









Cowpea leaf width correlates with above ground biomass across diverse environments

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Funding information

University of Illinois, Grant/Award Number: OPP1172157

Abstract

Cowpea (*Vigna unguiculata* [L.] Walp.) yields within the dry savannahs of Sub-Saharan Africa are low. Given the contribution of cowpea to food security in this region, it is essential that high-yielding varieties are developed to improve crop productivity in a sustainable manner. Identifying morphological or physiological traits that correlate with biomass could assist breeders with rapid screening of diverse germplasm. This study investigated 23 diverse Nigerian cowpea lines in an environmentally controlled greenhouse and 50 diverse lines from a Multiparent Advanced Generation Intercross (MAGIC) population in the field to identify easily measurable traits associated with high above ground biomass. Correlation analyses found that leaf traits were significantly and positively correlated with above ground biomass, and the leaf width of the youngest fully expanded leaf was the best indicator of biomass yield. Analysis of variance identified significant differences among the genotypes for all measured traits, indicating that there is genetic variation among these varieties and opportunity for selection. These results from the greenhouse and the field indicate that the leaf width of the youngest fully expanded leaf can be used to rapidly select cowpea lines with high biomass production potential.

KEYWORDS

biomass yield, crop productivity, path analysis, phenotypic marker, *Vigna unguiculata*

1 | INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is an annual, herbaceous, warm-season legume. The versatility of cowpea makes it one of the most economically important grain legumes cultivated globally (Langyintuo et al., 2003), and it is a major contributor to food security in sub-Saharan Africa (SSA), where it is predominantly grown by resource-limited subsistence farmers (Gomez, 2004; Owusu, 2018). West Africa is the leading producer and consumer of cowpea, generating about

95% of global cowpea production in 2017 (Food and Agricultural Organization, 2021). Cowpea is widely cultivated for its grain, which contains 20% to 30% of protein by dry weight (Herniter et al., 2020; Timko & Singh, 2008). Furthermore, cowpea provides essential nutrients such as iron, calcium and zinc (Carvalho et al., 2017), with reported ranges of 2.0–2.4, 7–8 and 90–360 mg kg⁻¹ for iron, zinc and calcium, respectively (Gondwe et al., 2019). The haulms can also be used as a nutritious fodder for livestock (Singh et al., 2013), making it a valuable and nutritious key feed resource in crop-livestock

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systems, and an important source of income for value chain actors, as the price of cowpea haulms is reported to range from 50% to 80% of the grain price (Singh et al., 2003). In Nigeria, farmers who cut and store cowpea fodder for sale at the peak of the dry season increased their annual income by 25% (Dugie et al., 2009). Cultivars that offer the opportunity to be used both for food and fodder are often referred to as 'dual purpose cultivars' (Tarawali et al., 2005; Omokanye et al., 2003).

Cowpea breeding efforts in SSA have focused on developing varieties with desired seed quality, grain yield, maturation time, improved growth habit (erect), photoin sensitivity and resistance to drought (Boukar et al., 2018; Kamai et al., 2014; Mofokeng et al., 2020). These efforts have largely been achieved through conventional breeding methods such as germplasm collection, evaluation, maintenance and selection for desired traits. Still, the use of improved varieties is not widespread among smallholder farmers (Horn et al., 2015). While grain yields above 3000 kg ha⁻¹ were reported in Egypt, North Macedonia, Palestine and Iraq in 2019, the average yield in 18 SSA countries was 606 kg ha⁻¹ during the same year (Food and Agricultural Organization, 2021). Reasons for the low yields obtained in these regions include the use of low yielding varieties, poor soil conditions, unfavourable weather conditions, such as drought events, and insect pests and diseases (Bolarinwa et al., 2021; Langyintuo et al., 2003; Sindhu et al., 2019). In addition, even though cowpeas can also be cultivated for fodder production, there has been limited research aimed at improving total biomass produced per plant, with only a few landraces being adapted for biomass production rather than seed production (Kristjanson et al., 2002; Mary & Gopalan, 2006). This emphasizes the need for easy and fast approaches to improve the productivity of the crop across multiple regions, which can eventually contribute to global food and nutrition security.

The morphological diversity within cowpea germplasm offers opportunities that could be exploited through breeding (Egbadzor et al., 2014). Fortunately, morphological characterization is a simple approach based on visible traits and requires minimal resources to evaluate the genetic diversity of crops (Hallauer, 2011; Ntundu et al., 2006; Perry & McIntosh, 1991; Singh, 1988; Stoilova & Pereira, 2013). Traits that are simple to measure and that, at an early stage of plant development, correlate with plant biomass could assist plant breeders and accelerate breeding programmes in the development of cultivars with enhanced biomass production.

Due to the extreme variability in leaf shape, different varieties of cowpea can be classified and distinguished based on this trait (Buleti et al., 2020). The trifoliolate leaf of cowpea contains two asymmetrical lateral leaflets and one symmetrical (central) terminal leaflet. Due to the variability in the shape of the lateral leaflets, which are asymmetrical, the central leaflet is used to classify leaf shape (Pottorff et al., 2012), and leaf shape can be classified into four categories: subglobose, subhastate, globose and hastate (Egbadzor et al., 2014). The ease of identifying variation in leaf shape makes it a good candidate for selection if it is associated with greater biomass.

In this study, we explored the natural diversity of 73 cowpea lines (23 elite breeding lines or commercial varieties of cowpea and

50 genotypes from the cowpea Multiparent Advanced Generation Intercross [MAGIC] population) and tested for phenotypic traits that were easy to measure and correlated with above ground biomass (AGB). Experiments were conducted in both the field and in controlled environments and at different developmental stages to test the consistency of phenotypic associations with biomass production.

2 | MATERIALS AND METHODS

2.1 | Greenhouse experiment
























A 21-day experiment conducted in an environmentally controlled greenhouse tested phenotypic associations in 23 cowpea (*V. unguiculata* [L.] Walp.) genotypes (Table 1), including 21 lines obtained from Nigerian breeders' germplasm collections and two IITA breeding lines IT86D-1010 and IT82E-16 obtained from the USDA. Eight biological replicates of each genotype were grown in a randomized complete block design using 0.6 L pots (D40H, Stuewe & Sons Inc., Tangent, Oregon, USA), containing a 1:1 mixture of compost (Petersfield Growing Mediums, Leicester, UK) and silver sand (horticultural grade, Royal Horticultural Society, London, UK). Plants were maintained well-watered throughout the experiment and each plant was given 100 ml of fertilizer (MiracleGro, Evergreen Garden Care, Surrey, UK) 2 weeks after planting. The day and night temperatures were maintained at 28°C and 19°C, respectively (Figure S1a), with a 16 h photoperiod and relative humidity of 34% ± 1.5%. Daylight was supplemented by high-pressure sodium lamps (SON-T 400 W, Philips Lighting, Eindhoven, The Netherlands) to ensure a minimum photosynthetic photon flux density (PPFD) of 500 μmol m⁻² s⁻¹.

All measurements were made 21 days after planting. The stem of each plant was cut at the base of the soil, and the plant height was measured with a ruler, from the base of the stem to the top leaf. The youngest fully expanded trifoliolate leaf from the third node was digitized using a multimode scanner to measure the leaf length (Figure 1; length from the tip to the base of the central leaflet) and leaf width (Figure 1; widest section across the central leaflet perpendicular to its length). All leaves and the stem, including petioles and petiolules, from individual plants were placed into separate paper bags and dried in an oven at 60°C for 48 h before determining the total AGB.

2.2 | Field experiment

Fifty lines from a cowpea MAGIC population (8 founder parents plus 42 recombinant inbred lines) developed by Huynh et al. (2018) were planted at the University of Illinois Energy Farm Facility in Urbana, IL (40.06°N, 88.21°W) on 26 June 2019, as described in Digrado et al. (2020) (Table 2). Each line was planted in a single 133.5 cm row oriented north-south. Among the lines tested, two were in common with the greenhouse experiment, namely, IT89KD-288 and IT93K-503-1. The cowpea lines were planted in a 5 ranges × 10 rows grid, with 152.5 cm between rows and a 3.8 cm spacing between plants

TABLE 1 List and description of genotypes used in the greenhouse experiment

Genotype	Determinacy	Leaf shape		Characteristics
IT82E-16	Determinate	Hastate		IITA breeding line, early maturity (60–70 days), high yielding, drought tolerant.
IT86D-1010	Determinate	Hastate		Reference IITA breeding line, medium maturity (70–75 days), photoperiod insensitive, semi-erect plant growth habit.
IT93K-503-1	Determinate	Hastate		Breeding line from IITA, drought tolerant.
58-77	Determinate	Hastate		Landrace from Senegal, poor performing under low-P soil.
524B	Determinate	Hastate		Breeding line from UCR-USA, large seed, <i>Fusarium</i> wilt resistance.
Danila	Indeterminate	Hastate		Landrace among Northern Nigeria farmers, photoperiod sensitive, late maturing (over 100 days), drought tolerant.
DanMisra	Indeterminate	Hastate		Landrace (cultivar) among Northern Nigeria farmers, popular in local markets, photoperiod sensitive.
Kanannado	Indeterminate	Subglobose		Landrace (cultivar) among Northern Nigeria farmers for high fodder yield, photoperiod sensitive, drought tolerant.
IAR-09-1009-6	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-07-1058	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-07-1050	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-07-1040	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-09-1030-6	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-011-151	Indeterminate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IAR-09-1151-2-1	Determinate	Subglobose		Advanced breeding line from IAR/ABU Nigeria.
IT89KD-288 (Sampea-11)	Indeterminate	Subglobose		Commercial variety, popular in Northern Guinea Savannah of Nigeria, photoperiod sensitive.
IT99K-573-1-1 (Sampea-14)	Determinate	Subglobose		Commercial variety, high yielding, Striga resistant.
IT08K-150-12 (Sampea-19)	Determinate	Subglobose		Commercial variety, early maturity, drought tolerant.
Yacine	Determinate	Subglobose		Landrace from Senegal, P use efficient.
TVu-297	Indeterminate	Subhastate		Accession from IITA collection, white large seed size, preferred by local consumers.
IT99K-573-1-1 (Sampea-15)	Determinate	Subhastate		Commercial variety, high yielding, drought tolerant, Striga resistant.
IT07K-318-33 (Sampea-17)	Determinate	Subhastate		Commercial variety, brown seed coat, early maturity, drought tolerant.
IT07K-297-13 (Sampea-18)	Determinate	Subhastate		Commercial variety, brown seed coat, early maturity, drought tolerant.

within a row. No fertilization or pesticide treatments were applied. Seeds were watered after planting to promote seed emergence. Meteorological data for the duration of the experiment are provided

in Figure S1b. The lines had all reached reproductive developmental stages between R1 (early bloom) and R3 (early pod set) at the time of measurements.

Measurements were performed across a 4-day period, 54 days after planting. Leaf length and width were measured on five leaves per genotype using a ruler (Figure 1). Leaf chlorophyll content was estimated with a chlorophyll meter (SPAD-502, Konica Minolta Sensing) by averaging six measurements per leaf from five leaves per genotype (Murillo-Amador et al., 2004). All leaf measurements were performed on the most recently fully developed, nonsenescent central leaflet at the fifth to seventh node down from the top of the canopy depending on the cowpea line. The number of nodes and the height of the canopy (i.e., height from the ground to the top leaf) were also measured. A length of 1 m per row of plants was harvested for each line 64 days after planting, with sampling of all the lines completed across a 2-day period. Leaves and stems were dried separately in an oven at 60°C for 3 weeks. Samples were weighed for total leaf biomass and total stem biomass. The total AGB was obtained from the sum of total leaf mass and total stem mass. Data describing this population were analysed in a previous publication (Digrado et al., 2020) and were reanalysed in the present study to test the specific hypothesis that the correlation between leaf dimension traits

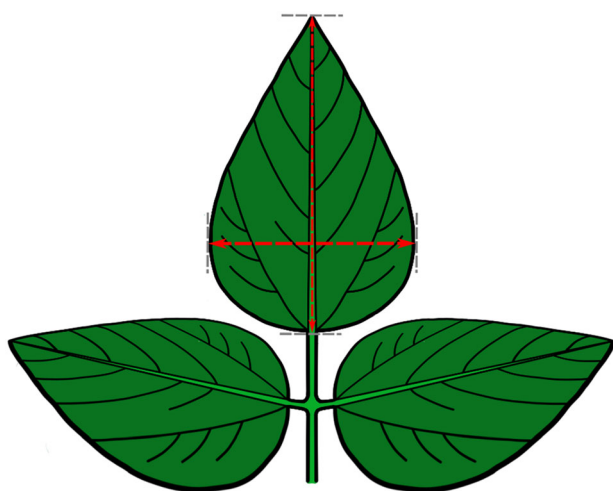


FIGURE 1 Guide to how leaf length and leaf width were measured in the greenhouse and field experiments. The terminal leaflet was measured for length and width at the widest part of the leaf

and AGB would be maintained in different cowpea genotypes across different environments.

2.3 | Statistical analysis

Correlation analyses were performed in R version 4.1.2 (R Core Team, 2016) and RStudio version 2021.09.1 (RStudio Team, 2015) on the individual and combined datasets. Pearson correlation coefficients for the combined dataset were calculated after data from the greenhouse, and field experiments were centred and scaled separately prior to merging. Pearson correlation coefficients for all measured traits were computed and visualized in R using the stats package version 3.5.2.

Path analysis is an extension of multiple regression approaches and allows examination of complex relationships among variables and comparison of different models to determine which fits the data best (Streiner, 2005). In this study, path analysis was used to quantitatively partition direct and indirect effects of different plant traits on AGB and to investigate whether traits that are rapid and easy to measure such as leaf size can be used as proxies for AGB. To this end, rapid and easy traits to measure (i.e., leaf length, width and plant height) were used as entries for the path model to predict AGB. Canopy height, the number of nodes, SPAD/chlorophyll content, total leaf mass and leaf length and width were considered in our path analysis as important components explaining the observed variations in AGB in cowpeas. Data were centred and scaled for the analysis. The path diagram was designed as follows: (1) SPAD/chlorophyll content, canopy height, leaf length, leaf width and leaf biomass directly affect AGB; (2) leaf biomass is related to the leaf length and width, plant height and the number of nodes; and (3) the number of nodes is directly related to the plant height. Leaf length and leaf width were tested together and separately as variables describing leaf size in the model. The models were built using the greenhouse and field datasets separately. Only the significant paths were kept in the final models. While other path diagrams could conceptually be feasible, the purpose of this analysis was not to explore the relative goodness of fit of different models but instead to identify the primary interaction among the different plant architectural traits and how they affect AGB. The

TABLE 2 List of cowpea lines measured in the field experiment in Illinois in 2019

CB27	MAGIC027	MAGIC066	MAGIC138	MAGIC222
IT00K-1263	MAGIC028	MAGIC076	MAGIC151	MAGIC223
Suvita2	MAGIC029	MAGIC084	MAGIC152	MAGIC229
IT89KD-288	MAGIC030	MAGIC086	MAGIC162	MAGIC231
IT84S-2246	MAGIC034	MAGIC095	MAGIC178	MAGIC236
IT84S-2049	MAGIC036	MAGIC112	MAGIC188	MAGIC250
IT82E-18	MAGIC046	MAGIC113	MAGIC189	MAGIC268
IT93K-503-1	MAGIC052	MAGIC119	MAGIC197	MAGIC280
MAGIC001	MAGIC059	MAGIC124	MAGIC200	MAGIC287
MAGIC025	MAGIC060	MAGIC127	MAGIC208	MAGIC291

analysis was performed in R using the Lavaan package v0.6-3 (Rosseel, 2012).

3 | RESULTS

Seventy-three cowpea cultivars were grown under two different environmental conditions and phenotypic traits were measured at different growth stages. In 23 cowpea genotypes grown in the greenhouse

and measured 21 days after planting, plant height ranged from 33 to 104 cm (Table S1). Leaf width and leaf length varied between 6.0 and 8.9 and 11.3 and 15.8 cm, respectively. Total leaf mass and AGB varied from 0.9 to 2.0 and 1.5 to 3.2 g per plant, respectively. In this experiment, IT82E-16 and Kanannado were the least productive cultivars (AGB lower than 1.6 g per plant) while IT86D-1010 and 524B were the most productive cultivars (AGB higher than 3.0 g per plant).

Cowpeas grown in the field were phenotyped 54 days after planting and, across the 50 genotypes, canopy height ranged from 30 to

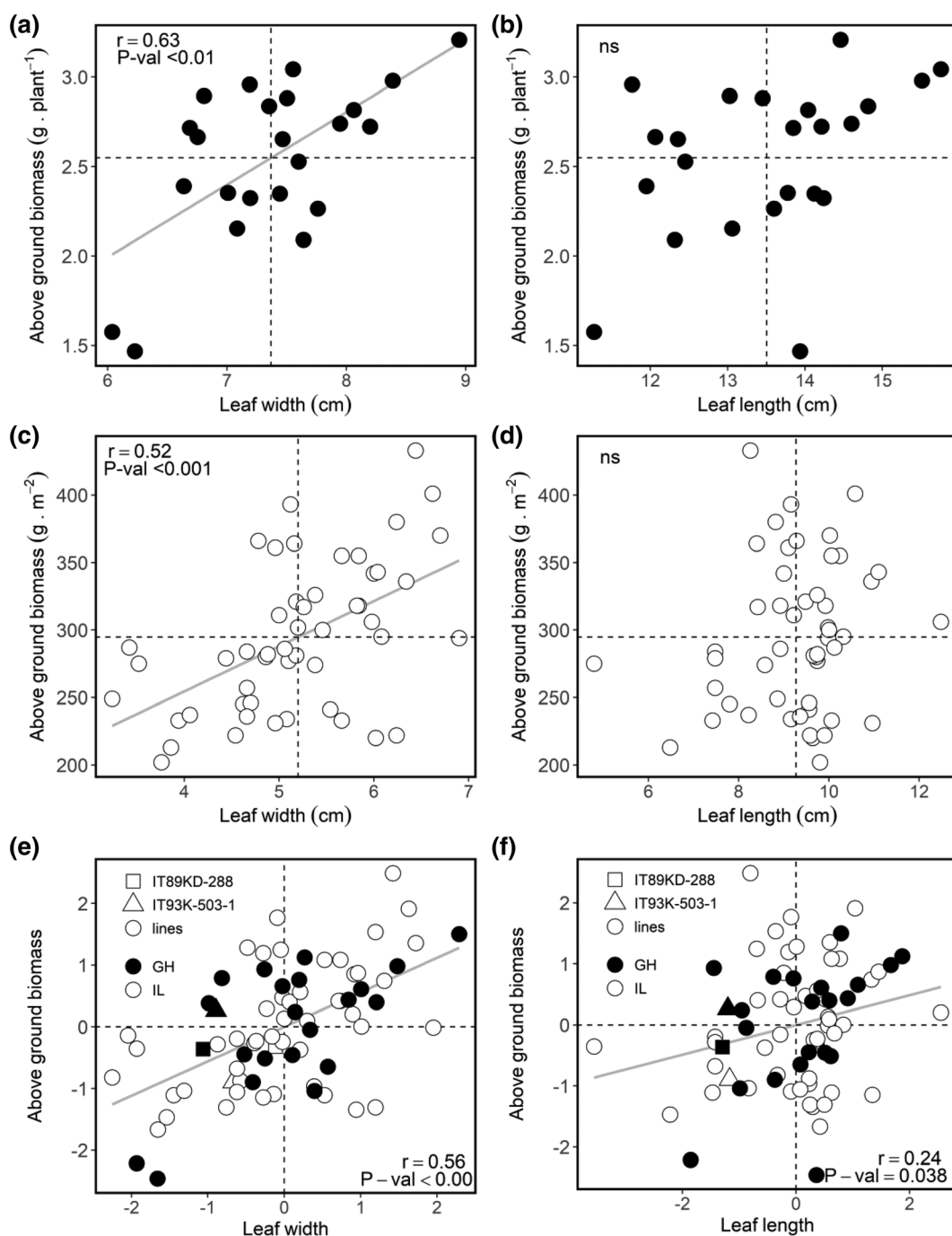


FIGURE 2 Correlation between (a,c,e) leaf width or (b,d,f) leaf length and above ground biomass measured (a,b) in the greenhouse trial, (c,d) in the field and (e,f) with the combined datasets. The Pearson correlation coefficients (r) and the p values are shown when significant

64 cm (Table S1). Leaf width and leaf length varied from 3.2 to 6.9 and 4.8 to 12.5 cm, respectively. Total leaf mass and AGB varied from 74 to 207 and 202 to 433 g m⁻². MAGIC231 and MAGIC101 were the least productive genotypes (AGB lower than 215 g m⁻²) while MAGIC138 and MAGIC060 were the most productive genotypes (AGB higher than 400 g m⁻²).

We examined the correlation between leaf length and width and indicators of plant productivity, including AGB (Figure 2), total leaf biomass (Figure 3) and plant/canopy height (Figure 4). Leaf width was

positively correlated with AGB measured in cowpea lines from the greenhouse experiment ($p < 0.01$, Figure 2a) and from the MAGIC population in the field experiment ($p < 0.001$, Figure 2c). In the greenhouse experiment, the correlation was mainly driven by two lines with low AGB. No significant correlation was observed between leaf length and AGB in either environment (Figure 2b,d).

Leaf biomass was also positively correlated with both leaf width ($p < 0.001$, Figure 3a) and leaf length ($p < 0.05$, Figure 3b) for cowpea lines measured in the greenhouse experiment. Those relationships

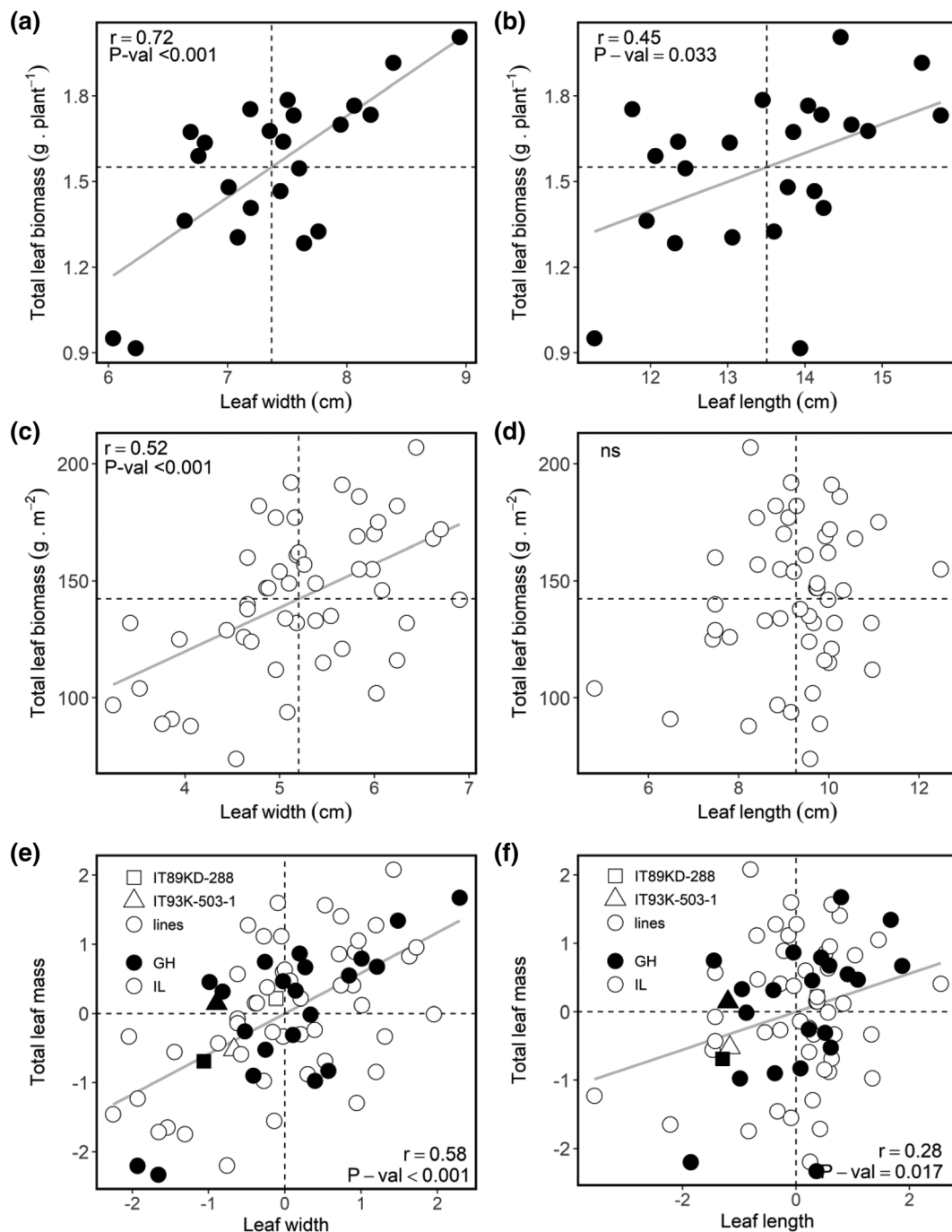


FIGURE 3 Correlation between (a,c,e) leaf width or (b,d,f) leaf length and total leaf biomass measured (a,b) in the greenhouse trial, (c,d) in the field and (e,f) with the combined datasets. The Pearson correlation coefficients (r) and the p values are shown when significant

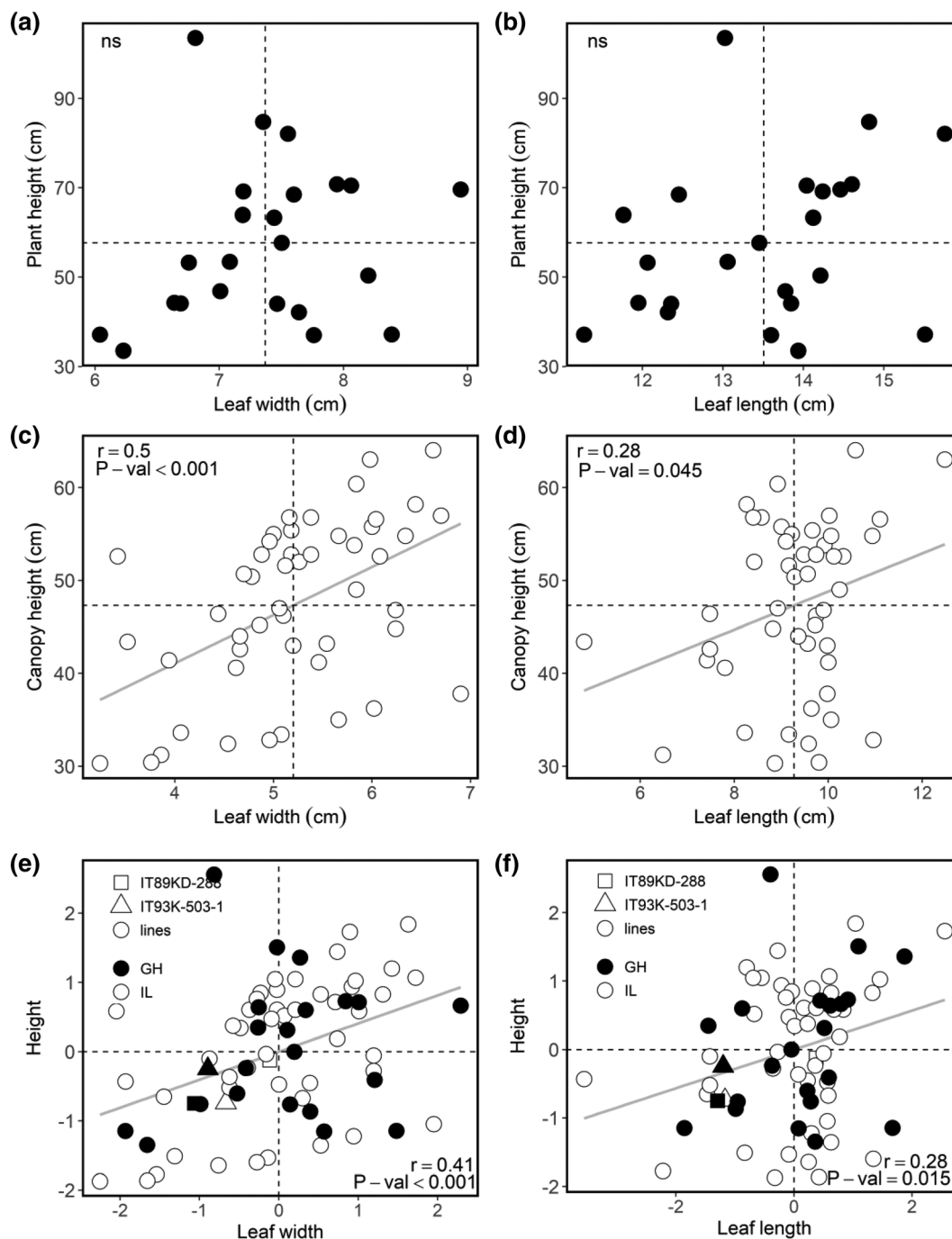


FIGURE 4 Correlation between (a,c,e) leaf width or (b,d,f) leaf length and plant or canopy height measured (a,b) in the greenhouse trial, (c,d) in the field and (e,f) with the combined datasets. The Pearson correlation coefficients (r) and the p values are shown when significant

remained significant even when the two low-biomass lines were removed from the dataset ($p < 0.05$ for both leaf width and leaf length). In the cowpea MAGIC population analysed in the field experiment, only leaf width was positively correlated with the leaf biomass ($p < 0.001$, Figure 3c), and no significant correlation was observed with leaf length (Figure 3d).

Canopy height measured in the MAGIC population was positively correlated with leaf width ($p < 0.001$, Figure 4c) and leaf length ($p < 0.05$, Figure 4d). No significant correlation was observed

between plant height and leaf length or width in the young cowpeas measured in the greenhouse experiment (Figure 4a,b). The full correlation analyses for the greenhouse and the field experiments are shown in supplementary Figures S2 and S3, respectively. Overall, these results revealed that leaf width, rather than leaf length, was consistently associated with indicators of plant biomass productivity.

After normalizing the data to enable a comparison across environments, leaf width was more strongly correlated with AGB, the total

leaf biomass and height (Figures 2e, 3e and 4e) than leaf length (Figure 2f, 3f and 4f).

Path analysis was conducted to explore which rapidly and easily measurable traits exhibited the greatest control on cowpea biomass productivity (Figure 5). Leaf width explained 61.8% of the variance in AGB in the greenhouse experiment and 20.6% of the variance in AGB in the field. In both datasets, leaf width showed a stronger path coefficient contribution toward total leaf biomass than AGB (Figure S4). Leaf length showed no direct or indirect effect on AGB (Figure S4). When both leaf length and leaf width were used in the path analysis, only the path between leaf width and total leaf biomass was significant (Figure S4). Hence, only the direct path between leaf width and total leaf biomass was kept in the final models (Figure 5a,b). Leaf chlorophyll content showed a direct effect on plant AGB only for the field experiment (Figure S4).

Plant and canopy height also predicted total AGB in the path analyses (Figure 5). In the proposed model, plant height was related both directly and indirectly to total AGB. The direct impact of plant height on total AGB is related to stem and branch biomass, with taller plants having more branches and stem biomass. The direct path between plant height and the total leaf biomass may indicate that taller plants tend to have faster growth rates, eventually producing more leaf biomass. Plant height can also serve as a proxy for the number of nodes on the main stem, which determines the total leaf biomass. Using data from the greenhouse trial, plant height showed a significant path coefficient to AGB and the number of nodes on the main stem (Figure 5a). Using data from the MAGIC population, canopy height showed a significant path coefficient with the number of nodes on the main stem, leaf biomass and total AGB (Figure 5b). Overall, plant height explained 36.2% and 67.5% of the variance in AGB in the greenhouse trial and in the MAGIC population in the field, respectively.

4 | DISCUSSION

This study investigated the relationship between cowpea biomass productivity and easily measured traits related to plant architecture such as height, the number of nodes and leaf size, across 73 cowpea cultivars grown under different environments and measured at different growth stages. The relationships between leaf size, plant height and AGB were consistent across experiments. In particular, correlation analysis showed that leaf width was a good predictor for biomass accumulation (Figures 2–4). The path analysis also revealed that leaf width had an indirect effect on plant AGB that was mediated by total leaf biomass (Figure 5). Importantly, the correlation between leaf size and AGB was observed across two growth stages and two environmental conditions.

The positive correlation observed in the cowpea populations between leaf size and AGB could be explained by a greater light interception in plants with wider leaves. Greater early interception could in turn support a higher initial growth rate and hence greater biomass production (Hammer et al., 2009). While positive correlation coefficients with AGB were observed for both leaf width and leaf length, only the correlations between AGB and leaf width were significant (Figure 2a,c). An alternative explanation for the correlation observed between leaf size and AGB may be that common physiological processes determine leaf lateral growth and biomass production such as cell division (Li et al., 2005; Rojas et al., 2009). Because of its important variability among varieties and the interest of this trait in allometry studies (Shi et al., 2019), the correlation between the ratio of leaf length to leaf width (L:W) and biomass was also explored. While no significant correlation between leaf L:W and biomass was observed in the greenhouse dataset, significant negative correlations were observed in the field and combined datasets between leaf L:W and

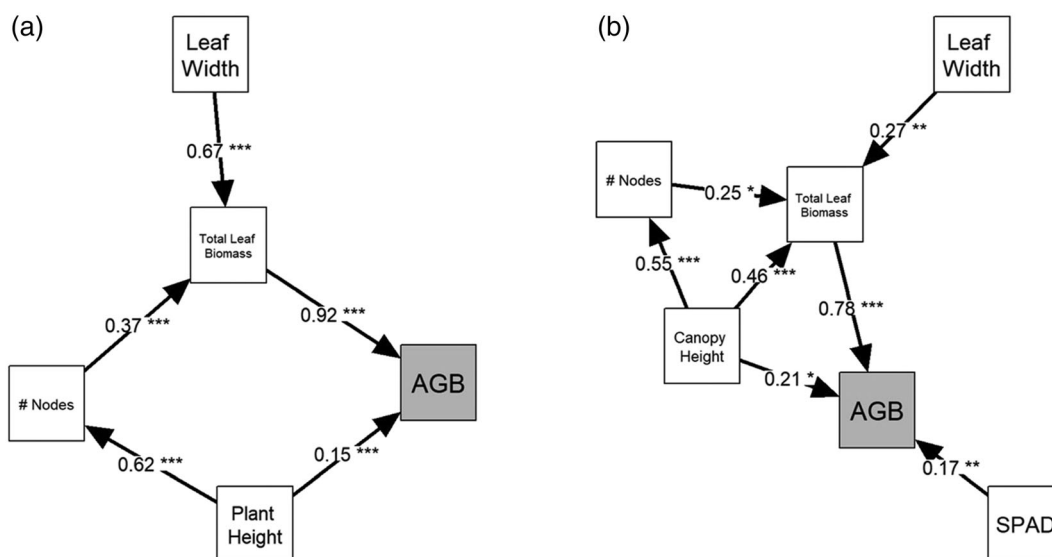


FIGURE 5 Path analysis of total above ground biomass (AGB) in cowpea using (a) the greenhouse trial dataset or (b) the field dataset. Data are centred and scaled. (a) Greenhouse trial: goodness of fit = 0.920, comparative fit index = 0.985, standardized root mean square residual = 0.038. (b) Field: goodness of fit = 0.925, comparative fit index = 0.955, standardized root mean square residual = 0.075. Standardized path coefficients are shown. **p* value < 0.05; ***p* value < 0.01; ****p* value < 0.001. Only significant paths were included in the final model

AGB and total leaf biomass (Figure S5). This supports the hypothesis that narrower leaves are disadvantaged at an early growth stage by intercepting less light than broader leaves. Those relationships, however, were mostly driven by extreme phenotypes with narrow leaves ($L:W > 2.0$), and only the correlations between AGB and leaf $L:W$ remained significant after removing those lines from the analysis. Overall, the correlations between biomass and leaf $L:W$ were weaker than the correlation with leaf width.

Our results argue that leaf width could be used as a proxy for biomass production, and hence fodder production, in cowpea breeding programmes. Utilization of leaf width in breeding has been illustrated in another study. Recurrent selection for wide leaves in wheat germplasm across six cycles over 15 years led to a 33% to 55% increase in leaf width after six cycles (Zhang et al., 2015). This was accompanied by a 35% to 155% increase in total leaf area and a 21% to 109% increase in total biomass. The study also reported high genetic correlation between leaf width and total leaf area ($r = 0.93$) and total biomass ($r = 0.76$). Interestingly, while leaf length increased along with leaf width during the first two cycles, no further increase was observed in the subsequent cycles of breeding. The study suggested that breeding for leaf width in wheat could indirectly select for higher plant biomass production either by selecting for greater light interception and/or for processes associated with cell division. In the present study, cowpeas leaf width stood out as a very good proxy for AGB for cowpeas, especially at a younger developmental stage, when leaf biomass accounted for the largest portion of AGB.

The positive correlation observed in the two experiments between leaf width and AGB under different environmental conditions suggests that leaf width could be a valuable trait for breeding programmes. A positive correlation between leaf dimension traits and AGB under different growth environments was also observed in a study performed on 10 sweet pepper cultivars grown at different temperatures (De Swart et al., 2010). Results showed that sweet pepper relative leaf growth rate (the increase in leaf area per unit of leaf area per unit of time) was positively correlated to the plant relative growth rate (the increase in dry mass per unit biomass per unit of time), which was in turn highly correlated to the total biomass under the different treatments. However, correlations between physiological or morphological traits and productivity are rarely consistent across environments (Asiwe et al., 2021; Martos-Fuentes et al., 2017; Mbuma et al., 2021). For instance, Mbuma et al. (2021) investigated 75 cowpea cultivars grown across four locations and measured pod length, pod width, the number of seeds per pod, individual seed weight and the number of pods per plant. Only the latter was found to be significantly correlated to seed yield across all four locations. This further emphasizes the importance of identifying traits that consistently predict productivity across different environmental conditions.

While selection for leaf width may benefit fodder production, the impact of selection for wider leaves on grain yield in cowpea remains to be determined. Zhang et al. (2015) reported higher 100-grain weight in wheat after recurrent selection for leaf width.

However, they did not report data on the total yield, measured on a land area basis. In a replicated field trial conducted on 22 cowpea genotypes in two different locations in South Africa, leaf width was found to be positively correlated with grain yield (Gerrano et al., 2019). A linkage mapping and quantitative trait loci (QTL) analysis performed on a biparental mapping population of 215 F8 recombinant inbred cowpea lines also found two shared QTL on chromosomes 1 and 8 for leaf width and 100-grain weight (Lo et al., 2018). Together, those studies suggest that selection for leaf width may benefit grain yield in cowpea. Further studies will be required to determine whether this remains true under diverse environments.

Path analysis showed that canopy height could also be considered as a proxy for total AGB (Figure 5). The greenhouse experiment did not show a significant correlation between plant height and AGB, but plants were only grown for 21 days, which may have limited the ability to detect a direct correlation. Plant height was a good predictor of AGB in the MAGIC population grown in the field despite the presence of lines characterized by a spreading growth habit. Breeding for taller plants and canopies may help select for high fodder producing varieties. Other studies have also reported a positive correlation between plant height and grain yield in cowpeas (Thapa et al., 2021), suggesting scope for targeting plant height as a trait to select for grain yield improvement. Past breeding efforts have aimed at reducing plant height in many important crops such as rice (Sakamoto & Matsuoka, 2004), wheat (Khush, 1999), maize (DuVick & Cassman, 1999) and soybean (Poehlman, 1987; Rincker et al., 2014; Specht & Williams, 1984; Ustun et al., 2001) to reduce the sensitivity of these crops to lodging while concomitantly improving their harvest index. In the case of cowpea, however, it should be noted that many existing varieties have a spreading growth habit, making lodging sensitivity irrelevant in this context. Only in some erect varieties produced for grain could lodging become an issue (Cui et al., 2020; Sindhu et al., 2019). The path analysis also showed that chlorophyll content estimated by a SPAD meter had positive direct effect on plant AGB when measured in the field (Figure 5). This suggests that greater chlorophyll content supports higher rate of photosynthesis and more AGB. This was not observed in greenhouse condition, however, where plants were grown at a lower light intensity.

5 | CONCLUSION

Leaf width was positively correlated to AGB at both an early developmental stage when grown under controlled conditions and at a flowering stage when grown under field conditions. This correlation was, in both cases, mediated by the total leaf biomass. Plant height was also a good proxy for AGB. Because these traits are quick and easy to measure, they could be immediately used for selection in breeding programmes for cowpea biomass. Future studies are needed to determine if the relationship between leaf width and biomass holds for seed yield.

ACKNOWLEDGEMENTS

Any opinions, findings, conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. The authors thank those who helped in the field: Christopher Moller, Duncan Martin, David Drag, Ben Harbaugh and Benjamin Thompson. This research was supported by a subaward from the University of Illinois as part of the research project Realizing Increased Photosynthetic Efficiency (RIPE) that is funded by the Bill & Melinda Gates Foundation, Foundation for Food and Agriculture Research and the UK Government's Foreign, Commonwealth & Development Office under Grant OPP1172157.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

AD, EAA and ECS conceptualized research. NO performed the greenhouse experiment with help from RP and EGE. AD performed the field experiment. SBM, MLU and OB provided seed and information about each genotype used in the greenhouse experiment. AD analysed data. AD, EGE, EAA and ECS wrote the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in the Lancaster University Research Directory at <http://www.research.lancs.ac.uk/portal/en/>.

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How to cite this article: Digrado, A., Gonzalez-Escobar, E., Owston, N., Page, R., Mohammed, S. B., Umar, M. L., Boukar, O., Ainsworth, E. A., & Carmo-Silva, E. (2022). Cowpea leaf width correlates with above ground biomass across diverse environments. *Legume Science*, 1–12. <https://doi.org/10.1002/leg3.144>