Effects of shading on the growth, development and yield of winged bean (*Psophocarpus tetragonolobus*)

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**ABSTRACT:** An experiment was conducted to investigate the effects of different shading regimes [i.e., 60% (heavy), 30% (moderate), and 0% (control)] on 25 traits associated with the morphological features, photosynthetic gas exchange and agronomic characteristics of winged bean (*Psophocarpus tetragonolobus*), an underutilized protein-rich legume from the tropics. Collectively, 80% of the studied variables displayed significant differences (P<0.05) between at least two shade treatments. Shading generally showed most pronounced effect on the physiological traits of the legume, whereby the stomatal conductance, photosynthetic and transpiration rate differed significantly among plants for all treatments. The non-shaded plants were observed to have superior growth and physiological responses than the shaded plants. Interestingly, the moderately shaded plants exhibited the highest yield per plant, which significantly differed from the non-shaded and heavily shaded plants. This indicated that winged bean can adapt to partial canopy cover, making it a potential nitrogen-fixing cash crop which can be planted together with fruit or oil trees in commercial plantations.

**Key words:** morphology, physiology, shading, winged bean, yield.

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**INTRODUCTION**

In recent years, global research on underutilized crops has continued to flourish following the call to action to promote sustainable agriculture through crop diversification (MASSAWE et al., 2016). The development of underutilized protein-rich legumes is one of the several momentous solutions in addressing the impending protein shortages and food crisis around the world, especially in the developing countries where high-protein sources like meat and grains are scarce and expensive (CHENG et al., 2019). Winged bean (*Psophocarpus tetragonolobus*), for example, is a promising future crop in the tropics owing to its hardiness and high protein content (MOHANTY et al., 2013; MASSAWE et al. 2016; CHENG et al., 2019). Native to Papua New Guinea, this diploid species from the Fabaceae (or Leguminosae) family is grown mainly by subsistence farmers in its native home and many hot and humid countries, particularly those in South and Southeast Asia. This self-fertilizing leguminous plant has multi-purpose uses as human food, livestock feed, and environmental conservation (PEYACHOKNAGUL et
The protein content of winged bean is equivalent to that of soybeans (32-38%), which is about 50% higher than most of the other edible legumes (NRC, 1975; AMOO et al. 2006). Additionally, its seeds contain a good balance of amino acids, including lysine, making them an excellent complement for cereal-based diets typically deficient in lysine (SHURTLEFF & AOYAGI, 1979).

Like other leguminous crops, the winged bean has the ability to fix atmospheric nitrogen, which consequently increases soil fertility and production of other crops such as rice (RAHMAN et al., 2014). It is a potential cash crop in the tropics as its cultivation requires low external inputs (MASSAWE et al., 2016). However, the winged bean has not been planted on a large scale due in part to its relatively low yield potential (MOHANTY et al., 2013). One of the most challenging aspects of winged bean cultivation is the management of its vigorous vining and indeterminate growth habits (STEPHENSON et al., 1981; WONG et al., 2015). Although, studies on winged bean’s growth and development flourished briefly during the 1970s and 1980s (STEPHENSON et al., 2015). However, the winged bean has not been studied in detail under different environmental conditions, particularly in the tropics, while 7th internode length (InL) was taken at the 7th internode from the soil reference point. Number of nodes, leaves, and branches per plant (NoN, NoL, and NoB, respectively) were recorded on weekly basis up to 9th weeks. Days to flowering (DTF) was the number of days recorded from germination to first flowering. All analyses were made in triplicate.

On the 16th week, winged bean plants with their pods from each treatment were harvested and oven-dried at 65 °C for 72 h to measure yield-related traits such as pods per plant (NoP), seed diameter (SD), and hundred-seed weight (HSW). The number of seeds per pod (NoS), pod diameter (PodD), pod length (PodL), and pod width (PodW) were recorded using five dried pods. The weight of harvested seeds per plant was measured to obtain the yield per plant (YPP). The total aboveground plant biomass was recorded as the average aboveground biomass of five dried plants, while the harvest index (HI) was calculated as the ratio of harvested seeds mass to the total aboveground plant biomass.

In-situ chlorophyll (SPAD) was measured using SPAD-502 (Minolta, Japan) on the 3rd, 6th, 9th, and 12th weeks. The LI-6400XT Portable Photosynthesis
System (LI-COR, USA) was used to determine photosynthetic gas exchange parameters, including photosynthetic rate (A), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E). The physiological measurements were taken under standard conditions [i.e. leaf temperature of 30 °C, PAR of 1,200 µmol m^{-2} s^{-1}, and adjusted CO_2 flux at 400 µmol s^{-1} inside the chamber] between the 7th and the 8th week on three individual plants; three leaves for each plant from each shading treatment between 1000 h and 1400 h.

**Evaluation of assimilation pigments and crude protein content**

Upon completion of yield analysis, concentrations of assimilation pigments, including chlorophyll A (ChlA) and chlorophyll B (ChlB), and carotenoids (Car), were determined following the methods by SUMANTA et al. (2014) with minor modifications. Concentrations of ChlA, ChlB, and Car were measured based on the wavelengths of the spectral absorbance, i.e., 663.2 nm, 646.8 nm and 470.0 nm, respectively. Additionally, the crude protein content of both winged bean seed (ProteinS) and leaf (ProteinL) was also being determined using the modified Kjeldahl method (AOAC, 1980). All analyses were made in triplicate.

**RESULTS AND DISCUSSION**

We reported here, for the first time, the effects of different intensities of shades towards the growth, development, and yield of winged bean.

| Trait | Non-shaded Plants (Control) | Moderately shaded plants (30% shade) | Heavily shaded plants (60% shade) |
|-------|-----------------------------|--------------------------------------|----------------------------------|
| Growth |                             |                                      |                                  |
| PH (cm) | 374.9 ± 15.61 \( ^{a} \)     | 333.0 ± 3.83 \( ^{b} \)              | 290.2 ± 3.93 \( ^{c} \)          |
| NoB | 47.9 ± 3.10 \( ^{a} \) | 35.7 ± 0.81 \( ^{b} \) | 33.6 ± 2.44 \( ^{b} \) |
| NoL | 104.0 ± 7.06 \( ^{a} \) | 90.9 ± 1.69 \( ^{b} \) | 76.0 ± 2.67 \( ^{c} \) |
| NoN | 16.6 ± 0.94 \( ^{a} \) | 15.4 ± 0.78 \( ^{ab} \) | 13.6 ± 0.38 \( ^{b} \) |
| InL (cm) | 14.3 ± 0.13 \( ^{a} \) | 12.0 ± 0.45 \( ^{b} \) | 10.9 ± 0.46 \( ^{b} \) |
| DTF | 48.9 ± 2.28 \( ^{a} \) | 63.9 ± 0.34 \( ^{b} \) | 67.1 ± 0.56 \( ^{c} \) |
| Yield |                             |                                      |                                  |
| NoP | 7.7 ± 0.36 \( ^{b} \) | 10.3 ± 0.57 \( ^{a} \) | 7.6 ± 0.53 \( ^{b} \) |
| PodD (cm) | 9.4 ± 0.88 \( ^{a} \) | 9.9 ± 0.91 \( ^{a} \) | 8.0 ± 0.61 \( ^{a} \) |
| PodL (cm) | 18.5 ± 1.70 \( ^{a} \) | 26.9 ± 2.46 \( ^{a} \) | 17.1 ± 2.36 \( ^{b} \) |
| PodW (g) | 6.3 ± 0.79 \( ^{a} \) | 7.5 ± 0.43 \( ^{a} \) | 5.3 ± 0.97 \( ^{b} \) |
| NoS | 10.6 ± 1.34 \( ^{a} \) | 13.5 ± 0.63 \( ^{a} \) | 8.9 ± 1.41 \( ^{b} \) |
| SD (cm) | 0.8 ± 0.11 \( ^{a} \) | 1.0 ± 0.03 \( ^{a} \) | 0.7 ± 0.08 \( ^{b} \) |
| HSW (g) | 20.8 ± 0.05 \( ^{a} \) | 25.0 ± 0.05 \( ^{a} \) | 19.6 ± 0.08 \( ^{b} \) |
| YPP (g) | 23.8 ± 1.06 \( ^{a} \) | 28.9 ± 1.47 \( ^{a} \) | 21.4 ± 1.48 \( ^{b} \) |
| HI | 0.2 ± 0.03 \( ^{a} \) | 0.3 ± 0.02 \( ^{a} \) | 0.2 ± 0.02 \( ^{b} \) |
| In-situ chlorophyll | SPAD | 36.17 ± 0.65 \( ^{a} \) | 28.18 ± 2.18 \( ^{ab} \) | 25.03 ± 2.00 \( ^{a} \) |
| Pigments |                             |                                      |                                  |
| ChlA (ug/mL) | 9.5 ± 1.06 \( ^{a} \) | 13.0 ± 2.22 \( ^{a} \) | 9.6 ± 1.87 \( ^{a} \) |
| ChlB (ug/mL) | 13.3 ± 0.92 \( ^{a} \) | 13.6 ± 3.13 \( ^{a} \) | 15.0 ± 1.81 \( ^{a} \) |
| Car (ug/mL) | 0.66 ± 0.06 \( ^{a} \) | 0.92 ± 0.09 \( ^{a} \) | 0.98 ± 0.10 \( ^{a} \) |
| Protein content | ProteinL (%) | 15.8 ± 0.28 \( ^{b} \) | 17.6 ± 0.30 \( ^{a} \) | 14.6 ± 0.25 \( ^{b} \) |
| ProteinS (%) | 31.7 ± 3.88 \( ^{a} \) | 35.4 ± 1.04 \( ^{a} \) | 23.2 ± 1.86 \( ^{b} \) |

Means followed by the same letter within rows do not differ significantly between shade treatments (\( P > 0.05 \)).
Table 1 shows that all the studied variables, with the exception of PodD, Ci, ChlA, ChlB, and Car, displayed significant differences between at least two of the light treatments (P<0.05).

Based on the morphological parameters tested in this study, winged bean plants revealed greater growth responses towards non-shaded condition (Table 1). Non-shaded plants had higher PH, DTF, NoB, NoL, and InL (P<0.05) than both the moderately shaded and heavy shaded plants, indicating that winged bean has an optimum growth without shade. This outcome is consistent with the studies conducted on other legumes such as chickpea (LAKE & SADRAS, 2014) and soybean (IQBAL et al., 2018). The non-shaded plants in this study were taller and took a shorter period to flower than the moderately shaded and heavily shaded plants (Table 1), supporting the preliminary conclusion made by HERATH & ORMROD (1979) that flowering in winged bean could be delayed by low light intensity.

Interestingly, the yield of the moderately shaded plants was generally higher than both the non-shaded and heavily shaded plants (Table 1). Moderately shaded plants also recorded higher NoP, PodL, NoS, and HSW (P<0.05) than the non-shaded and heavily shaded plants (Table 1). This can perhaps be explained using the recent findings from a study conducted by KHALID et al., (2019) on soybean. They reported that plants grown under light shading could yield better because the stomata of these plants may perform more optimally during the physiological processes, besides the possibility of increased availability of primary bioactive compounds for their seed formation. The heavily shaded plants; conversely, exhibited the lowest yields with the shortest plant stature (Table 1). These results indicated that heavy shading adversely affects the yield of winged bean, just as shown in many other crop plants, such as soybean and sage (Salvia officinalis) (ZERVOUDAKIS et al., 2012; WU et al., 2016; IQBAL et al., 2018).

The non-shaded plants showed higher physiological responses than the shaded plants, recording greater values for A, g, and E (P<0.05) (Figure 1). The rate of photosynthesis in non-shaded

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Figure 1 - Effects of shading treatments on photosynthetic gas efficiency: net photosynthesis rate, A (a); stomata conductance, gs (b); intercellular CO2, Ci (c); transpiration rate, E (d). Bars represented standard error of differences between means.
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-plants was between two and four times higher than moderately shaded and heavily shaded plants, respectively (Figure 1). This outcome is expected given that the rate of photosynthesis in most plants will increase if the light availability or intensity increases (ATHANASIOU et al., 2010; ZHU et al., 2017). This notion; however, can only be applied to cases where other major limiting factors, including carbon dioxide concentration and temperature, are negligible (KAISER et al., 2014). Regression analysis showed that A had significantly increased PH (\(r^2=0.6449**\)) and NoL (\(r^2=0.6012**\)) of winged bean (Figure 2), displaying the strength of source and sink relationship with the increasing of photosynthesis rate. The results are in agreements with studies on some other plant species such as neotropical species (RIJKERS et al., 2000) and dipterocarp species (KENZO et al., 2006).

As for the concentrations of assimilation pigments, there were no statistically significant differences (\(P>0.05\)) between plants grown under all shading treatments for ChlA, ChlB, and Car. Additionally, the in-situ chlorophyll showed non-significant differences among treatments. These results suggested that winged bean is capable of adjusting their photosynthetic mechanism to accommodate different levels of light. Several studies on algae species, including kleptoplastic benthic foraminifer (*Haynesina germanica*) (JAUFFRAIS et al., 2017) and red seaweed (*Pyropia haitanensis*) (WU, 2016) also found that there were no significant differences of pigment contents when the algae were grown in various light conditions.

Nevertheless, shading was reported to affect the protein content of winged bean, both on its leaves and seeds (Table 1). Moderately shaded plants recorded the highest Protein S (35.4% ± 1.04), which was similar to the non-shaded plants (31.7% ± 3.88) but much higher (\(P<0.05\)) than the heavily shaded plants (23.2% ± 1.86). This is a good indication that winged bean plants are well-suited to be grown under tree canopies in commercial plantations as secondary or cash crops which is high in protein. Winged bean has recently been promoted as one of the potential crops for the future due mainly to its exceptionally high protein content (CHENG et al., 2019). Being a nitrogen-fixing plant, winged bean has a symbiotic relationship with the motile bacteria *rhizobia* which can help to replenish the soil (SOMASEGARAN & HOBEN, 2012). Monoculture tree plantation has long been a prime issue in the tropical regions (PUTZ & REDFORD, 2010). As such, planting cash crops like winged bean which can supply natural nitrogen fertilizer together with these trees may benefit both the plantation company and the environment. Nonetheless, it should be noted that the winged bean seeds obtained from heavy shading condition in

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**Significant at \(p \leq 0.01\).**

Figure 2 - Relationship between plant height, number of leaves and photosynthesis rate of winged bean during the 8th week.
this study recorded only about 70% of the ProteinS produced by those under light shading. This implied that winged bean is not a suitable cash crop for plantations with a high percentage of canopy cover.

CONCLUSION

Winged bean, being hailed as a new soybean of the tropics, has the potential to become one of the affordable plant-based protein sources in the near future and its improvement is therefore crucial. Based on our overall finding, light does play a crucial role in the growth and development of winged bean. The outcomes of the present study, for the most part, demonstrated that winged bean grows well and yields better under light shading condition. The release of this new insight can benefit the producers, consumers, as well as the environment. Further research is recommended to assess the crop’s response to other important exogenous factors such as temperature and water availability.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

Conceptualization, A.C.; data curation, N.A.M.Z and N.O.; methodology, N.A.M.Z, N.O. and A.C.; Formal analysis, M.N.R. and N.A.S.; investigation, M.N.R. and N.A.S.; resources, A.C.; software, N.A.M.Z, validation, N.A.M.Z and N.O.; writing—original draft, M.N.R. and N.A.S.; writing—review & editing, N.A.M.Z, N.O., and A.C.; supervision, N.A.M.Z. and A.C.; project administration, A.C.; funding acquisition, A.C.

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