Research article

Leaf growth, gas exchange and assimilation performance of cowpea varieties in response to *Bradyrhizobium* inoculation

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ARTICLE INFO

Keywords:
Internal CO₂ concentration
Leaf area index
Stomatal conductance
Net photosynthesis
Transpiration efficiency

ABSTRACT

Supplying nitrogen to crops through selecting high N fixing legumes and effective inoculant is one of the key strategies to improve crop productivity. However, studies related to the effect of Bradyrhizobial inoculation on leaf growth, its functioning in relation to photosynthesis, and transpiration efficiency (WUE) of cowpea (*Vigna unguiculata* (L.) Walp) varieties in the tropics were inadequate. A two-year field experiment was conducted at three sites to evaluate the effect of inoculation on leaf growth, gas exchanges and photosynthetic efficiency of cowpea varieties. The study treatments were composed of four varieties, Keti (IT99K-1122), TVU, Black eye bean, and White wonderer trailing and three levels of inoculation (non-inoculated or inoculated with *Bradyrhizobium* strains CP-24 or CP-37). Gas exchange was measured on live plants at 67–77 days after sowing, between 8:00 to 11:00 a.m. and 14:00 to 16:00 p.m. Leaf growth parameters (leaf number and leaf area) were measured by destructive sampling, and the yield data was determined by harvesting plants in the three central rows at physiological maturity. Variety TVU performed best in terms of leaf number, photosynthesis rate, and WUE. Whereas, Black eye bean revealed superior performances for leaf area, leaf area index, and stomatal conductance compared with the rest two varieties. The effect of inoculation was significant with 14.0, 23.8, 13.7, and 11.0% advantage in leaf area, leaf area index, net photosynthesis, and WUE, respectively. Moreover, the performance of cowpeas of the 2018 cropping season showed a relative advantage over 2019 in terms of leaf number, leaf area, leaf area index, net photosynthesis, and stomatal conductance. Therefore, inoculating cowpea varieties with effective *Bradyrhizobium* strain can be a viable alternative to enhance growth, gas exchange, photosynthetic efficiency, and grain yield.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most popular grain legumes grown in Africa and some regions of America and Asia (Boukar et al., 2011). It has multiple purposes serving as an affordable source of quality protein for both rural and urban dwellers in high undernourished areas (Antova et al., 2014). Its leaves and green pods are used as vegetables, and dried grains are used in many different dishes (Kyei-Boahen et al., 2017). The protein concentrations of cowpea leaves and dry grains range from 27 – 43% and 21–33%, respectively (Abudulai et al., 2016). In Ethiopia, cowpea is cultivated mainly for food in dry grain forms since very long time, but utilization of its leaf as a green vegetable has been recently gotten attention (Mulugeta et al., 2016). Cowpea has many non-food desirable benefits in the farming system including supporting sustainability due to its excellent N₂-fixing ability (Appiah et al., 2015; Bittenbender 1990). Moreover, its tolerance to drought and adaptability to stressful environments make it an ideal crop under changing climate conditions (Bisikwa et al., 2014).

The growing impact of climate change on crop production calls for the need of improved agronomic practices, including the use of inoculants and improved crop varieties (Olivares et al., 2013). Moreover, low N contents of most tropical soils are among the various factors that limit the growth and photosynthesis rates of crops (Belane and Dakora 2010; Hailu et al., 2015). Legume inoculation with effective inoculants can be an alternative for the sustainable improvement of soil fertility to enhance crop yields (Samago et al., 2018). Thus, supplying N to crops and cropping systems through either inoculation or utilization of legumes is expected to increase photosynthetic processes, leaf area production, leaf

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https://doi.org/10.1016/j.heliyon.2022.e08746
Received 29 September 2021; Received in revised form 27 November 2021; Accepted 10 January 2022
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area duration as well as net photosynthesis rate (Ahmad et al., 2009). In this regard, N\textsubscript{2}-fixing legumes, including cowpea, have an advantage over non-legumes in their ability to meet their photosynthetic N requirements from symbiotic N\textsubscript{2}-fixation and thereby fulfill the N requirement for morpho-physiological functions of plants (Hikosaka and Terashima 1995). Among the legumes, cowpea, which is widely grown in sub-Saharan Africa, including Ethiopia, is a major player in sustaining the fertility of soils under smallholder farms. It is estimated that cowpea can fix up to 200 kg ha\textsuperscript{-1} of N (Dakora et al., 1987) and can contribute up to 92 kg ha\textsuperscript{-1} of N to the soil (Chikowo et al., 2004).

Leaf functioning regulates leaf transpiration rates, metabolite flux, long-distance signaling, and the influx of CO\textsubscript{2} and water (Lawson 2009). Thus, selecting crop varieties having a high carbon yield in stressing environments would require a prior understanding of leaf growth, stomatal functioning in relation to photosynthetic gas exchange, and leaf water relations (Lawson 2009; Wang et al., 2005). On the other hand, the threat of climate change has affected important natural resources, including water (Chatterjee et al., 2012). In this regard, crop varieties having higher water use efficiency (WUE) are preferred which in turn could be improved via the N\textsubscript{2} – fixation (Xu and Hsiao 2004). Evidently, Batterman et al. (2013) have reported that N\textsubscript{2}-fixing plants alleviate nitrogen shortage, thereby responding to climate change by increasing the capacity to fix CO\textsubscript{2}. Similarly, N\textsubscript{2}-fixing plants demonstrated higher WUE compared with the non-fixing plants (Adams et al., 2016). N\textsubscript{2} – fixation influences WUE because leaf transpiration and carbon fixation are related to leaf nitrogen (Osnas et al., 2013). Thus, increasing leaf nitrogen can increase consumption of intercellular CO\textsubscript{2} because a strengthened CO\textsubscript{2} diffusion gradient helps to maintain the supply of CO\textsubscript{2} to Rubisco (Adams et al., 2016). Therefore, leaf nitrogen and photosynthetic rates have strong positive relationships (Evans, 1989). Collectively, these pieces of evidence highlight the demands for the establishment of a link between N\textsubscript{2}-fixation and thereby fulfills the N requirement for morpho-physiological functions of plants (Hikosaka and Terashima 1995). Among the legumes, cowpea, which is widely grown in sub-Saharan Africa, including Ethiopia, is a major player in sustaining the fertility of soils under smallholder farms. It is estimated that cowpea can fix up to 200 kg ha\textsuperscript{-1} of N (Dakora et al., 1987) and can contribute up to 92 kg ha\textsuperscript{-1} of N to the soil (Chikowo et al., 2004).

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2. Materials and methods

2.1. Description of the experimental sites

The experiment was conducted during the main rainy season of the years 2018 and 2019 at three sites in southern Ethiopia. The three sites are situated at: Hawassa (7º 3’ N; 38º 30’ E, 1700 m a.s.l.), Boricha (6º 17’ N; 38º 04’ E, 1866 m a.s.l.) and Dore (7º 3’ N; 38º 28’ E, 1694 m a.s.l.). They are selected as they are among the vulnerable areas in southern Ethiopia to climate change. In addition, the population is prone to food insecurity (Atara et al., 2019). It is worth to noting that the plots used in the 2018 cropping season were changed during 2019 in order to avoid the residual effects of inoculants.

2.2. Soil and climate characteristics

Soil and climatic data are summarized in Tables 1 and 2, respectively. The soil texture of the study sites was clay loam for Boricha and Hawassa, and loam for Dore sites. The pH of the experimental soil was found suitable for cowpea production as it is characterized by a pH of about neutral (Onyibe et al., 2006). As per the limit set by Havlin et al. (1999) and (Herrera (2005)), the total N ranges from low to moderate and the organic carbon very low to low, respectively. The soils were not out of range for legume production in terms of the available P, cation exchange capacity, and exchangeable cations content (FAO 2006) (Table 1). During the 2018 experimental season Boricha, Dore, and Hawassa sites received a mean monthly rainfall of 116, 115 and 107 mm, respectively. However, the sites were characterized with 169, 164, and 141 mean monthly rainfall, respectively, during the second season. The mean atmospheric temperature were within the range of 19.0–20.3 °C for both years (Table 2). A detailed description has been given in Ayalew et al. (2021).

2.3. Experimental material sources and experimental design

The seeds of four cowpea varieties, Keti (IT99K-1122), TVU, Black eye bean, and White wonder trailing, were provided by Melkassa Agricultural Research Center, Ethiopia. These cowpea varieties are under production in Ethiopia, which were released for commercial production between the years 1970–2012 (MaA 2018). They differ regarding the time required to reach maturity at this experiment locations with the range of 121–129 days (Ayalew et al., 2021). CP-24 and CP-37 Bradyrhizobium strains were provided by Hawassa University, Agriculture College. The strains, after isolation, were subjected to authentication and characterization (Degefè et al., 2018). Furthermore, the symbiotic effectiveness was confirmed using sand culture, before use in field conductions (Ayalew and Yoseph 2020).

The treatments consisted of four cowpea varieties and three inoculation doses in Randomized Complete Block Design with a factorial arrangement, replicated four times. A spacing of 0.5, 0.2, 0.5, and 1 m were used for row - row, plant - plant, plot – plot, and block – block arrangements, respectively, with 3.5 × 3.4 m individual plot size. The seeds were inoculated with 10 g of peat-based inoculant per kg of seeds (Rice et al., 2001) containing 6.5 × 10\textsuperscript{10} viable bacterial cells g\textsuperscript{-1} peat of Bradyrhizobium strain CP-24 and CP-37. The seeds were inoculated under shade and air dry for some minutes prior to planting to maintain cells viability and avoid fungal growth, respectively (Rice et al., 2001). The plots used in 2018 were changed in 2019 to avoid the residual effects of inoculants.
Table 2. Monthly rainfall and temperature records at Hawassa, Boricha and Dore sites during cropping seasons of 2018 and 2019. Adapted from Ayalew et al., (2021).

| Month | Precipitation (mm) | Average temperature (°C) |
|-------|-------------------|------------------------|
|       | Boricha 2018 | Boricha 2019 | Dore 2018 | Dore 2019 | Hawassa 2018 | Hawassa 2019 |
| June  | 133            | 125            | 154         | 141         | 142          | 128          |
| July  | 50             | 168            | 68          | 178         | 67           | 158          |
| Aug.  | 135            | 161            | 145         | 175         | 150          | 160          |
| Sept. | 122            | 128            | 107         | 139         | 91           | 128          |
| Oct.  | 140            | 263            | 102         | 196         | 83           | 133          |
| MA    | 116            | 169            | 115         | 166         | 107          | 141          |
| GMT   | 580            | 844            | 575         | 829         | 533          | 706          |

MA = monthly average, GMT = total for the growing months.

inoculants). The detailed design and procedures are described in Ayalew et al. (2021).

2.4. Photosynthetic rate and gas exchanges

Net photosynthesis rate ($P_n$) [μmol (CO$_2$) m$^{-2}$ s$^{-1}$], transpiration rate ($E$) [mmol (H$_2$O) m$^{-2}$ s$^{-1}$], internal CO$_2$ concentration (Ci) and stomatal conductance ($g_s$) were measured using a portable infrared gas analyzer (IRGA) (ADC BioScientific LCI-SD System Ltd., UK, 2011) at 70–72 and 75–77 days after sowing, at flowering, during 2018 and 2019 crop seasons, respectively. Measurements were made between 8:00 to 11:00 a.m. and between 14:00 to 16:00 p.m. During the measurement, the system was calibrated with the ambient carbon dioxide concentration ($C_{ref}$) and between 14:00 to 16:00 p.m. During the measurement, the system was calibrated with the ambient carbon dioxide concentration ($C_{ref}$) and between 14:00 to 16:00 p.m. The leaf area was measured using an automatic leaf area meter (model LI 31000A Li-Cor, Lincoln, USA). Then, leaf area index (LAI) and the leaf area ratio (LAR) were calculated using Eq. (1) and Eq. (2), respectively.

$$\text{LAI} = \frac{\text{Total green leaf area of the sampled plant}}{\text{Ground area occupied by the sampled plant}}$$

(1)

$$\text{LAR} = \frac{\text{Leaf area of the sampled plants}}{\text{Shoot dry weight of the sampled plants}}$$

(2)

Plant height was measured from the ground level to the tip of the plants from those plants uprooted for nodulation data determination.

2.6. Nitrogen content and grain yield

The yield data was determined by harvesting plants in the three central rows at physiological maturity as described in detail by Ayalew et al. (2021), a paper published from the same experiment with this, and in this paper the yield data was used to show the relation between leaf growth, gas exchange and photosynthesis with that of yield performance of cowpea.

2.7. Statistical analysis

The dataset was subjected to a generalized linear model procedure of SAS software version 9.4 (SAS Institute, 2012). Inoculants and cowpea varieties were considered as fixed factors and growing season and location as random variables. The two seasons were combined in the analysis when the test for variances was homogeneous, and separate analysis was done when the variances between seasons were heterogeneous. Variety responses and inoculation effects on leaf number, leaf area, leaf area index, plant height, net photosynthesis, stomatal conductance, internal CO$_2$ concentration and shoot nitrogen content were analyzed for the 2018 and 2019 growing seasons separately. Since the data for those parameters were heterogeneous upon testing homogeneity of variance over seasons, it helped to compare inter-annual variation. Again, since the data for these parameters were homogeneous over seasons, the analysis of interaction effect was conducted for the varieties response and inoculation effect on leaf area ratio, leaf transpiration rate, water use efficiency and grain yield. The data from the three sites were combined as the variances among locations were homogenous. Treatments were compared by the least significant difference (LSD) method for a significance level of $P \leq 0.05$. Correlation analysis was done using Pearson's simple correlation coefficients to test the relationships between leaf development, gas exchanges and yield.

3. Results

3.1. Varietal effects on growth traits

Leaf number plant$^{-1}$ was significantly influenced by cowpea variety (Table 3). Variety TVU produced the greatest number of leaves plant$^{-1}$ followed by White wonderer trailing and Keti (IT99K-1122), while the lowest number of leaves plant$^{-1}$ were recorded in the Black eye bean variety (Table 3). The relatively broad-leaved Black eye bean variety produced the largest LA, while White wonderer trailing variety presented the lowest LA in both years (Table 3). However, Black eye bean and TVU in 2018, and Keti and White wonderer trailing in 2019, did not significantly ($P \geq 0.05$) differ for LA. Similarly, Black eye bean recorded the highest LA, followed by TVU and Keti (IT99K-1122) followed by White wonderer trailing. The LAR was numerically higher for Black eye bean followed by TVU with the lowest from White wonderer trailing (Table 4). Plant height was significantly influenced by the cowpea varieties, being highest for variety of Black eye bean, and lowest for Keti (Table 3).

3.2. Inoculation effects on growth traits

Plants inoculated with either of the *Bradyrhizobium* strains produced more leaves plant$^{-1}$ compared with the non-inoculated control (Table 3).
However, the two strains did not significantly differ for their effects on the number of leaves plant$^{-1}$. Inoculation of cowpea with Bradyrhizobium strains showed a significant effect on LA and LAI in the 2018 cropping season compared with the control, however, the effect was not significant in 2019. The two strains did not differ for LA and LAI in the 2018 cropping season (Table 3). LAR was also significantly influenced by inoculation with Bradyrhizobium strains, with the higher LAR from CP-24 inoculated plants (Table 4). However, the strain CP-37 did not differ from the control for LAR.

### 3.3. Varietal effect on physiological gas exchanges

There was no difference in net photosynthesis among varieties (Table 5). However, there was a significant difference among cowpea varieties for stomatal conductance ($g_s$) during 2018 (Table 5). Among the varieties, the White wonderer trailer and Black eye bean exhibited the highest $g_s$ in 2018. However, the varieties Black eye bean and White wonderer trailing did not significantly differ for leaf $g_s$. The variety Keti recorded the lowest value for stomatal $g_s$ compared with the other three varieties in 2018 but not 2019. Similarly, the varieties differed significantly for internal carbon dioxide concentration ($C_i$) in 2018 cropping season. As indicated in Table 5, the variety Black eye bean (274.2 µmol mol$^{-1}$) and TVU (274.0) exhibited higher $C_i$ followed by Keti (269.0 µmol mol$^{-1}$). The lowest $C_i$ was recorded for the White wonderer trailing variety. The effect of varieties on $C_i$ was not significant in 2019, however, the relative performance among the varieties followed the same trend for $C_i$ in 2018 (Table 4). The highest $E$ was recorded in the variety Black eye bean which significantly differed from TVU. Regarding the WUE generally, TVU variety recorded the highest values for WUE, while the other varieties did not differ one another (Table 4).

### 3.4. Effects of inoculation on physiological gas exchanges

Inoculation with Bradyrhizobium significantly increased net photosynthesis during both 2018 and 2019 cropping seasons compared with the control but without significant differences between the Bradyrhizobium strains (Table 5). Stomatal conductance ($g_s$) also markedly affected with Bradyrhizobium inoculation in 2018 with both strains (Table 5) and resulted in increased transpiration efficiency, WUE, than non-inoculated plants (Table 4). However, the influence of the two strains on WUE did not differ significantly.

### 3.5. Correlation analysis

The Pearson’s correlation coefficients for comparison of the degree of association between leaf growth, gas exchange and/or seed yield are presented in Table 6. There were significant positive correlation between yield and its components and all the leaf development parameters. For example, significant positive correlations occurred between number of pods and leaf number (0.83**), leaf area (0.69**), and LAI (0.65**). Similarly, the biological yield (the aboveground biomass including the straw and pods yields) was significantly positively correlated with leaf number (0.66**), leaf area (0.62**) and LAI (0.58**) indicating the contribution of leaf growth performance for better biomass accumulation. More importantly, grain yield showed significant positive correlation with leaf number (0.90***), leaf area (0.74**) and LAI (0.72**), indicating the importance of leaf growth performance for grain yield improvement. Grain yield and WUE were positively correlated although not significantly (0.55ns), whereas, grain yield correlated negatively with leaf transpiration (−0.57*). Biological yield had highly significant positive correlation with number of pods (0.91**), and grain yield also

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**Table 3. Effects of cowpea varieties and Bradyrhizobium strains inoculation on leaf number (LN), leaf area (LA), leaf area index (LAI) and plant height (PH) of cowpea grown at three sites during the cropping seasons of 2018 and 2019.**

| Treatments | LN | LA [cm$^{-2}$] | LAI | PH [cm] |
|------------|----|---------------|-----|---------|
|            | 2018 |                | 2019 |                |
| Variety    |      |                |      |            |
| Keti       | 100b | 1919b          | 1.9b | 15.8c    |
| TVU        | 124a | 1947b          | 1.9b | 16.8bc   |
| Black eye bean | 91b  | 2066b          | 2.1b | 28.0a    |
| White wonderer trailing | 117b | 1600b          | 1.6b | 22.4ab   |
| Bradyrhizobium |     |                |      |          |
| Non-inoculated | 91b  | 1729b          | 1.6b | 20.2     |
| CP-24      | 122a | 2005b          | 2.1a | 21.5     |
| CP-37      | 111ab| 1920a          | 2.0a | 20.5     |

Means followed by different letters in a column are significantly different at *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001 by the LSD test.

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**Table 4. Effect of cowpea varieties and Bradyrhizobium strains inoculation on leaf area ratio (LAR), leaf transpiration rate ($E$) and water use efficiency (WUE) of cowpea grown at three sites for the pooled results of the cropping seasons 2018 and 2019.**

| Treatments | LAR [cm$^2$ g$^{-1}$] | $E$ [mmol (H$_2$O) m$^{-2}$ s$^{-1}$] | WUE [µmol (CO$_2$) mmol (H$_2$O)$^{-1}$] |
|------------|-----------------------|-------------------------------------|----------------------------------------|
| Variety    |                       |                                     |                                        |
| Keti (IT99K-1122) | 91.1 | 6.1ab                  | 3.6b                                  |
| TVU        | 94.8                  | 5.8b                  | 4.0b                                  |
| Black eye bean | 100.0 | 6.4b                  | 3.6b                                  |
| White wonderer trailing | 82.0 | 6.1ab                  | 3.6b                                  |

Means followed by different letters in a column are significantly different at *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001 by the LSD test.
Table 5. Effects of cowpea varieties and *Bradyrhizobium* inoculation on net photosynthesis ($P_{n}$), stomatal conductance ($g_{s}$) and internal carbon dioxide concentration ($C_{i}$) of cowpea grown at three sites during the cropping seasons of 2018 and 2019.

| Treatments | Variety | 2018 | 2019 | 2018 | 2019 |
|------------|---------|------|------|------|------|
|            |         | $P_{n}$ [μmol (CO$_2$) m$^{-2}$ s$^{-1}$] | $g_{s}$ [mmol m$^{-2}$ s$^{-1}$] | $C_{i}$ [μmol mol$^{-1}$] | $P_{n}$ [μmol (CO$_2$) m$^{-2}$ s$^{-1}$] | $g_{s}$ [mmol m$^{-2}$ s$^{-1}$] | $C_{i}$ [μmol mol$^{-1}$] |
|            | Keti (IT99K-1122) | 21.3 | 1.3$^{b}$ | 269.9$^{ab}$ | 19.8 | 1.3 | 246 |
|            | TVU | 22.1 | 1.5$^{b}$ | 274.0 | 20.7 | 1.0 | 247 |
|            | Black eye bean | 21.1 | 1.9$^{a}$ | 274.2 | 20.2 | 1.2 | 248 |
|            | White wonderer trailing | 20.9 | 1.9$^{a}$ | 259.9$^{b}$ | 20.6 | 2.0 | 236 |
|            | *Bradyrhizobium* Non-inoculated | 19.5 | 1.3 | 265.7 | 19.4 | 1.3 | 246 |
|            | CP-24 | 22.6 | 1.8$^{a}$ | 271.8 | 21.1 | 1.5 | 244 |
|            | CP-37 | 22.0 | 1.8$^{a}$ | 270.4 | 20.5 | 1.3 | 243 |
|            | Variety x *Bradyrhizobium* | 8.9 | 0.30$^{a}$ | 280.4 | 5.6 | 0.74 | 735 |

Means followed by different letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$ by the LSD test.

demonstrated significant positive correlation with pod numbers per plant (0.93***), and biological yield (0.86***).

There was significant positive correlation between net photosynthesis and leaf number (0.60***), and LAI (0.62**), while positive non-significant correlation with leaf area. WUE exhibited a significant positive correlation with leaf number (0.61*), leaf area (0.64**) and leaf area index (0.70**). Furthermore, WUE showed a positive significant (0.62**) and a negative significant (0.75*) correlation with net photosynthesis and leaf transpiration, respectively.

4 Discussion

4.1 Effects of inoculation on plant development and photosynthesis

The significant improvement in leaf number per plant at flowering due to *Bradyrhizobium* inoculation of the 2018 observation, can be associated with the improved vegetative growth because of enhanced nitrogen supply by biological nitrogen fixation. In this experiment, inoculation with *Bradyrhizobium* demonstrated an advantage of about 41% $N$ contribution compared with the non-inoculated control (Ayalew et al., 2021). The subsequent improvement in LAI with inoculation is associated with the advantage in LA development due to inoculation. In agreement with these observations, a higher leaf growth in inoculated cowpea plants was also reported by Agba et al. (2013). Furthermore, Pereira et al. (2019) reported the LA improvement for inoculated cowpea varieties. Agba et al. (2013) and Kasewski (1994) and Okon (1989) have concluded that $N_2$-fixation by Rhizobia inoculated legumes enhances leaf expansion. Plant nutrition management practices like inoculation with elite strains aim to optimize photosynthesis rate and yield, mainly by optimizing LAI (Mitchell and Hardy 2000), due to the fact that leaf area growth determines the light interception capacity and thereby the resource use efficiency of crops (Sarathi et al., 2015).

It has been indicated that total leaf photosynthesis increases with an increase in LAI (Mitchell and Hardy 2000). Connected to this, the correlation analysis found a strong and significant relationship between LAI and net photosynthesis, and grain yield (Table 6), indicating a link between LAI with capacity of assimilation and yield (Mitchell and Hardy 2000). Higher yield recorded from this experiment (Ayalew et al., 2021) was attributed to the inoculation driven improvement in leaf characteristics. The advantage for LAR due to inoculation is associated with a corresponding leaf growth improvement, as inoculation enhanced leaf area. In agreement, Pereira et al. (2019) also reported significant increase in leaf area ratio due to inoculation of faba bean (*Vicia faba* L.) plants. However, during the cropping season of 2019, inoculation did not lead to...
a significant effect on most of the leaf growth characteristics. Rainfall was higher at all sites during 2019, with 141–168 mm monthly range, what may have led to lower grain yield response of cowpea to inoculation (Ayalew et al., 2021). Higher rainfall during the 2019-cropping season, might have affected the survival, establishment and symbiotic properties of Bradyrhizobium strains (Hamdi 1999). Climatic condition are among the most important factors affecting inoculant performance (Kunert et al., 2016) and the high rainfall in 2019 (Table 2) could explain the non-significant effect of inoculation on leaf development. High precipitation can affect microbial respiration, biomass nutrition and enzyme activities (Odette et al., 2016), and thus impair the performance of the inoculated Rhizobia.

The improvement in net photosynthesis with inoculation (Table 5) might be due to the improved supply of N nutrition from symbiosis (41% more fixed N was achieved compared with the non-inoculated control) and thereby improved leaf metabolic processes. Nitrogen is the major nutrient required for the synthesis of the macromolecules responsible for CO₂ assimilation and radiation interception (ribulose-1, 5-bisphosphate carboxylase-oxygenase -Rubisco), thereby improving the rate of net photosynthesis when enough N is available for plants. N₂ fixation and net photosynthesis are, therefore, metabolically interlinked in nodulating legumes. In agreement with the current result, Belane and Dakora (2015) have reported improvement of net photosynthesis in cowpea due to inoculation.

The net photosynthesis response was relatively higher in 2018 compared with 2019, what might be related with the poor crop-inoculant performance in 2019 due to high rainfall. Supporting the current result, Pule-Meulenberg et al. (2010) reported differences in net photosynthesis due to environmental effects on the host plant performance. A significant improvement with Bradyrhizobium inoculation in leaf gs was recorded. The increased gs might be associated with the increased N concentration in the leaf due to N fixation that reached 104.9 kg ha⁻¹ (Ayalew et al., 2021, submitted). In accordance with the findings of this experiment, Augé et al. (2015) reported improvement in stomatal functioning due to symbioses. Furthermore, the higher LAI due to inoculation might play a role in the modification of the stomatal conductance (Blatt et al., 2017), because symbiosis modifies stomatal behavior (Augé et al., 2015). A change in leaf gs and E in cowpea due to rhizobial inoculation was also reported elsewhere (Matiru and Dakora 2005) where stomatal conductance increased. Similarly, the improvement in WUE of cowpea due to inoculation highlights the importance of the cowpea-Bradyrhizobium symbiosis for enhanced water relationships. The improvement in WUE with inoculation could be related to the improved nitrogen availability; supporting the optimal physiological functions. The symbiosis-triggered enhancement in cowpea WUE was also reported by Tankan et al. (2019) and Yahaya et al. (2019) supporting the current findings. Generally, the current study is innovative, because crop production has becoming vulnerable to climate changes and the rural community is under food insecurity pressure (Atara et al., 2019). Thus, the legume and its interaction with Rhizobia brings a potential alternative to face the climate changes due to biological nitrogen fixation capacity (Ayalew et al., 2021).

4.2. Varietal effect on plant development and photosynthesis

Significant genotypic variations and inoculation effect on leaf growth and photosynthetic gas exchanges were observed, which could give indications of superiorities when comparing cowpea genotypes and Bradyrhizobium strains for agronomic fitness during the era of climate changes (Anyia and Herzog 2004). For example, a large number of leaves, as observed in variety TVU, could be an important attribute from the viewpoint of yield and microclimate improvement. Corroborating this statement, variety TVU having higher leaf number results with higher grain yield (Ayalew et al., 2021). The significant positive correlation between leaf number and grain yield confirmed this relationship (Table 6). Moreover, it will serve for a more effective light interception, thereby improving net photosynthesis (Mitchell and Hardy 2000). This is also confirmed by the significant positive correlation (r = 0.6, p = 0.001) between net photosynthesis and leaf number (Table 6). Increase in leaf area heads to improved photosynthesis (Wenshi et al., 2020). According to Liu et al. (2014), net photosynthesis rate is directly proportional to the leaf light intercepting capacity. This is supported by the significant positive correlation between LAI and net photosynthesis in this study. Furthermore, the higher yield observed for the variety TVU over the years in three sites (Ayalew et al., 2021) validates the link between leaf development and photosynthetic properties with enhancing crop yield were also observed by Wenshi et al. (2020). Similarly, Malone et al. (2002) reported that yield increases with the increase in LAI, which is in agreement with our study manifested by the significant positive relationship between LAI and grain yield.

Black eye bean variety performed best for LA and LAI over the years and seasons and this was associated with its broad leaf nature compared with the other three varieties. For instance, calculating the leaf size as LA over leaf number, Black eye bean leaf size exceeded that of the small leaf producing variety White wonderer trailing by 39%. In agreement with this result, Pereira et al. (2019), Peksen et al. (2005) and Bisiikiwa et al. (2014) reported a difference in leaf growth among cowpea varieties including leaf number and area. The improved LAI observed for the Black eye bean variety could be associated with its larger LA and the strong positive (r = 0.99, p = 0.001) correlation between LAI and LAI (Table 5). Agba et al. (2013) and Toker et al. (2002) also observed differences in LAI among cowpea varieties. The higher plant height from the Black eye bean variety is attributed to the growth behavior of the variety. Kyel-Boahen et al. (2017) found the differences in shoot performance among cowpea varieties. In agreement with the current result, significant difference in plant height among cowpea varieties were reported also by Bisiikiwa et al. (2014) and El Naim and Jaberdeldar (2010).

The relatively higher photosynthesis rate in 2018 might explain the poor performance of the varieties caused by higher precipitation in 2019. The subtleties in host plant performance could account for the differences in net photosynthesis rate between genotypes in time and space (Pule-Meulenberg et al., 2010). Martínez-Acosta et al., 2020 reported negative effect of excess water supply on stomatal conductance, photosynthesis and transpiration. Varieties of White wonderer trailing and Black eye bean exhibited the highest leaf gs compared with the other three varieties. The variability for stomatal gs among varieties could be attributed to the genetic variation among the tested varieties. The higher leaf gs for White wonderer trailing might also be associated with the relatively low LAI demonstrated by this variety, which could increase the conductance per unit of leaf area. Processes such as canopy interception, evapotranspiration and photosynthesis are affected by the LAI (Liu et al., 2014). In agreement with the current result, Augé et al. (2015) reported a variation in leaf stomatal conductance among cowpea varieties.

The highest and the lowest transpiration rate exhibited by Black eye bean and TVU, respectively, might be related to the leaf growth characteristics of each variety. The higher LA and resulting LAI for the Black eye bean might be the cause for higher transpiration rate observed in this variety, which exposes the leaf to more water loss at the expense of CO₂ diffusion in the process of photosynthesis. Leaf area contributed for variation in leaf water loss (Wang et al., 2019). The negative correlation between transpiration with leaf number and leaf area in this study (Table 6), indicates the association amongst leaf growth characteristics and leaf transpiration rate. The current result also agreed with the previous findings of Driss and Bill (2001). Similarly, the variation in WUE among the varieties might be due to the variation in genetic characteristics of the varieties and their interactions with environment. For instance, the higher WUE for TVU is linked with the lower stomatal gs and leaf E (Tables 4 and 5). The significant negative correlation between WUE and leaf transpiration rate, and the negative correlation between the WUE and leaf gs confirms the inverse association between higher leaf transpiration rate and stomatal gs with WUE (Tables 4 and 6). In
confirmation, the variety Black eye bean with the highest leaf E and stomatal gs recorded the lowest WUE. High WUE can be achieved either through lower stomatal conductance or higher photosynthetic capacity or a combination of both (Condon et al., 2002). In line with the current findings, Damba et al. (2019) presented a variation among cowpea varieties for WUE. In good agreement with the current findings, a previous study by Tomas et al. (2012) confirmed the existence of variation in WUE among cowpea varieties. The internal CO2 concentration was lower for the variety White wonderer trailing compared with the other three varieties in 2018, which might be attributed to the relatively lower net photosynthetic rate during the same year. In accordance with this, Janoudi et al. (1993) reported a reduction in net photosynthesis due to a limited CO2 concentration.

5. Conclusion

Inoculation with Bradyrhizobium strains CP-24 and CP-37 significantly increased leaf growth performance of cowpea in terms of leaf number, leaf area, leaf area index and leaf area ratio. The tested varieties also showed a significant difference regarding to leaf growth characteristics except leaf area ratio, whereby variety TVU exhibits higher performance for number of leaf per plant. Whereas, the variety Black eye bean performed best for LAI, LAI and plant height. Inoculation improved net photosynthesis rate, stomatal conductance, and WUE of cowpea varieties. The varieties also differed for physiological parameters with a high performance of TVU for WUE, and White wonderer trailing for physiological performance and associated yield advantage of cowpea varieties.

Declarations

Author contribution statement

Tewodros Ayalew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tarekegn Yoseph; Petra Högy; Georg Cadisch: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by a PhD scholarship at the Hawassa University, in the framework of the German-Ethiopian SDG Graduate School “Climate Change Effects on Food Security (CLIFOOD)” between the Food Security Center, University of Hohenheim (Germany) and the Hawassa University (Ethiopia), supported by the DAAD with funds from the Federal Ministry for Economic Cooperation and Development (BMZ).

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors are grateful to Haimanot Beruk and Hussein MB for their support in statistical data management.

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