similar (Fig. 1A). The total precipitation during the growth period was 516 mm in 2018 and 557 mm in 2019, which was lower than the 30 year average (576 mm) for the same period. In 2018, higher precipitation was observed in the early to middle growth periods before 40 DAS. In 2019, precipitation mostly occurred in the middle growth periods (30–60 DAS). At 35 DAS in 2019, a heavy rain of 46 mm h<sup>-1</sup> was recorded.

The volumetric soil water content (%) was continuously recorded at 10-min intervals in each field using TDR probes (CS616, Campbell Scientific, Logan, UT, USA) installed at depths of 0–10 cm and 10–25 cm. The obtained data were calibrated and used to calculate the plant-available soil water content (SWC; mm) (> pF 3.0) at 0–25 cm deep according to our previous study (Iseki et al., 2021). Soil air volume (%) was calculated in each soil layer by subtracting volumetric soil water

**Table 1**  
Soil physical and chemical properties in the studied fields.

Field soil type	Depth cm	Coarse fragment (> 2 mm) weight %	Clay content (< 0.002 mm)	TN content <sup>†</sup> g kg <sup>-1</sup>	Amount of TN g m <sup>-2</sup>	Available P content <sup>†</sup> mg kg <sup>-1</sup>	Amount of available P mg m <sup>-2</sup>
Ferric Lixisols (LXfr)	0–5	2.0	4.9	0.3	21.2	4.8	374.3
	5–15	1.1	5.6	0.2	36.5	2.7	427.1
	15–30	1.3	17.8	0.3	79.2	0.5	133.9
	30–70	2.5	23.6	0.3	191.6	0.4	256.1
	70–97	17.4	21.3	0.2	83.9	0.7	238.9
	97–106	81.7	24.0	0.2	7.0	0.5	14.4
	106–130+	–	–	–	–	–	–
Petric Plinthosols (PTpt)	0–5	20.8	3.3	0.3	18.5	3.8	250.5
	5–18	34.8	8.3	0.3	42.8	2.1	289.8
	18–39	45.6	14.3	0.3	59.3	0.8	157.1
	39–45/50	73.8	23.4	0.3	16.5	0.4	23.0
	45/	–	–	–	–	–	–
	50–90+	–	–	–	–	–	–
Pisoplinthic Petric Plinthosols (PTpt.px)	0–5	49.7	1.8	0.3	12.3	2.6	108.9
	5–20/25	58.4	2.7	0.2	25.8	1.6	214.2
	20/	–	–	–	–	–	–
	25–50+	–	–	–	–	–	–

The data are cited from [Ikazaki et al. \(2018a, 2018b\)](#).

TN: total nitrogen; P: phosphorus.

–: A soil sample could not be taken from petroplinthite because it was consolidated.

<sup>†</sup> Content was measured for fine earth (< 2 mm) and that for coarse fragment was assumed zero in calculating the amount of TN and available P.

content from porosity (%) and then, soil air volume at 0–25 cm deep was estimated by their weighted average. Temporal changes in the available SWC and soil air volume reflected the precipitation pattern. Higher SWC and lower soil air volume were observed in the early growth periods of 2018 and in the middle growth periods of 2019 (Fig. 1B and C). At 60 DAS, the available SWC began to decrease as the precipitation frequency decreased in both years. Among the soil types, the available SWC was higher in LXfr and lower in PTpt.px throughout the growth period. This is mainly due to the higher water holding capacity (less gravels and higher clay content) of LXfr than PTpt.px. The lateral water flow in the soil may also have contributed to the difference in SWC since LXfr was located at the lower slope and PTpt.px at the upper slope, though there was drainage around the LXfr field. The soil air volume in LXfr was lower than that in the other soil types, especially immediately after rainfall, suggesting more severe excessive water stress in LXfr. This is because LXfr has finer pore size than PTpt and PTpt.px ([Ikazaki et al., 2018a](#)). The temporal changes in available SWC and soil air volume in PTpt were similar to those in LXfr under high precipitation and similar to those in PTpt.px under low precipitation. In PTpt, the poor drainage of the subsoil (25–50 cm deep) caused by petroplinthite (iron hardpan) starting at 50 cm deep would have pushed up the available SWC in the topsoil (0–25 cm deep) under high precipitation ([Supplemental Fig. S2A](#)). Differences in soil water conditions among the treatments of plant density and fertilization were negligible which was much smaller than the variations among the soil types ([Supplemental Fig. S2B](#)). Therefore, the effects of treatments on soil water conditions were not considered in this study.

### 2.3. Plant materials and growth conditions

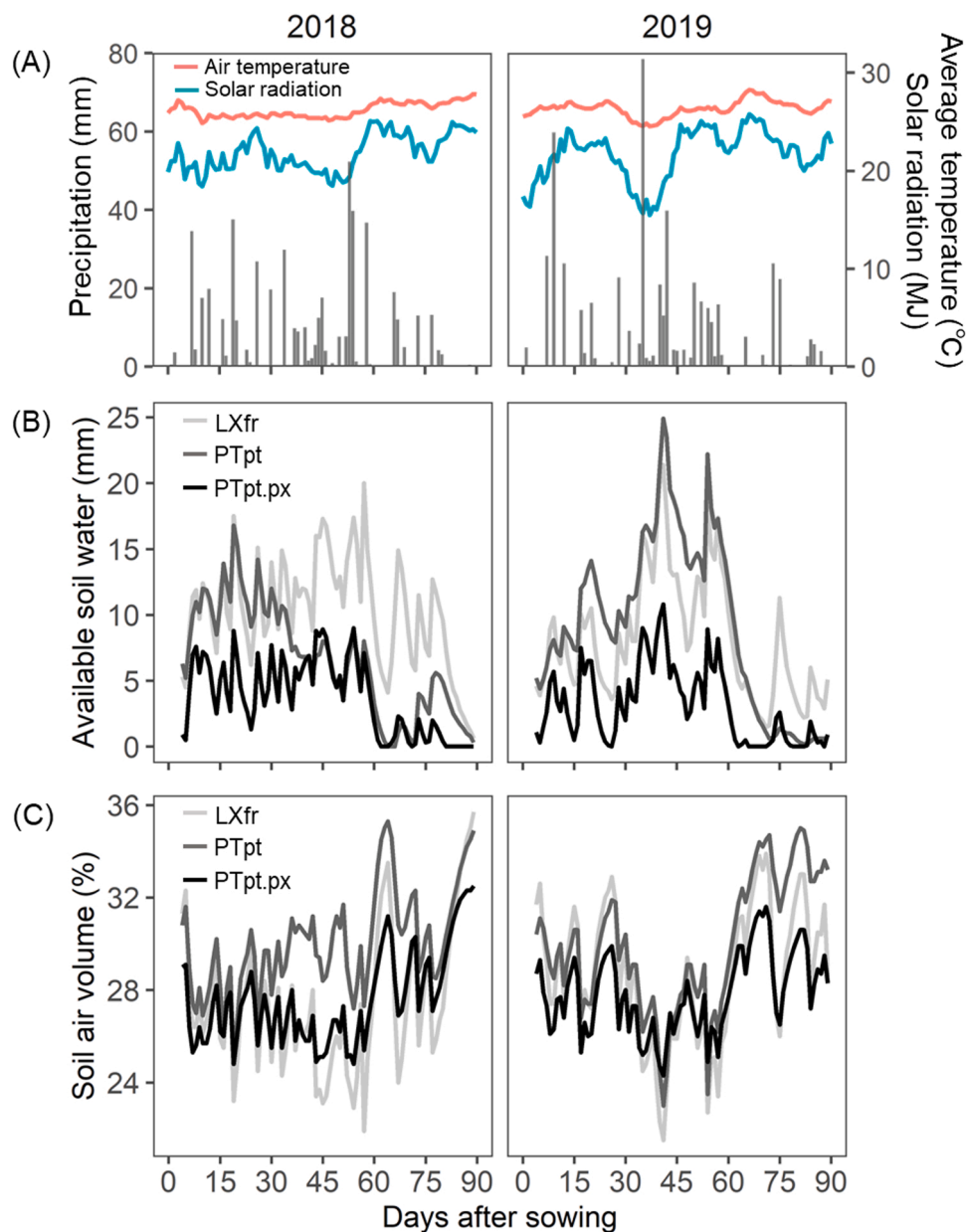
Three cowpea genotypes were used (KVx61-1, KVx421-2J, and Dja), which had different yield responses in different soil types in our previous study ([Iseki et al., 2021](#)). The former two are breeding lines with erect plant types and the third is a local variety with a prostrate plant type. KVx61-1 is a commonly cultivated genotype in this region. The flowering date was almost same for the varieties but was slightly earlier (2–3 days) for Dja. All genotypes completed their life cycle at the experimental site.

Sowing was performed on July 18, 2018, and July 19, 2019. For each soil type, six treatments consisting of combinations of two plant densities (low and high density) and three fertilizer applications (no

fertilizer, basal dose, and split dose) were conducted: low-density and no fertilizer (L0), high-density and no fertilizer (H0), low-density and basal dose (L1), high-density and basal dose (H1), low-density and split dose (L2), and high-density and split dose (H2). Basal and split doses of fertilizer application were compared because the effect of fertilization would be different depending on the timing of application. For the basal dose, 100 kg ha<sup>-1</sup> of chemical compound fertilizer with a composition of N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 14:23:14 (%) was applied two weeks after sowing, whereas for the split dose, a half of the total amount (50 kg) was applied at sowing and another half was at four weeks after sowing. For the split dose, the time of the first application was different from the basal dose to induce priming and boot effects on the shoot growth. The application rate and type of the fertilizer followed the INERA recommendations. Fertilizer was applied at 5 cm depth near the base of each plant hill and then covered by soil. The row and plant intervals for the low plant density were 80 and 40 cm, corresponding to 31,250 plants ha<sup>-1</sup>, and that for high plant density were 40 and 40 cm, corresponding to 62,500 plants ha<sup>-1</sup>. The low plant density was the same as that in the INERA recommendation. Each plot was 2.4 m wide and had four (low density) or seven (high density) 4.4 m rows with 12 plants in each row. The plot layout followed a randomized block design with five replicates. The total field size was approximately 0.15 ha per field. The same plot layout and management were applied to all three experimental fields; thus, the total number of plots was 270, comprising three varieties, six treatments, five replicates, and three soil types. Weeding was conducted weekly using a hand hoe. Insecticides were applied two to three times, as needed.

### 2.4. Shoot growth and yield evaluation

Shoot growth was evaluated by measuring the leaf coverage. Aerial imagery of the fields was captured using the built-in digital camera of an unmanned aerial system (UAV; Phantom4 Pro, DJI, Shenzhen, China). RAW images were taken 20 m above the ground, along with parallel flight lines determined in each field. The flight lines and photographing intervals were arranged to obtain a set of images covering entire field with forward and lateral overlap of 70 %. Flight was performed every 7 days from 14 to 90 days after sowing (DAS). RAW images were used to synthesize orthomosaic field images for each of the soil types and flight dates using MetaShape version 1.8.0 (Agisoft LLC, St. Petersburg, Russia). A series of field images at different time points were then processed to clip each plot area. The field image had six ground control



**Fig. 1.** Temporal changes in the meteorological and soil conditions. (A) Daily precipitation is shown by the bar plot. The air temperature and solar radiation were moving averages of 7 days. (B) The available soil water content above pF 3.0 at 0–25 cm deep was moving averages of 3 days. (C) The soil air volume ratio was moving averages of 3 days. The available soil water content and soil air volume ratio are shown separately for different soil types.

points (GCPs), with an accurate geographical position installed in each field. Before processing, geometric correction was applied to match the GCP positions of all the field images using ENVI version 5.6.2 (L3Harris Geospatial Solutions, Inc., Broomfield, CO, USA). Plot images were then clipped using shapefiles made from polygons of the plot areas in R (version 4.1.1; <https://www.r-project.org/>). The percentage of the area covered by leaves in a plot image was obtained as the leaf coverage (%) using ImageJ version 1.8.0 (NIH; <https://imagej.nih.gov/ij/>).

To evaluate plant phenology, 50 % flowering dates were recorded. The pods were harvested from whole plot area ( $2.4 \times 4.4$  m) for several times because of gradual maturation of the plant. Harvested pods were dried in the sun for a week until a constant moisture content was reached and then threshed. The total grain weight and seed moisture content were measured using an electronic weighing balance (GX-1000; A&D, Tokyo, Japan) and a grain moisture tester (MT-16; Agratronix, Streetsboro, OH, USA). Grain yield was represented as  $\text{g m}^{-2}$  at a 12 % seed moisture content.

## 2.5. Statistical analysis

Tukey's multiple comparison test was performed to detect significant differences in flowering date and grain yield among treatments with different plant densities and fertilizer applications using R version 4.1.1.

## 2.6. Yield prediction using Bayesian regression model

The grain yield was predicted using a Bayesian linear regression model as described in Iseki and Matsumoto (2019). A Bayesian model using genotype as the hierarchical factor was constructed separately for each combination of soil type and year (Eq. (1)). The number of data points for the model was  $n = 90$ , consisting of three genotypes, six treatments, and five replications. The maximum leaf coverage and 50 % flowering date were used as the input variables according to our previous study where the leaf coverage and flowering date were directly or indirectly correlated with the grain yield (Iseki et al., 2021). To evaluate

the parameter contribution to the prediction of grain yield, input variables were standardized with a mean of 0 and a standard deviation of 1 before the analysis. The model was applied as follows:

$$\text{Grain yield} = a[k] \times \text{maximum leaf coverage} + b[k] \times 50 \% \text{ flowering date} + c[k] \tag{1}$$

$$x[k] \sim \text{Normal}(x', \sigma_x) \tag{2}$$

where  $a[k]$ ,  $b[k]$ , and  $c[k]$  differ for each genotype. The number of  $k$  was three, corresponding to KVx61-1, KVx421-2J, and Dja. Here, we assumed that the differences in the coefficients of the models among the genotypes followed a normal distribution. In Eq. (2),  $x[k]$  represents  $a[k]$ ,  $b[k]$ , and  $c[k]$  in Eq. (1), and  $x'$  and  $\sigma_x$  represent the mean and standard deviation of the normal distribution of  $x[k]$ , respectively. Eq. (1) was used to predict the grain yield distribution. The posterior distributions of all coefficients were generated using the Markov chain Monte Carlo (MCMC) method. The MCMC algorithm was set at 3000 steps for iterations and 500 steps for warm-up; the number of chains was four and the total sample size was 10,000. Convergence was confirmed by visualizing a trace plot and 'R hat' (potential scale reduction factor on split chains). Bayesian analysis was performed using the statistical software R version 4.1.1 with the package 'rstan'. The accuracy of the constructed model was evaluated by calculating the root mean square error (RMSE).

### 3. Results

#### 3.1. Shoot growth

Leaf coverage began to increase around 30 days after sowing (DAS) and reached a maximum around 45–60 DAS (Fig. 2). However, the coverage did not reach 100 %, even for the high plant density and fertilized plots. An early decrease in leaf coverage that began after 45 DAS was only observed for LXfr in 2018.

As for the effects of plant density, averages of the maximum leaf coverage in high-density plots (H0: 40.5 % and H1: 68.4 %) was 1.4–1.6 times higher than that in low-density plots (L0: 24.9 % and L1: 48.0 %) under the same fertilizer application (Fig. 2). The increase in leaf

coverage due to high plant density tended to be larger in PTpt.px (1.5–2.4 times higher than that in low plant density) than in LXfr (1.2–1.8 times); moreover, it was greater in 2019 (1.7 times on average) than that in 2018 (1.4 times on average).

As for the effects of fertilization, the basal dose of fertilizer also increased leaf coverage (L1 and H1) but the effect was much higher in H1 than in L1. Averages of the maximum leaf coverage in the basal dose plots (L1 and H1) was 1.7–1.9 times higher than that in the unfertilized plots (L0 and H0) under the same plant density. The effect of fertilization on leaf coverage was greater in PTpt.px (1.9–3.5 times higher than that without fertilization) than in LXfr (1.5–1.9 times); additionally, it was greater in 2019 (2.2 times on average) than that in 2018 (1.7 times on average). A higher increase of leaf coverage due to fertilization was observed in high-density plots than low density plots.

The split dose of fertilizer (L2 and H2) had little effect on the maximum leaf coverage which was similar to that in basal dose treatments (L1 and H1). It accelerated early leaf development before 45 DAS, and the effect was greater in high-density plots than that in low-density plots; however, this was only observed in 2018. In 2019, the maximum leaf coverage in the split-dose plots was similar to (LXfr) or less (PTt and PTpt.px) than that in the basal-dose plots.

#### 3.2. Flowering date

In 2018, the flowering date differed among the treatments. Delayed flowering was frequently observed in all treatments, except in the H2 plots (Fig. 3). Late flowering was distinguished in the unfertilized plots (L0 and H0). The number of days to flowering was relatively lower in the fertilized plots and the earliest flowering was observed in the H2 plots. In 2019, averages of the days to flowering were almost same with that in 2018 but variations of the values in each treatment were smaller than that of 2018. The flowering date was similar for the treatments; however, it was a little earlier in the H2 plots than in the other treatments although the differences were not statistically significant.

#### 3.3. Grain yield

The grain yields for the soil types were largely different between the

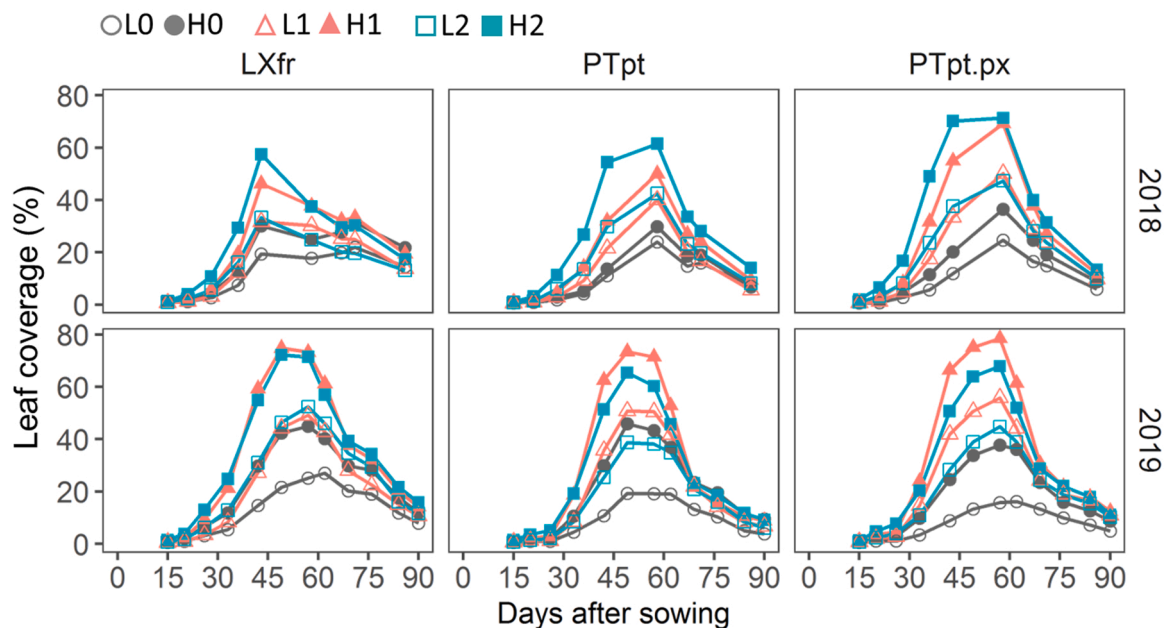
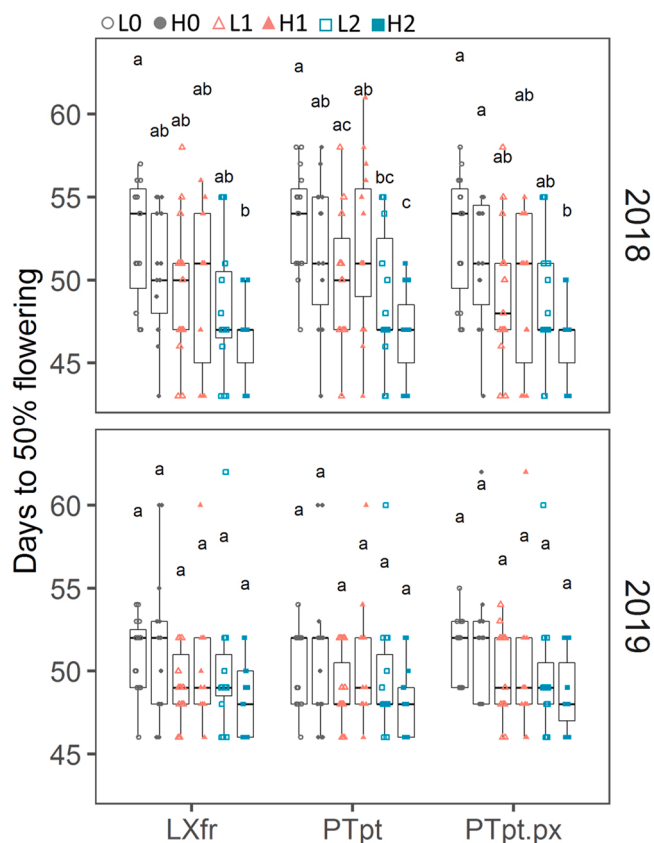


Fig. 2. Temporal changes in the leaf coverage. The leaf coverages are shown separately for the treatments. L0: low-density and no-fertilizer; H0: high-density and no-fertilizer; L1: low-density and basal-dose; H1: high-density and basal-dose; L2: low-density and split-dose; H2: high-density and split-dose. Each point is the average of 15 data comprising three genotypes and five replications.



**Fig. 3.** Distributions of the data for 50% flowering dates. A box plot is shown for each treatment, including 15 data points from three genotypes and five replications. Abbreviations for the treatment are same as shown in Fig. 2. The horizontal lines in the boxes are the median values. The height of the box represents the interquartile distance, indicating the distribution for 50 % of the data. Approximately 99 % of the data fall within the top and bottom of the lines extending from the box. Bars with different letters are significantly different at the  $P < 0.05$  level.

two years. The yield in LXfr was the lowest in 2018 but the highest in 2019 (Table 2). For the unfertilized and basal-dose plots (L0, H0, L1, and H1), grain yields in 2019 were similar to or higher than those in 2018 for all soil types. Except for the H0 plots of LXfr and PTpt in 2018, yields in the high-density plots (H0:  $37.7 \text{ g m}^{-2}$  and H1:  $67.8 \text{ g m}^{-2}$ ) were, on average, 1.2 times higher than those in the low-density plots (L0:  $33.1 \text{ g m}^{-2}$  and L1:  $55.6 \text{ g m}^{-2}$ ) under the same fertilizer treatment. Among the soil types, the effect of high plant density was the highest in PTpt.px, where the yield ratios to low-density plots were up to 1.5 and 1.7 in H0/L0 and H1/L1, respectively. Compared to the yield increase due to high plant density, the yield increase due to basal dose of fertilizer was much higher. The yield of the basal-dose plots was averagely 1.8 times higher than that of the unfertilized plots (L1/L0 and H1/H0) under the same plant density. In 2018, the effect of the basal dose was higher in high-density plots than that in low-density plots; additionally, it was higher in LXfr in high-density plots but higher in PTpt and PTpt.px in low-density plots. In 2019, the effect was higher in PTpt and PTpt.px, without any specific tendency within plant densities. The effects of the split dose differed between the years. In 2018, the yield of split-dose plots (L2 and H2) was, on average, 1.3 times higher than that of the basal-dose plots (L1 and H1) under the same plant density. This tendency was apparent in the high-density plots, where the average yield ratio of H2/H1 was 1.5, whereas that of L2/L1 was 1.1. Among the soil types, the effect of split dose on increased yield was the highest in PTpt and the lowest in LXfr. In contrast, in 2019, the yield was increased by split dose only in the L2 plot of LXfr. The yields in the other split-dose

**Table 2**  
Grain yields ( $\text{g m}^{-2}$ ) in all treatments of plant density and fertilizer application.

Year	Treatment	LXfr	PTpt	PTpt.px	Means for treatment	
2018	Low density + No fertilizer (L0)	29.4 ± 9.4 b	31.6 ± 8.1 cd	33.1 ± 5.6c	31.4	
	High density + No fertilizer (H0)	23.0 ± 8.7 b	30.9 ± 8.4 d	35.3 ± 8.1c	29.7	
	Low density + Basal dose (L1)	43.7 ± 10.5	49.8 ± 8.8 bd	51.4 ± 8.2 bc	48.3	
	High density + Basal dose (H1)	54.5 ± 12.6 a	60.0 ± 11.6	59.6 ± 9.6 b	58.0	
	Low density + Split dose (L2)	45.1 ± 9.0 ab	66.2 ± 9.4 b	53.4 ± 7.5 bc	54.9	
	High density + Split dose (H2)	64.3 ± 10.6 a	108.6 ± 21.0 a	85.0 ± 13.1 a	86.0	
	Means for soil type	43.3	57.9	53.0		
	2019	Low density + No fertilizer (L0)	42.9 ± 8.7 b	29.5 ± 6.2c	32.1 ± 8.0c	34.8
		High density + No fertilizer (H0)	49.1 ± 9.8 b	43.7 ± 8.8 bc	44.4 ± 6.9 bc	45.7
		Low density + Basal dose (L1)	60.1 ± 7.8 ab	66.5 ± 14.0	62.4 ± 8.1 ab	63.0
		High density + Basal dose (H1)	79.5 ± 13.9 a	72.5 ± 16.8 a	80.9 ± 14.1 a	77.6
		Low density + Split dose (L2)	72.6 ± 8.2 a	54.2 ± 12.3	57.7 ± 9.6 ab	61.5
High density + Split dose (H2)		77.3 ± 9.1 a	55.2 ± 8.8 ac	71.1 ± 10.5 a	67.9	
Means for soil type		63.6	53.6	58.1		

Values are the average ± standard error of 15 data samples comprising three genotypes and five replicates. Values with different letters are significantly different at the  $P < 0.05$ .

plots were lower than those in the basal-dose plots.

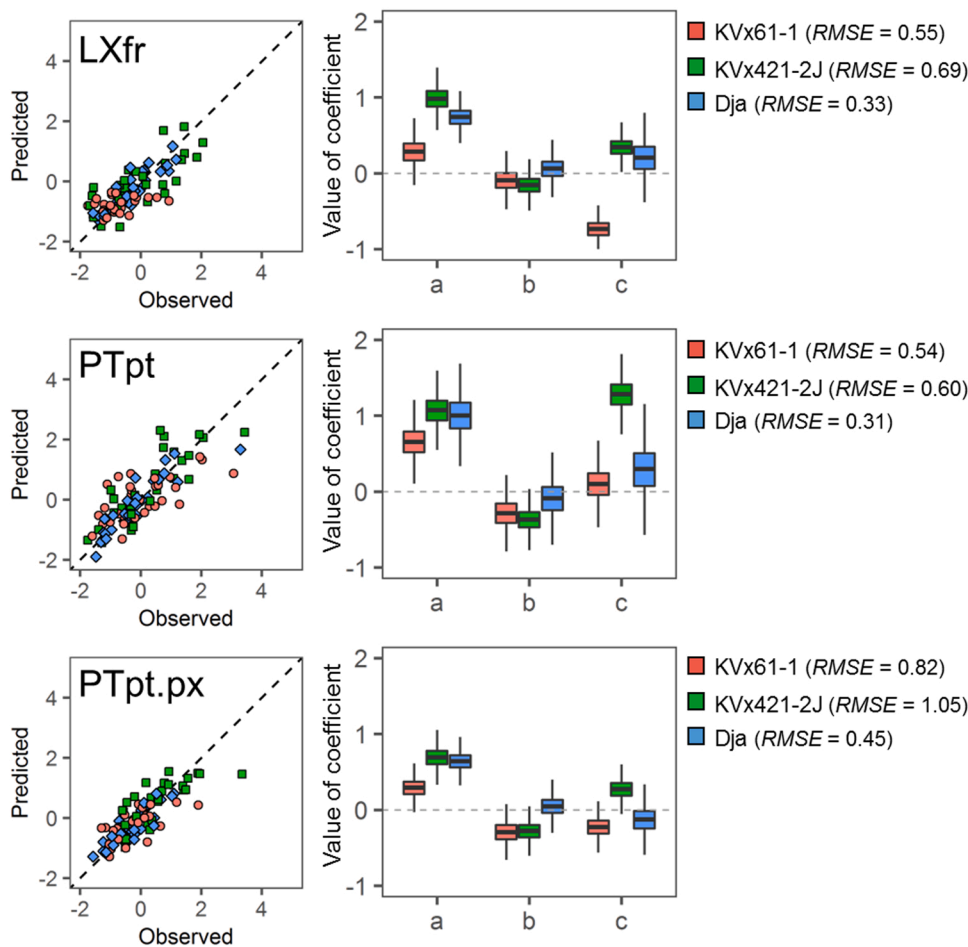
### 3.4. Genotypic differences in the yield prediction model

The yields were well predicted with an RMSE lower than 1.05 for all the obtained models, where the parameters were separately estimated for each genotype (Fig. 4 and Fig. 5). The RMSE values tended to be higher in 2018 than in 2019. Absolute values of the coefficient of maximum leaf coverage (coefficient a) were mostly larger than that of flowering date (coefficient b) and constant (coefficient c) for all genotypes. Among the genotypes, KVx421-2J showed the highest a-values. Dja had a-values similar to those of KVx421-2J in 2018. The coefficient of the flowering date tended to be in negative ranges for KVx61-1 and KVx421-2J in 2018, whereas neutral effects and little genotypic difference were observed in 2019. In 2019, 50 % range of the b-values distributed across zero indicated that the effects of flowering date on grain yield were not detected. The highest positive values of the constant were observed in KVx421-2J for all soil types in both years. High constant values of KVx61-1 and Dja were observed only in PTpt.px in 2019 and in LXfr in 2018.

## 4. Discussion

### 4.1. Effect of plant density and fertilizer application on grain yield under different soil types

The effects of plant density and fertilizer application are hereafter explained in terms of three different aspects: (i) increase in shoot biomass, (ii) promotion of flowering, and (iii) other. The interaction



**Fig. 4.** Predictions for grain yield in 2018 using hierarchical Bayesian regression model. The results of prediction model are shown separately for the three genotypes. Right boxes show the comparison of observed and predicted yields. The yield values were standardized with a mean of 0 and a standard deviation of 1. Left boxes show the distributions of the coefficients generated from 10,000 MCMC samples for each genotype. The horizontal lines in the boxes are the median values. The height of the box represents the interquartile distance, indicating the distribution for 50 % of the data. Approximately 99 % of the data fall within the top and bottom of the lines extending from the box. a: coefficient of maximum leaf coverage; b: coefficient of 50 % flowering date; c: constant. RMSE value of each model is shown inside the parenthesis with the genotype legend.

between these three aspects and soil types is also discussed, focusing on soil moisture/air volume conditions, in the following sections.

For the increase in shoot biomass, leaf coverage increased in both the high-density and fertilized plots, which indicated an increase in shoot biomass. Shoot biomass was detected as the major determinant of grain yield in our previous study conducted at same experimental site (Iseki et al., 2021). This was consistent with the higher values of the leaf coverage coefficient (a) in the yield prediction model (Figs. 4 and 5). However, the increase in leaf coverage due to high plant density and fertilization was small in LXfr (Fig. 2). This might be due to the more severe excessive moisture stress in LXfr than other soil types which was reflected in the poor root nodulation (Supplemental Fig. S3). The negative effect of excessive moisture stress on shoot growth was obvious in 2018 that the leaf coverage started to decrease after 45 DAS when soil air volume was largely depressed by heavy rainfall. In case of soybean, the plants were sensitive to excessive moisture stress during the early growth stage; once the plants were exposed to the stress at this stage, its effect continued throughout the growth period (Bajgain et al., 2015). As a similar phenomenon is expected in cowpea, the excessive moisture stress during this period irreversibly decreased the positive effects of high-density cropping and fertilization. Therefore, the treatments were more effective in PTpt and PTpt.px having a lower risk of excessive moisture stress than in LXfr. The occurrence of excessive moisture stress also caused the yearly difference of the effects of high plant density and fertilization on grain yield that was higher in 2019 than 2018 when large decreases in soil air volume were frequently observed.

For the promotion of flowering, the flowering date was shortened in the fertilized plots. The promotion of flowering date helps to avoid the drought stress during grain-filling periods and increase grain yield

(Bisikwa et al., 2014). This effect was only detected in 2018 when a delay in flowering date was observed. The delayed flowering reduced grain yield because plants with delayed flowering would have experienced severe drought stress at the grain-filling period. The delay in flowering date might be due to excessive moisture stress during the early growth periods (Garcia et al., 2020), which was recovered by fertilization in all soil types (Fig. 3). On the other hand, in 2019, the delay in flowering date was not observed because of the absence of excessive moisture stress representing by the frequent depression of soil air volume. In this case, the flowering date was determined by day length and basic vegetative growth of each genotype, and the pod maturity was completed before the rain stopping. Therefore, the flowering date was little affected to the grain yield in 2019.

In 2018, the contribution of early flowering to grain yield was higher in PTpt and PTpt.px than in LXfr (Fig. 4). Soil water in PTpt and PTpt.px rapidly decreased after the rain stopped at approximately 60 DAS but the grain filling had been continued for late flowering pods. In contrast, the available SWC in LXfr was as high as in the previous growth periods and thus the effect of drought stress on grain yield was limited. The absence of late drought stress in LXfr hindered the effect of early flowering on grain yield in the yield prediction model. Therefore, fertilizer should be preferentially applied in PTpt and PTpt.px to reduce the risk of late drought stress in the Sudan Savanna.

For other, grain yields in the fertilized plots were not fully explained by the changes in the shoot biomass and flowering date. This was apparent in LXfr, in which excessive moisture stress was the most severe among the soil types. In both years, the maximum leaf coverage was similar between H0 and L1 (Fig. 2), but the yield was higher in the latter. Because late drought did not occur in LXfr, factors other than shoot

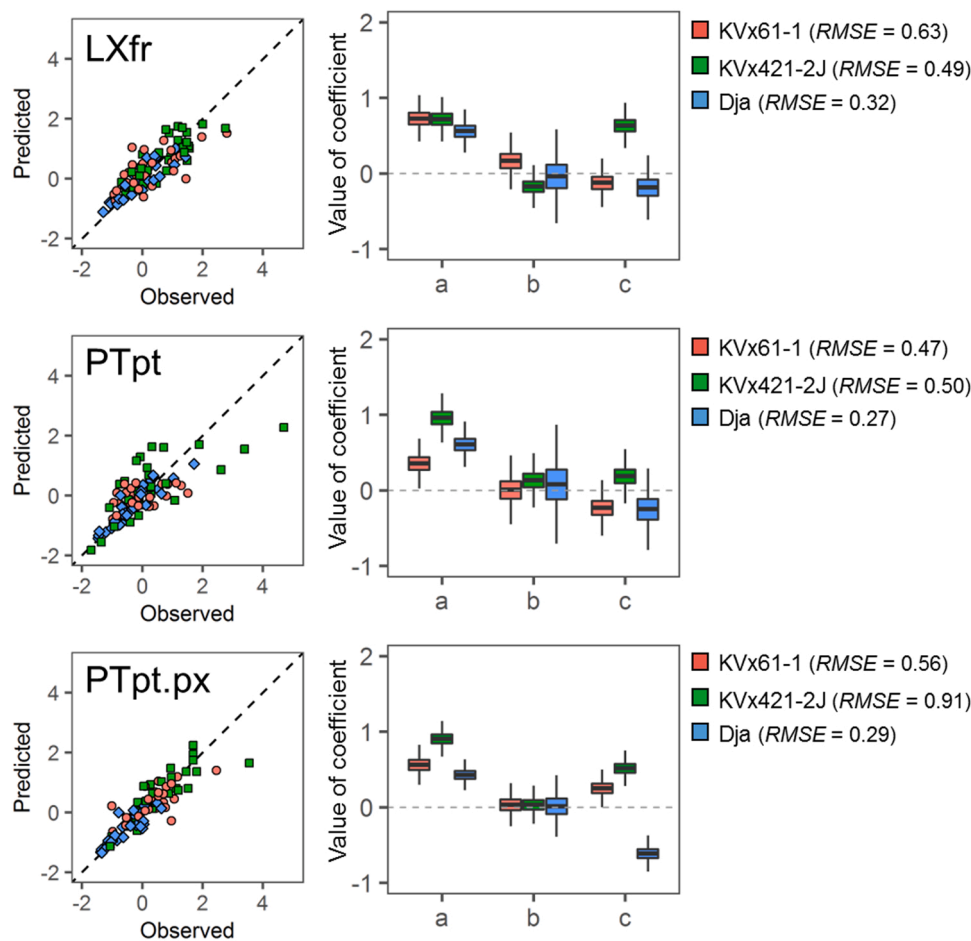


Fig. 5. Predictions of grain yield in 2019 using hierarchical Bayesian regression model. Explanations for the right and left boxes are the same as for Fig. 4.

biomass and flowering date may be responsible for the difference in yield. One possibility is that the phosphorus in the fertilizer helps in grain filling through the activation of root nodules (Ndor et al., 2012). Because symbiotic nitrogen fixation reached a maximum at the flowering stage (Elowad and Hall, 1987), an increase in the number of nodules before the flowering stage might be important for yield formation. Another possibility is the effect of nitrogen component of the fertilizer. Generally, it is believed that nitrogen fertilization reduces root nodulation (Chen et al., 1992). However, nitrogen application is still effective for nodulation when applied to low-fertility soil (Eaglesham et al., 1983). The inconsistency was also observed between H0 and L2 in PTpt in 2019, where leaf coverage was higher in the former, but the yield was higher in the latter. The effects of nitrogen fixation are also expected in this case but further research is needed to confirm its interaction with high plant density and fertilization.

#### 4.2. Effect of split dose of fertilizer on grain yield

A drastic increase in grain yield in the high-density and split-dose plots (H2) was observed in 2018. This can be explained by the following three factors. First, the split dose enhances fertilizer use efficiency and increases plant growth after the second dose (Baligar and Bennett, 1986). The second dose at 4 weeks after sowing (WAS) accelerated early shoot growth, which led to the elongation of early branches and an increase in the number of nodes and peduncles in the branches. Because approximately 65% of grain yield is obtained from the first three branches of the main stem (Iseki et al., 2020), elongation of early branches is particularly important for yield formation. In contrast, the advantage of early shoot growth in the H2 plot was diminished at 60

DAS, indicating that the maximum shoot biomass was not a cause of the yield difference. Second, symbiotic nitrogen fixation by root nodules is enhanced by high-density cropping (Makoi et al., 2009). This phenomenon was also reported for soybean, where high plant density increased interplant competition for soil nutrients, and thus the plants became more dependent on symbiotic nitrogen (Gan et al., 2002). Because the nitrogen fixation during the flowering period determines the number of flower buds (Peat et al., 1981), it is critical for grain yield during this period, especially under the conditions of low soil fertility. Third, the earliest flowering date was observed in the H2 plots. This resulted in the complete avoidance of drought stress during the grain-filling period, especially in PTpt and PTpt.px. Abubaker (2008) reported the relationship between plant density and flowering date as follows: greater availability of fertilizers per plant due to low plant density results in more vigorous vegetative growth and late flowering. However, this explanation was partly inconsistent with our results as the flowering date did not differ between the H0 and H1 plots, although the latter showed more vigorous shoot growth than the former. Therefore, reason for the earliest flowering in H2 plots was not fully addressed in this study. The more severe excessive moisture stress in LXfr also decreased the effect of split dose, which resulted in a higher yield of H2 plots in PTpt and PTpt.px than in LXfr, although the latter had higher soil fertility level.

The effect of the split dose on the increase in yield was not observed in 2019. This was because fertilizer leaching due to heavy rainfall occurred at 35 DAS, immediately after the second dose. Therefore, vigorous leaf development during the early growth period observed in 2018 in the H2 plots was absent in 2019. Instead, the maximum leaf coverage in the H2 plot was lower than that in the H1 plot for PTpt and



PTpt.px. Fertilizer leaching was expected to be more severe in PTpt and PTpt.px than in LXfr due to LXfr having soil crusts at the soil surface, which are hard thin layers with low infiltrability (Valentin and Bresson, 1992). Ikazaki et al. (2018b) reported that annual runoff coefficient in a Lixisol was as high as 28 %, suggesting that only 72 % of annual rainfall infiltrates into Lixisols in the Sudan Savanna. The lower risk of fertilizer leaching in LXfr than PTpt and PTpt.px was supported by the fact that the yields in split dose plots in LXfr were similar to (H2) or higher (L2) than those in the basal dose plots (H1 and L1, respectively) in 2019. The combination of high density and split-dose would have a synergistic effect on grain yield but also poses a risk of fertilizer runoff because the second dose will be applied after the full onset of the rainy season.

#### 4.3. Genotypic differences in the yield responses to plant density and fertilizer application

The effects of plant density and fertilizer application on shoot growth and flowering date were assumed to be similar for the genotypes used in this study because the results of analysis of variance showed little contribution of the interaction between the genotype and treatment on the total variances of shoot growth and flowering date (Supplemental Table S1). The regression models with low RMSE values indicated that the yield variation in each soil type was well explained by the selected factors of maximum leaf coverage and flowering date. When the RMSEs were compared between the years, the higher RMSE values in 2018 than in 2019 might be due to the severe effect of excessive soil moisture stress in 2018 disturbing the contribution of the factors on grain yields.

The higher a-values than other coefficients indicated that shoot biomass was a major yield limiting factor in this study. Among the cowpea genotypes, the higher a-values in KVx421-2J than the other two indicate that the shoot biomass was more efficiently linked to grain yield. In other words, KVx421-2J had a higher yield to biomass ratio (harvest index) which caused the higher grain yield in this genotype (Supplemental Fig. S4). In contrast, the lower a-values in KVx61-1 indicated a low yield to biomass ratio, implying a slight increase in grain yield even when the biomass increased. The differences in the harvest index might be related to the traits of branch number, node number, and pod number per reproductive node in each genotype (Board and Tan, 1995).

The b-values showed large negative values in KVx421-2J and KVx61-1, indicating that early flowering was more effective for increasing yield in them than in the local variety Dja. This might be related to the flowering period, which is short in breeding lines but long in local varieties. Drought avoidance due to early flowering was ensured by short flowering period, in which most flowers bloomed at once, and the subsequent grain filling was completed before soil drying. In 2019, the ranges of the b-values were almost neutral and the genotypic differences were small. This was because delayed flowering due to excessive moisture stress was absent in this year.

Genotypic differences in grain yield caused by factors other than shoot biomass and flowering date were explained by constant values. The genotype with a higher constant value indicated that the grain yield of this genotype was still higher than that of other genotypes with the same shoot biomass and flowering date. The higher constant values in KVx421-2J may be related to the environmental yield stability. KVx421-2J was the genotype with the lowest genotype-environment interaction, showing stable yield for the soil types under different precipitation conditions (Iseki et al., 2021). High environmental adaptability might be related to presence of tolerance mechanisms against excessive moisture conditions as well as drought conditions (Hall, 2012; Yamauchi et al., 2013). KVx61-1 and Dja showed higher constant values only for PTpt.px in 2019 and LXfr in 2018, respectively. The former was drought-prone and the latter had excessive moisture conditions during early growth periods. These genotypes were thought to be adaptable only to specific conditions of drought or excess moisture stress.

## 5. Conclusion

This study elucidated that the occurrence of excessive moisture stress and drought stress were the cause of the heterogeneity effect of plant density and fertilizer application in the dominant soil types in Sudan Savanna. This was determined according to the field observation that low soil air volume after heavy precipitation was frequently observed in LXfr and caused excessive moisture stress. However, PTpt.px was prone to drought stress after the rainfall stopped during the late growth period because of its lower water holding capacity (only 2–3 % of clay and abundant coarse fragments). These soil properties have a critical effect on plant growth and yield responses to plant density and fertilizer application. The yield variation can be minimized by utilizing appropriate cowpea genotypes with high yield stability to the varying soil water conditions. Plant density and fertilizer application should be optimized with appropriate variety selection according to the soil type of the target region. Its optimization would help to achieve food security and better economic income of small households of the region.

### CRedit authorship contribution statement

**Kohtaro Iseki:** Conceptualization, Data curation, Methodology, Writing – original draft. **Kenta Ikazaki:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Joseph B. Batiemo:** Conceptualization, Writing – review & editing, Supervision.

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### Declaration of Competing Interest

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

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fcr.2023.108825](https://doi.org/10.1016/j.fcr.2023.108825).

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