Photosynthetic Activities and Growth Rate of Cowpea (*Vigna Unguiculata* L. Walp) Inoculated with Rhizobia at Two Developmental Stages.

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Abstract

This study investigated the effect of symbiotic N-source on the photosynthetic activities of cowpea at different developmental stages. Treatments were two developmental stages (vegetative and reproductive) and five N-sources (control, inoculation with BR 3267, BR 3262, USDA 3451 rhizobia strains and 90kg N ha^{-1} application). Chlorophyll fluorescence parameters and crop growth rate were measured. All the treatments had significantly higher quantum yield of photosystem II at their vegetative stage than reproductive stage ($P<0.05$) except plants to which 90kg N ha^{-1} was applied which maintained similar values at both stages. Opposite trend was observed for quantum yield of non-photochemical quenching. Plants which received 90kg N ha^{-1} had significantly higher growth rate at the reproductive stage than others which had statistically similar growth rates. It can therefore be concluded that application of nitrogenous fertilizer sustained significantly high quantum yield of photosynthesis and dry matter production in cowpea at reproductive stage.

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Introduction

Nitrogen (N) is a major element that limits crop productivity in the tropics. Some major ways through which N is added to the soil for crop use include fertilizer application and biological N fixation. Rhizobia inoculation is done to ensure that compatible and effective rhizobia strain is available for symbiosis with the legume to bring about improved nodulation and N fixation. Many soils lack adequate amount of effective indigenous rhizobia (Slattery and Pearce, 2002) that will enhance nitrogen fixation to meet the N need of the crop. Hence the need for rhizobia inoculation. Rhizobia inoculation not only meet the N need of the legume but a reasonable amount of the N need of the succeeding crop if effective rhizobia strains form symbiosis with the legume. This cuts down the quantity of chemical fertilizer which is not always available to the resource-poor farmers and less environmentally friendly. Lupwayi et al. (2004) reported that increase in yield of crops sown after leguminous crops are equivalent to those expected from application of 30 to 80 kg N ha−1 of fertilizer.

Cowpea (*Vigna unguiculate* L. Walp) is a very important leguminous crop in Nigeria but it is often grown on depleted farm land without external input and it always receives little attention. This has contributed to the low yield obtained on farmers' field in this part of the world. Being a leguminous crop, cowpea naturally should be able to meet its N need through N fixation and soil N. Pule-Meulenberg *et al*. (2010) reported that cowpea is capable of deriving up to 99% of its nitrogen requirement from symbiotic fixation but this is not always the case due to ineffective indigenous rhizobia. The crop hardly meet its N need let alone meeting the N need of the succeeding crop. Abayomi *et al*. (2008) asserted that though cowpea fixes N, it may suffer from temporary N deficiency once the cotyledonary N reserve is exhausted before nodulation and N fixation starts. Also, at the reproductive stage, nodules' ability to fix N reduces because assimilate is preferably transferred to the pods rather than the nodules. Hence legumes could suffer N deficiency at this period especially if the soil N is low (Lindemann and Glover, 2015). Rhizobia inoculation with an effective strain that will fix more N and increase the productivity of cowpea could help bridge this gap. Studies have shown increase in N fixed and grain yield of other legumes as a result of rhizobia inoculation (Ndakidemi *et al*. 2006; Bambara & Ndakidemi, 2009; Schweiger *et al.,* 2012). However, unlike soybean, commercial cowpea inoculant is yet to gain popularity.

Photosynthesis and nitrogen fixation are the two major physiological processes of uttermost importance to legume growth and yield (Vollmann *et al*., 2011). The major measurements used to objectively evaluate the capability of rhizobia strains to affect photosynthesis in legume is chlorophyll fluorescence (Samaniego-Gámez *et al*., 2016)**.** There is an increasing need for a fast and non-destructive measurement of chlorophyll fluorescence through the use of steady-state illumination in the field.

Photosynthesis parameters are valuable descriptors for analyzing the sensitivity of plants to various biotic and abiotic conditions such as N deficiency, making plant improvement and development possible. The quantum yield of photosystem II (ΦII) has been used as a tool for investigating photochemical mechanisms underlying photosynthesis in addition to showing the efficiency of CO_2 assimilation (Bibi *et al*., 2008). It measures the ratio of light that is utilized in photochemistry by chlorophyll associated with photosystem II for the production of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) which are thus utilized in reduction of carbon dioxide (CO_2). For optimum performance of photosystems excess energy is either dissipated as heat through regulatory or non-regulatory non-photochemical quenching (NPQ) to avoid photo-damage (Lazar, 2015).

Linear relationship between N content of the plant and photosynthesis has been reported by many researchers (Nunes *et al*., 1993). Inoculation with rhizobia was been reported to have significant effects on photosynthesis of legumes (Han and Lee, 2005; Zhou *et al*., 2006; Bambara and Ndakidemi, 2009) but cowpea is generally reported as being poorly responsive to inoculation (Giller, 2001; Fening and Danso, 2002; Aliyu *et al*., 2013) hence, the need to understand the relationship between N-fixation and the photosynthesis functioning in cowpea. Measurement of photosynthetic performance are important for crop improvement purposes (Murchie and Lawson, 2013). To provide new perspective for the improvement of rhizobia inoculant use in cowpea, this work therefore aimed at determining the effect of rhizobia inoculation on photosynthesis of cowpea at different stages of development.

Materials and Methods

Study location

The trial was conducted in three locations in Minna (09° 28.026$^{'}$ N 006° 25.325$^{'}$ E, 09° 27.832$^{'}$ N   006° 25.375$^{'}$ E and 09° 31.203$^{'}$N   006° 27.678$^{'}$ E) in Nigeria's southern Guinea savanna. Minna's clime is sub-humid; the average maximum temperature recorded during the experiment was 32.4^{o}C, minimum temperature was 21.4^{o}C, relative humidity was 70% and rainfall was 607 mm. The soil type of all the sites was sandy loam with total N ranging between 0.10 to 0.18 g kg^{-1}, organic carbon ranged between 0.6 -1.35 g kg^{-1}, and pH between 5.99 and 6.50.

Treatments and experimental design

The treatments were five levels of N- sources viz: control -without any input, inoculation with BR 3262, BR 3267, and USDA 3451 rhizobia strains, and 90 kg N ha^{-1} as a positive control at two developmental stages (vegetative and reproductive). A split plot design was adopted where the N-sources were assigned to the main plot and the developmental stages to the sub-plot. These treatments were in three replicates.   The gross plot (15 m^2) consisted five rows while the net plot (9 m^2) consisted three rows. Intra-row spacing of 20 cm was maintained.

Plant material, rhizobia inoculation and fertilizer application

An early maturing cowpea variety (IT93K-452-1) was used in this study. Five grams inoculant were applied per kg seed using the slurry method (IITA & N2Africa, 2014). The seeds were coated with a solution of 85 ml water and 15 g sugar

(sticker) after which the inoculant was applied. Two seedlings were maintained per stand and the plants obtained 40 kg K_2O and 20 kg P ha^{-1} at planting. For the 90 kg N ha^{-1} treatment, One-third (30 kg N ha^{-1}) was applied at two weeks after planting. (WAP) and two-third (60 kg N ha^{-1}) at flowering initiation.

Data collection

At two weekly intervals, plants within one meter were uprooted and the dry mass obtained at 65°C until constant weight was obtained. The Crop growth rate was calculated using equation i below:

$$ \text{CGR (g/m}^2\text{/day)} = \frac{W_2 - W_1}{T_2 - T_1} \quad \text{-----------------------------(equation i)} $$

W_1 and W_2 are total dry mass at time T_1 and T_2 respectively (Das 2011). At 3 (early vegetative stage) and 7 (flowering stage) weeks after planting, chlorophyll fluorescence measurement (quantum yield of photosystem II (ΦII), quantum yield of non-photochemical quenching (ΦNPQ), quantum yield of non-regulatory processes (ΦNO), relative chlorophyll content) and environmental measurements (photosynthetic active radiation (μmol photons m^{-2} s^{-1}), leaf temperature °C, ambient temperature °C) were obtained using the MultispeQ 1.0 device (Photosynq, Michigan, USA).

Data were taken on fully opened uppermost trifoliate leaves of five tagged plant before noon using the MultispeQ v1.0 leaf photosynthesis protocol. Collected data were subjected to analysis of variance (ANOVA) using Minitab 17 software General Linear Model procedure. At 5% level of probability, means differences were separated using Least Significant Difference (LSD).

Results and Discussion

Photosynthetic active radiation (PAR), leaf temperature and chlorophyll

Higher PAR values were recorded at reproductive stage (mean value of 1142.0 μmol (photons) m^{-2} □$^{-1}$ and interquartile range of 730.0 -1573.0 μmol (photons) m^{-2} □$^{-1}$) than at vegetative stage (mean value of 620.7 μmol photons m^{-2} s^{-1} with interquartile range of 480 - 800 μmol (photons) m^{-2} □$^{-1}$). (Figure 01). Similar trend was observed for leaf temperature with the mean value of 29.54°C and interquartile range of 27.36-31.12°C at vegetative stage, and mean value of 30.86°C with interquartile ranging 28.57-32.85°C at reproductive stage (Figure 02). The relative chlorophyll content was not significantly affected by the treatments (Table 01).

Quantum Yield of Photosystem II (Φii)

The quantum yield of photosystem II was significantly affected by developmental stage ($P<0.001$), rhizobia inoculation ($P<0.01$) and interaction between the two ($P<0.05$) (Table 01). The ΦII observed at vegetative stage was significantly higher than at reproductive stage of the plant (Table 02). Plants which received 90 kg N ha^{-1} had significantly higher ΦII than the inoculated and uninoculated plants which had statistically similar quantum yields except for plants inoculated with BR 3267 rhizobia strain (Table 03). The interaction between rhizobia inoculation and developmental stage revealed that all the plants had similar quantum yield in the vegetative phase which reduced significantly in plants without adequate N at the reproductive phase (Figure 03). A negative linear correlation exists between ΦII and PAR; as PAR increases, ΦII reduces (Figure 04). Similar trend was observed between ΦII and ΦNPQ (Figure 05).

Quantum yield of non-photochemical quenching (ΦNPQ)

Ratio of light that goes towards non-photochemical quenching was significantly affected by the developmental stage ($P<0.001$), rhizobia inoculation ($P<0.05$) and interaction between the two ($P<0.05$) (Table 01). In contrast to ΦII, ΦNPQ was significantly higher at reproductive stage of the plant than at vegetative stage (Table 02). Statistically similar values were observed in inoculated and uninoculated plants. These were statistically higher than the values recorded in plants that obtained 90 kg N ha^{-1} except for plants inoculated with BR 3267 rhizobia strain (Table 03) which had similar values with 90 kg N ha^{-1} fertilized plants. The interaction effect showed that ΦNPQ was generally higher at reproductive stage than at vegetative stage except in 90 kg N ha^{-1} fertilized plants. The difference was however not significant except in plants inoculated with BR 3262 (Figure 06).

Crop growth rate

The growth rate was significantly affected by rhizobia inoculation, developmental stage ($P<0.01$) and interaction between the two (Table 01). Plants which obtained 90 kg N ha^{-1} had significantly higher growth rate than plants inoculated with BR 3267 and USDA 3451 (Table 03). The interaction effect of developmental stage and rhizobia inoculation on crop growth rate of cowpea revealed that significant difference did not exist between the crop growth rate of the plants at the two developmental stages except in plants that obtained 90 kg N ha^{-1} and USDA 3451 inoculated plants which had 236% and 150% increase in growth rate respectively at reproductive stage. Plants that obtained 90 kg N ha^{-1} had significantly higher growth rate in their reproductive stage than all other treatment combination (Figure 07).

Discussion

The significantly lower ΦII values recorded at reproductive stage of the plant compared to the vegetative stage except in 90 kg N ha^{-1} fertilized plant in this study could be partly attributed to the higher values of PAR recorded at the reproductive stage. The reproductive stage of the plant coincided with period of higher solar radiation with an average

photosynthetic active radiation (PAR) of 1142.0 µmol photons m^{-2} $\square^{-1}$ compared to the average of 620.7 µmol photons m^{-2} $\square^{-1}$ recorded at the vegetative stage. Also, N supply increases photosynthesis at high irradiance by increasing photoprotective pigments and reducing the damage caused by high light intensity (Nunes *et al.*, 1993). Thus, nitrogen deficient plants have lesser capacity to use solar radiation captured for Co_2 assimilation.

The higher PAR at the reproductive stage could have also accounted for the significantly higher ratio of light that goes towards non photochemical quenching (ΦNPQ) at reproductive stage than vegetative stage. Plants dissipate excess energy as heat through non-photochemical quenching. This may as well be responsible for the increased leaf temperature recorded at the reproductive stage in this study. Dissipation of excess energy is necessary for optimum performance of photosystems and to avoid photo-inhibition. Photosynthetic photo inhibition occurs under excess light condition; when the irradiance is higher than the acclimation capacity, causing a decrease in photosynthesis (Hou and Hou, 2013). According to Barker (1996), a constant feature of photo inhibition is a reduction in quantum efficiency of photosynthesis. Photosensitivity, photo tolerance, photo-inhibition, and photo-damage processes could occur when the energy received is more than the demand or dissipation capability of plants (Hanelt and Figueroa, 2012).

Many light use efficiency models measure plant productivity based on amount of intercepted solar radiation (Rosati and Dejong, 2003). The result obtained in this study suggests that positive linear relationship does not always exist between PAR and photosynthesis. Harb *et al.* (2018) similarly reported reduction in ΦII and electron transport rate in *Pterocladiella capillacea* as irradiance increased. The authors attributed this to energy associated with high PAR exceeding the demand or dissipation capacity of the plant. Under high light intensity condition, an accumulation of excess excitation energy occur, therefore inducing photo-stress condition. Under this condition, plants pigments such as carotenoids and chlorophyll are destroyed (Beach *et al.*, 2000).

In this study, significantly high ratio of light that goes towards photochemistry was sustained at reproductive stage in 90 kg N ha^{-1} plants compared to other plants at reproductive stage. This suggests that nitrogen content was higher in the fertilized plants and further confirms the importance of nitrogen in sustaining the photosynthetic tissue, increasing the efficient use of solar radiation and delaying senescence. Rosati & Dejong (2003) similarly reported that photosynthetic radiation use efficiency increased with nitrogenous fertilizer.

Early in their reproductive stage, annual plant initiates the process of senescence (Bieker & Zentgraf, 2013). At the initiation of senescence there is a shift from anabolic to catabolic processes and reduced synthesis of chlorophyll and carotenoid. Furthermore, carbon assimilation is suppressed while expression of cysteine-aspartat proteases increases. Consequently, there will be reduction in photosynthetic activity and degradation of the photosynthetic apparatus (Bieker & Zentgraf, 2013). Aguera *et al.* (2010) reported that plants grown under low nitrogen condition showed a higher reduction in photosynthetic activity and more pronounced senescence symptoms than plants grown under sufficient nitrogen supply.

In this study, monotonic inverse relationship was observed between ΦII and ΦNPQ. This indicated that if the light energy was more than what could be harvested by photosystem II for manufacture of food, there was efficient regulated non-photochemical quenching of excess energy to prevent photo damage. This confirms the photo tolerance efficiency of cowpea plants as a typical tropical crop. Photosynthesis is often used to assess high temperature tolerance in plant. Bukhov *et al.* (1998) asserted that increase in ΦNPQ may be a mechanism to moderate electron transport such that production of ATP and NADPH would be equal with the demand in Calvin cycle and to also prevent over-reduction of quinone acceptor (QA) which controls photo-damage.

The significant increase in growth rate in plants that received 90 kg N ha^{-1} is an indication that cowpea growth is limited by nitrogen in the study area. Although cowpea fixes N, and it is expected to meet its N need through N fixation, it can also benefit from external N supply especially in the tropical region where N is inherently low in the soil (Abayomi *et al.,* 2008). Enhanced nitrogen fixation through rhizobia inoculation provides a sustainable substitute to the application of chemical nitrogenous fertilizers which is more expensive and not environmentally friendly. The fact that the growth rate and quantum yield of the rhizobia inoculated plants were similar to the uninoculated plant in this study could be an indication that the introduced strains did not enhance the nitrogen fixation of the crop; both the indigenous rhizobia and the introduced rhizobia strain having similar capacity for fixing nitrogen for the plant. However, both have not efficiently met the N need of the crop. Zhou *et al.* (2006) similarly reported that the positive effects of rhizobia inoculation on growth and photosynthesis of soybean were much less than those obtained when 5 mM $(NH_4)_2SO_4$ fertilizer was applied. Some researchers have however reported an increase in plant nitrogen accumulation, crop growth rate, leaf chlorophyll content, and photosynthesis as a result of rhizobia inoculation (Clayton *et al.*, 2004; Sogut, 2006; Bambara & Ndakidemi, 2009; Namvar *et al.,* 2011). Samaniego-Gámez *et al.* (2016) also reported that *Bacillus spp.* inoculation improved the photosystem II efficiency and enhanced photosynthesis in pepper plants. The difference in the result might be as a result of the difference in the component of the symbiosis and environment. The effectiveness of a symbiotic relationship not only depend on the rhizobium strain but also on the cultivar (Fall *et al.*, 2003).

Conclusions and Recommendation

Quantum yield of photosystem II was significantly higher at vegetative stage than reproductive stage but nitrogen sustained significantly high quantum yield of photosynthesis in cowpea at reproductive stage. Furthermore, application of nitrogen significantly increased the growth rate of cowpea than the inoculated and uninoculated plants which had similar growth rate suggesting that the N need of the crop was not sufficiently met by the indigenous rhizobia and the introduced rhizobia strains used in this study could also not improve N fixation in the study area. Hence, there is the need for the development and testing of more effective rhizobia strains to improve the nitrogen fixation and productivity of cowpea in the study area.

References

Abayomi, Y.A., Ajibade, T.V., Samuel, O.F., Sa'adudeen, B. F. (2008). Growth and yield responses 0f cowpea (*Vigna unguiculata* (L.) Walp) genotypes to Nitrogen fertilizer application in the Southern Guinea Savanna Zone of Nigeria. *Asian Journal of Plant Science*. 7(2), 170-176.https://doi.org/10.3923/ajps.2008.170.176

Aguera, E., Cabello, P., de la Haba, P. (2010). Induction of leaf senescence by low nitrogen nutrition in sunflower (*Helianthus annuus*) plants. *Physiologia Plantarum*. 138(3), 256-267. https://doi.org/10.1111/j.1399-3054.2009.01336.x

Aliyu, A., Yusuf, A.A., Abaidoo, R.C. (2013). Response of grain legumes to rhizobia inoculation in two savanna soils of Nigeria. *African Journal of Microbiology Research*. 7(15), 1332-1342. https://doi.org/ 10.5897AJMR12.1127

Bambara, S. and Ndakidemi, P.A. (2009). Effects of Rhizobium inoculation, lime and molybdenum on photosynthesis and chlorophyll content of *Phaseolus vulgaris* L. *African Journal of Microbiology Research*. 3(11), 791-798. https://doi.org/10.1007/978-1-4613-0409

Barker, N.R. (1996). Photoinhibition of photosynthesis, in: Jennings, R.C., Zucchelli, G., Ghetti, F., Colombetti, G. (eds.), Light as energy source and information carrier in plant physiology. NATO ASI series (Series A, Life sciences), Pp 287. Springer, Boston.

Beach, K.S., Smith, C.M., Okano, R. (2000). Experimental analysis of rhodophyte photoacclimation to PAR and UV-radiation using in vivo absorbance spectroscopy. *Botanica Marina*. 43,525-536. https://doi.org/10.1515/B0T.2000.052

Bibi, A.C., Oosterhuis, D.M., Gonias, E.D. (2008). Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. *The Journal of Cotton Science*. 12,150–159. https://journal.cotton.org

Bieker, S. and Zentgraf, U. (2013). Plant senescense and Nitrogen mobilization and signaling, in: Wang, Z., Inuzuka, H. (Eds), Senescence and senescence related disorders. Intech open. https://doi.org/10.5772/54392

Bukhov, N.G., Boucher, N., Carpentier, R. (1998). Loss of the precise control of photosynthesis and increased yield of non-radiative dissipation of excitation energy after mild treatment of barley leaves. *Physiologia Plantarum*. 104, 563-570. https://doi.org/10.1034/j.1399-3054.1998.1040407.x

Clayton, G.W., Rice, W.A., Lupwayi, N.Z., Johnston, A.M., Lafond, G.P., Grant, C.A., Walley, F. (2004). Inoculant formulation and fertilizer nitrogen effects on field pea, Nodulation, N2 fixation and nitrogen partitioning. *Canadian Journal of Plant Science*. 84, 79-88. https://doi.org/10.4141/P02-089

Das, T.K. (2011). Weed science basics and applications. Pp 791-797. Jain brothers, New Delhi.

Fall, L., Diouf, D., Fall, M.A., Badiane, F.A., Gueye, M. (2003). Genetic diversity in cowpea (*Vigna unguiculata* (L.) Walp) varieties determined by ARA and RAPD techniques. *African Journal of Biotechnology* 2(2), 48–50. https://doi.org/10.5897/AJB2003.000-1009

Fening, J.O. & Danso, S.K.A. (2002). Variation in the symbiotic effectiveness of cowpea Bradyrhizobia indigenous to Ghananian soils. *Applied Soil Ecology*. 21, 23-29. https://doi.org/10.1016/S0929-1393(02)00042-2

Giller, K.E. (2001). Nitrogen fixation in tropical cropping systems. 2nd edition, CAB International, Wallingford, United Kingdom. https://doi.org/10.1079/9780851994178.0000

Han, H.S. and Lee, K.D. (2005). Physiological responses of soybean to inoculation with *Bradyrhizobium japonicum* in saline soil conditions. *Research Journal of Agriculture and Biological Sciences*. 1(3), 216-221.

Hanelt, D. and Figueroa F.L. (2012). Physiological and Photomorphogenic Effects of Light on Marine Macrophytes, in: Wiencke C., Bischof K. (eds) Seaweed Biology. Ecological Studies (Analysis and Synthesis). Springer, Berlin, Heidelberg, 219, 3-23. https://doi.org/10.1007/978-3-642-28451-9_1

Harb, T.B., Nardell, A.I., Chow, F. (2018). Physiological responses of *Pterocladiella capillacea* (rhodophyta, gelidiales) under two light intensities. *Photosynthetica*. 56(4), 1093-1106 https://doi.org/10.1007/s11099-018-0805-9

Hou, X. and Hou, H.J. (2013). Roles of manganese in photosystem II dynamics to irradiations and temperatures. *Frontiers in Biology*. 8, 312-322. https://doi.org/10.1007/s11515-012-1214-2
    https://doi.org/10.1080/01140671.2006.9514395

IITA and N2Afica (2014). Legume inoculation technology. A manual prepared by International Institute for Tropical Agriculture and nitrogen to Africa. Retrieved from, http://bibli0.iita.0rg/d0cuments/U14ManIitaLegumeN0th0mN0dev.pdf-fb3408313162eaa6a34bbff5e9664684.pdf

Lazar, D. (2015). Parameters of photosynthetic energy partitioning. *Journal of Plant Physiology*. 175, 131-147. https://doi.org/10.1016/j.jplph.2014.10.021

Lindemann, W.C. and Glover, C.R. (2015). Nitrogen fixation by legumes. College of agricultural, consumer and environmental sciences, New Mexico State University. http://aces.nmsu.edu/pubs/_a/A129/ (accessed 7th December 2016).

Lupwayi, N.Z., Clayton, G.W., O'donovan, J.T. Harker, K.N., Turkington, T.K. and Rice, W. A., (2004). Decomposition of crop residues under conventional and zero tillage. *Canadian Journal of Soil Science*. 84, 403-410. https://doi.org/10.4141/S03-082

Murchie, E.H. and Lawson, T. (2013). Chlorophyll fluorescence analysis, a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, 64 (13), 3983-3998. https://doi.org/10.1093/jxb/ert208

Namvar, A., Seyed, S.R. and Teymur, K. (2011). Growth analysis and yield of chickpea (*Cicer arietinum* L.) in relation to organic and inorganic nitrogen fertilization. *Ekologija*, 57, 97–108. https://doi.org/10.6001/ek0l0gija.v57i3.1915

Ndakidemi, P.A., Dakora, F.D., Nkonya, E.M., Ringo, D., Mansoor, H. (2006). Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in northern Tanzania. *Animal Production Science*. 46, 571-577. https://doi.org/10.1071/EA03157

Nunes, M.A., Ramalho, J.C., Dias, M.A. (1993). Effect of nitrogen supply on the photosynthetic performance of leaves from coffee plants exposed to bright light. *Journal of Experimental Botany*. 44 (262), 893-899. https://doi.org/10.1093/jxb/44.5.893

Pule-Meulenberg, F., Alphonsus, K.B., Kroswade, T., Dakora F.D. (2010). Symbiotic functioning and bradyrhizobia biodiversity of cowpea (*Vigna unguiculata* L. Walp) in Africa. *Microbiology*. 10, 89-101. https://doi.org/10.1186/1471-2180-10-89

Rosati, A. and Dejong, T.M. (2003). Estimating photosynthetic radiation use efficiency using light and photosynthesis of individual leaves. *Annals of Botany*. 91(7), 869-877. https://doi.org/10.1093/aob/mcg094

Samaniego-Gámez, B.Y., Garruña, R., Tun-Suárez, J., Kantun-Can, J., Arturo Reyes-Ramírez, A., Cervantes-Díaz, L. (2016). *Bacillus spp*. inoculation improves photosystem II efficiency and enhances photosynthesis in pepper plants. *Chilean Journal of Agricultural Research*. 76, 4. https://doi.org/10.4067/S0718-58392016000400003

Schweiger, P., Hofer, M., Hartl, W., Wanek, W., Vollmann, J. (2012). N$_2$ fixation by organically grown soybean in Central Europe, Method of quantification and agronomic effects. *European Journal of Agronomy*. 41, 11–17. https://doi.org/10.1016/j.eja.2012.01.011

Slattery, J.O. and Pearce, D. (2002). The impact of background rhizobia populations on inoculation response, in: Herridge, D. (ed), Inoculants and nitrogen fixation of legumes in Vietnam. ACIAR proceedings, 109c. Pp 37-45.

Sogut, T. (2006). Rhizobium inoculation improves yield and nitrogen accumulation in soybean (*Glycine max*) cultivars better than fertilizer. *New Zealand Journal of Crop and Horticultural Science*. 34, 115-120.

Vollmann, J., Walter, H., Sato, T., Schweiger, P. (2011). Digital image analysis and chlorophyll metering for phenotyping the effects of nodulation in soybean. *Computers and Electronics in Agriculture*.75(1), 190-195. https://doi.org/10.1016/j.compag.2010.11.003

Zhou, X.J., Liang, Y., Chen, H. Shen, S.H., Jing, Y. X. (2006). Effects of rhizobia inoculation and nitrogen fertilization on photosynthetic physiology of soybean. *Photosynthetica* 44(4), 530-535. https://doi.org/10.1007/s11099-006-0066-x

Tables

Table 1. Mean square values for cowpea responses to rhizobia inoculation at different developmental stages.

Source of Variation	ΦII	ΦNPQ	ΦNo	Relative chlorophyll	CGR
Rep	0.0019	0.0096	0.0032	4.398	1.66
Developmental stage (D)	0.0862***	0.1235***	0.0030	197.67	102.83***
Error I	0.0034	0.0055	0.0016	50.52	15.39
Inoculation (I)	0.0114**	0.0149*	0.0012	56.58	31.87**
D x I	0.0090*	0.0148*	0.0014	50.93	37.92***
Error II	0.0021	0.0028	0.0008	78.20	5.42

*ΦII - Quantum yield of photosystem II, ΦNPQ - Quantum yield of non-photochemical quenching, **ΦNo** - Quantum yield of non-regulatory processes, CGR-crop growth rate,*, **, ***- significant at 5, 1 and 0.1 percent respectively*

Table 2. Photosynthetic activities and growth rate of cowpea at two developmental stages.

| Treatments	ΦII	ΦNPQ	CGR (g/m^2/day)
Developmental stage			
Vegetative	0.53	0.18	5.49
Reproductive	0.43	0.30	9.19
LSD $_{(0.05)}$	0.04	0.05	1.87

ΦII- Quantum yield of photosystem II, ΦNPQ- Quantum yield of non-photochemical quenching, CGR-crop growth rate, LSD-least significant difference at P=0.05

**Table 3.** Effect of rhizobia inoculation on photosynthetic activities and growth rate of cowpea.

Treatments	ΦII	ΦNPQ	Crop Growth Rate (g/m²/day)
Uninoculated	0.45	0.27	8.27
90 kg N ha^{-1}	0.55	0.16	10.17
BR 3262	0.44	0.27	7.40
BR 3267	0.50	0.23	3.86
UDSA 3451	0.47	0.27	7.03
LSD$_{(0.05)}$	0.06	0.08	2.96

ΦII- Quantum yield of photosystem II, ΦNPQ- Quantum yield of non-photochemical quenching, CGR-crop growth rate, LSD-least significant difference at P=0.05

Figures
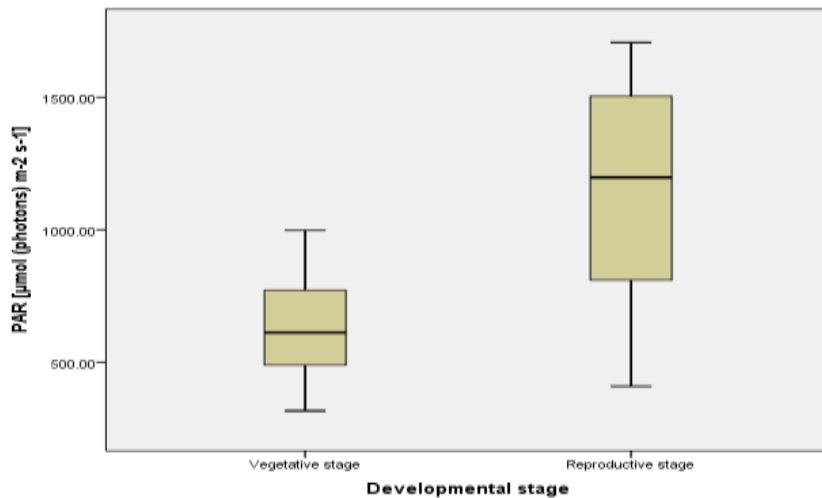
Figure 1: Photosynthetic active radiation at two developmental stages of cowpea
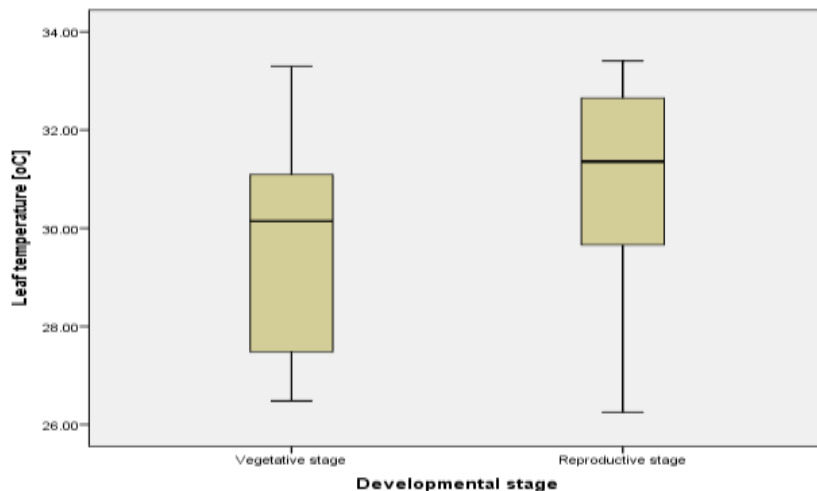
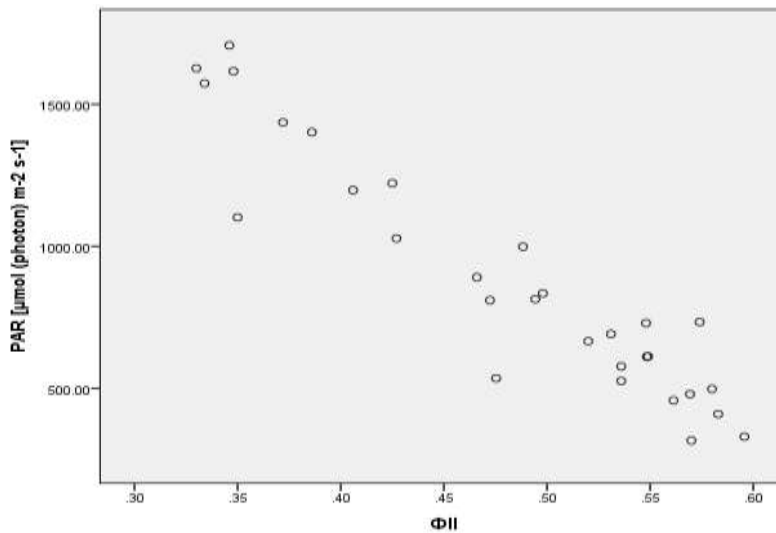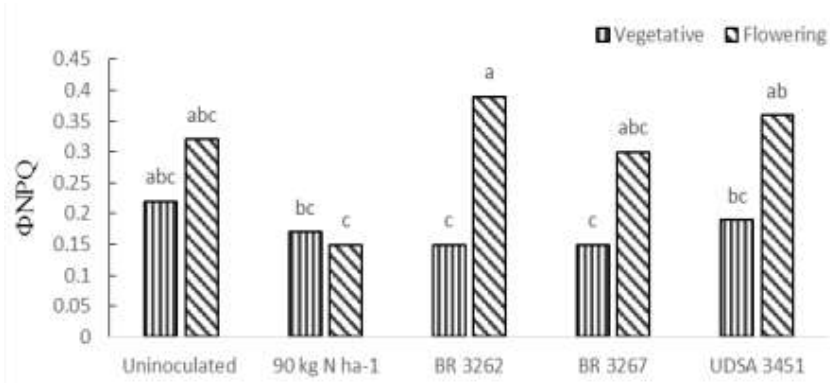**Figure 2:** Leaf temperature of cowpea at two developmental stages

Adediran *et al (2022).*
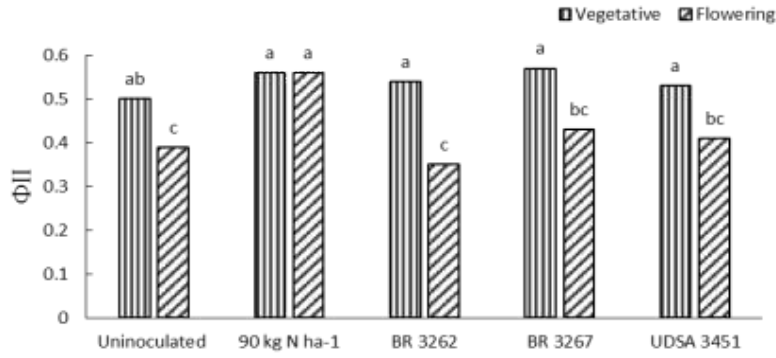
Figure 03. Interaction effect of rhizobial inoculation and plant developmental stage on ΦII of cowpea
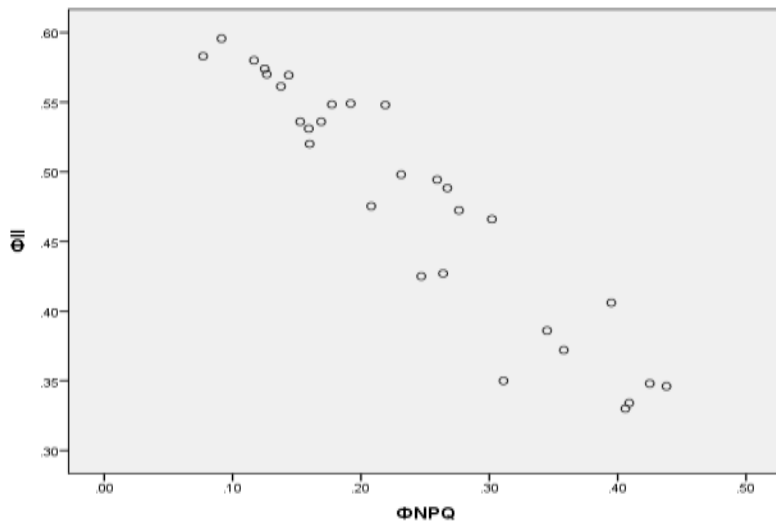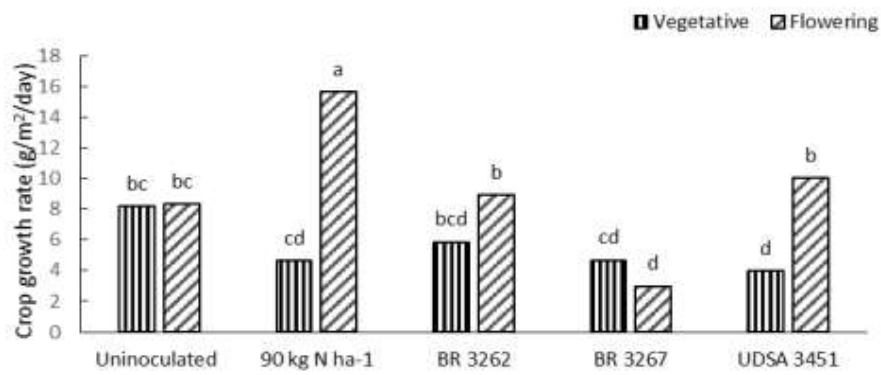

Figure 4: Relationship between ΦII and ΦNPQ

Figure 5: Relationship between ΦII and PAR

Figure 06. Interaction effect of rhizobial inoculation and plant developmental stage on ΦNPQ of cowpea

Figure 07. Interaction effect of rhizobial inoculation and plant developmental stage on crop growth rate of cowpea