GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF WINGED BEAN (*Psophocarpus tetragonolobus)* TOWARDS DIFFERENT SHADE LEVELS

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GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF WINGED BEAN (*Psophocarpus tetragonolobus)* TOWARDS DIFFERENT SHADE LEVELS

ABSTRACT

Ambiguous evidence suggests that shade could regulate the indeterminate growth habit of winged bean (*Psophocarpus tetragonolobus*), an underutilized protein-rich legume from the tropics. This study was conducted to examine the effects of three different shade levels, including 60% (heavy shade), 30% (moderate shade), and 0% (as control) on 25 traits associated with morphological features, photosynthetic and agronomic characteristics of winged bean. The shade-house experiment was conducted using a completely randomized design. Collectively, approximately 80% of the studied variables displayed significant differences ($P<0.05$) between at least two shade treatments. Shading generally showed the most pronounced effect on the physiological traits of the legume, whereby the stomatal conductance, photosynthetic and transpiration rates differed significantly ($P<0.05$) among plants for all treatments. Overall, non-shaded plants were observed to have superior growth and physiological responses than the shaded plants, especially being significantly taller with higher stomatal conductance, photosynthetic and transpiration rates. Interestingly, the moderately shaded plants exhibited the highest yield per plant (28.9 g ± 1.47), which significantly differed from the non-shaded (23.8 g ± 1.06) and heavily shaded (21.4 g ± 1.48) plants. These findings suggested that winged bean plants can adapt to partial shaded conditions or canopy cover, making it a potential nitrogen-fixing cash crop which can be planted together with fruit and oil trees in commercial plantations. Given that there were no significant differences between the moderately shaded and heavily plants with respect to their seed protein content ($P>0.05$),

this project also demonstrates that winged bean can grow and yield well while maintaining its protein content under moderately shaded condition.

Keywords: Biochemical responses, morphology, physiology, shade, winged bean

PERTUMBUHAN, FISIOLOGI DAN RESPONS BIOKIMIA KACANG BOTOR (*Psophocarpus tetragonolobus*) TERHADAP PARAS TEDUH YANG BERBEZA

ABSTRAK

Bukti samar menunjukkan bahawa teduhan dapat mengawal pertumbuhan kacang botol (*Psophocarpus tetragonolobus*), satu tumbuhan kekacang kaya protein dari kawasan tropika yang masih tidak mendapat perhatian yang sewajarnya. Kajian ini dijalankan untuk mengkaji kesan tiga rejim teduhan yang berlainan, iaitu 60% (teduhan berat), 30% (teduhan sederhana) dan 0% (sebagai kawalan) ke atas 25 sifat ciri morfologi, fotosintesis dan agronomi kacang botol. Eksperimen rumah teduh ini dijalankan dengan menggunakan rekabentuk penuh rawak. Secara kolektif, 80% daripada ciri-ciri kajian menunjukkan perbezaan yang signifikan ($P < 0.05$) antara sekurang-kurangnya dua teduhan yang berbeza. Teduhan secara amnya menunjukkan kesan paling ketara terhadap ciri fisiologi kekacang, di mana konduksi stomata, kadar fotosintesis dan transpirasi berbeza dengan signifikasi ($P < 0.05$) di antara tumbuhan untuk semua teduhan. Secara keseluruhannya, kacang ditanam tanpa teduhan mempunyai pertumbuhan dan tindak balas fisiologi yang terbaik berbanding tumbuhan di bawah teduhan, terutamanya untuk ciri konduksi stomata, kadar fotosintesis dan transpirasi. Apa yang menarik adalah tumbuhan yang ditanam dibawah teduhan sederhana mempamerkan hasil tertinggi (28.9 g ± 1.47) berbanding daripada tumbuhan ditanam tanpa teduhan (23.8 g ± 1.06) dan teduhan tinggi (21.4 g ± 1.48). Penemuan ini menunjukkan bahawa kacang botol dapat menyesuaikan diri dengan keadaan separa teduh atau penutup kanopi, menjadikannya tanaman ini berpotensi ditanam bersama dengan pokok-pokok buah-buahan dan minyak di ladang komersial. Memandangkan tiada perbezaan yang ketara di antara pokok-pokok bawah teduhan sederhana untuk ciri protein benih mereka ($P > 0.05$), projek ini menunjukkan bahawa kacang botol boleh tumbuh dan berhasil dengan baik sambil mengekalkan kandungan protein di bawah keadaan sederhana teduh.

Kata kunci: Fisiologi, kacang botol, morfologi, respons biokimia, teduhan

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree Celsius
cm	:	Centimetres
mm	:	Millimetres
g	:	Grams
mg	:	Milligrams
mL	:	Millilitres
L	:	Litres
min	:	Minutes
ANOVA	:	Analysis of variance
CO_2	:	Carbon dioxide
$CuSO_4$	:	Cupric sulphate
GI	:	Glycemic index
GHG	:	Greenhouse gas
H_3BO_3	:	Boric acid
NIDDM	:	Non-insulin dependent diabetes
HCL	:	Hydrochloric acid
NaOH	:	Sodium hydroxide
PAR	:	Photosynthetically active radiation
SGD	:	Sustainable Development Goal
K_2SO_4	:	Potassium sulphate
rpm	:	Revolutions per minute

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research background

The global population is expected to reach approximately 10 billion by 2050, and the global food demand is projected to increase by about 50% (Godfray et al., 2010; United Nations, 2019). Although the Green Revolution initiated in the 1960s has increased the productivity of major crops because of better irrigation and the use of chemical fertilizers and pesticides (Foley et al., 2011), the technology has caused the growing dependence of humans on about 1% of the 250,000 edible species of plant in the world (Cheng, 2018). This can be one of the causes of future food insecurity, given that the world grain production per capita for the major cereals such as maize, wheat, and rice will likely decline more than 10% between 2008 and 2030 (Funk and Brown, 2009). As such, considerable attention has been given to promoting crop diversification in the past decade, whereby many research groups throughout the world have started to develop and improve the lesser-known crops (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. This is especially true in the developing countries where high-protein sources like meat and grains are scarce and expensive (Cheng et al., 2019). These legumes include winged bean (*Psophocarpus tetragonolobus*). Winged bean has recently been hailed as one of the most promising future crops in the tropics owing to its hardiness and high nutritional value (Mohanty et al., 2013; Massawe et al., 2016). It is a diploid legume species of the family Fabaceae (or Leguminosae) and subfamily Faboideae (Harder and Smartt, 1992). Native to Papua New Guinea, winged bean 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 is an underutilized species with multi-purpose uses as human food, livestock feed, and environmental conservation (Peyachoknagul et al., 1989). The protein content of winged bean is equivalent to that of soybeans (Cheng et al., 2019). Its seeds have a balance amount of amino acids, for example lysine, which makes them an excellent complement for cereal-based diets typically deficient in these amino acids (Shurtleff and Aoyagi, 1979). Moreover, its seeds are also rich in oil, mainly unsaturated oil that contains vitamins such as vitamin E (Salunkhe et al., 1992; Mohanty et al 2015). Winged bean flour has been found suitable to be utilized as a milk substitute to treat children suffering from kwashiorkor (Shurtleff and Aoyagi, 1975).

Like other leguminous crops, the winged bean has the ability to fix atmospheric nitrogen that can increase soil fertility and also the production of other crops, including rice (Rahman et al., 2014; Cheng et al., 2019). It is a potential cash crop in the tropics as its cultivation requires low external inputs (Massawe et al., 2016). This makes it a suitable cash crop for small or marginal farmers in areas that face increased temperatures or water scarcity because of climate change. Despite its outstanding nutrition and versatility benefits, winged bean is usually being planted on a small scale due in part to its relatively low yield potential (Mohanty et al., 2013). One of the most challenging aspects of winged bean growing is the management of its vigorous vining and indeterminate growth habits (Stephenson et al.,1981; Tanzi et al., 2019). To avoid two-winged bean plants from intertwining with each other, the sowing distance between the plants must be at least 60 cm apart (Heralth and Omron. 1979). Although studies on winged bean's growth and development flourished briefly during the 1970s and 1980s, some of the reported findings remain largely inconclusive. Early studies on its growth and development have focused primarily on its vegetative growth and flowering induction. Winged bean is considered a short-day species with flowers setting within critical day length regimes, and its optimal day length is about 12 hours (Herath and Omron. 1979; Schiavinato et al., 1996).

Light is one of the most significant exogenous (or environmental) factors that regulate plant photosynthesis, survival, growth and development. In general, modification of light intensity has been reported to change morphological and physiological responses of many plant species, leading to the changes of certain biochemical responses (Gonçalves et al., 2005; Kumar et al., 2012). Most studies pointed that plant biomass and physiological traits, such as photosynthetic and transpiration rates, would decrease under low light intensity (Wang et al., 2009; Mielke and Schaffer 2010).

A multitude of studies has been carried out to examine light on the growth and development of some leguminous species, such as the lentils (*Lens culinaris*), chickpeas (*Cicer arietinum*) and soybeans (*Glycine max*) (Bayahi et al., 2015; Mondal et al., 2013; Van Roekel et al., 2015). Moreover, some of the legume studies have been done intensively on their morphological, physiological and biochemical responses with manipulation of different light intensities. Some studies showed that there was a significant relationship between light intensity and legume productivity (Baligar et al., 2017; Pang et al., 2019). However, this is not the case for winged bean. The utilization of this high-protein legume is still low, and the existing information or knowledge on its growth patterns under different environmental conditions remains limited (Raai et al., 2020). Thus, a concerted effort to better understand the relationships between its growth and development is required to develop and improve this high-protein underutilized legume (Vatanparast et al., 2016; Tanzi et al., 2019) .

1.2 Problem Statement

While winged bean is one of the most nutritious, versatile and inexpensive leguminous species, the utilization of winged bean is relatively low, and the existing knowledge on its growth and development patterns under different environmental conditions remains limited. Understanding the relationships between its morphophysiological and biochemical responses with relevant exogenous factors, such as light, would provide a more in-depth insight into its growth and development patterns, and subsequently pave the way for the breeding of self-supporting and improved winged bean cultivars.

1.3 Research Objectives and Hypotheses

1.3.1 Objectives

i. To examine the effects of three different shade levels on morphological, physiological and biochemical responses of winged bean

ii. To determine the optimum shade levels for winged bean growth and development.

1.3.2 Hypotheses

i. Winged bean shows different morphological, physiological and biochemical responses to shading variability.

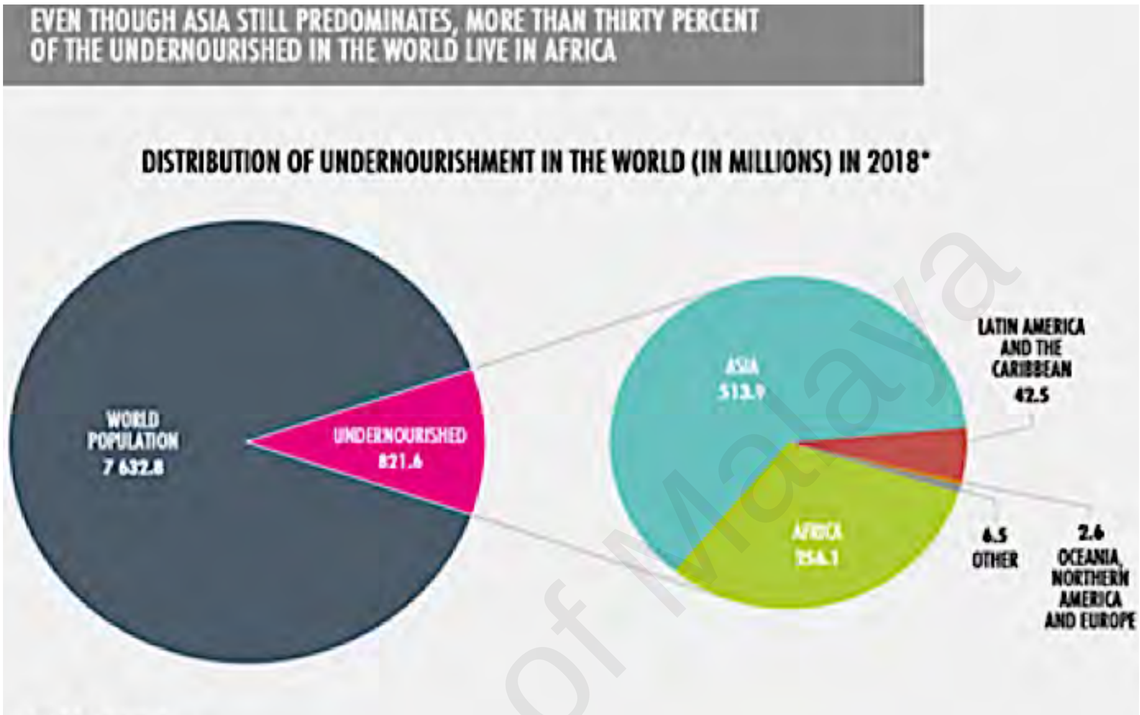
ii. Winged bean plants grown and develop better under non-shaded conditions.

CHAPTER 2: LITERATURE REVIEW

2.1 Global Food Security

Green Revolution was launched in the 1960s to improve global food productivity through several specific approaches, including the development of high-yielding seeds for some major crops and the use of more inputs such as synthetic fertilizers (Ameen and Raza, 2017; Mohd Hanafiah et al., 2020). Since the commencement of the Green Revolution, the productivities of the world's major staple crops, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), and maize (*Zea m*improved drastically and this has helped to combat food insecurity for the past few decades (Massawe et al., 2016; Mohd Hanafiah et al., 2020). Although the Green Revolution has achieved its ultimate goal to improve the productivity of certain crops (Foley et al., 2011), the approaches involved have brought some negative consequences such as increased imputs and monoculture (Massawe et al., 2016).

In 2018, the Food and Agriculture Organization (FAO) highlighted that there has been a rise in global hunger and malnutrition, especially in past couple of years. For instance, about 821 million of peoples were reported to be undernourished in 2018, and the distribution is shown in Figure 2.1 (FAO, 2019). Malnutrition is one of the largest contributors to child mortality worldwide, with more than 150 million affected children under 5 years old (Black et al., 2013; FAO, 2018; Cheng et al., 2019). Coordinated planning and effective efforts are required across multiple sectors to address food insecurity and malnutrition (Massawe et al., 2016; De Jager et al., 2017). The Sustainable Development Goals (SGDs) with 17 goals have recently been developed and announced by the United Nations, and achieving zero hunger is the second goal of the global initiative (Salvia et al., 2019). This specific goal was set to address the challenges of global hunger, food insecurity and malnutrition in all its forms. Achieving this goal can directly and indirectly help to achieve other SGDs such as no poverty and good health and well-being (Sharuzaini et al., 2020).



Source: FAO, 2019

Figure 2.1: Distribution of undernourished people (in millions) worldwide in 2018.

Food insecurity exists when people lack of access to sufficient amounts of safe and nutritious food (FAO, 2013). It would soon be one of the major global issues if sustainable food systems are not being developed for the rapidly changing world with soaring population growth (Godfray, 2010; Massawe et al., 2016). One of the primary means of combating food insecurity is through the diversification of food sources (Massawe et al., 2016; Cheng, 2018; Cheng et al., 2019). This can be done by promoting and developing the potential underutilized (or neglected and orphan) species across the globe (Cheng et al.,, 2019; Hunter et al., 2019).

2.2 Underutilized Species and Their Importance

Among the approximately 30,000 plant species known to be edible, about 7000 of them have been cultivated as food for centuries (Smil. 2001; Kiple. 2007). However, approximately 20 of these spesies have been reported to account for 90% of global food supply, with two-thirds being dominated by the big three cereals (rice, wheat, and maize) (Massawe et al., 2016). Most of the other edible species have only been grown and used in their native homes, and these species are termed underutilized species (Hughes. 2008; Padulosi et al., 2013). Underutilized crops by and large are applied to a wide range of crops that used to a small degree of their potential (Padulosi et al., 2011). They can be found all over the world especially in the rural regions where they play important roles in providing food and incomes for small farmers (Dansi et al., 2012; Ameen and Raza. 2017). The past two decades have witnessed a growing interest from the scientific community in promoting and developing potential underutilized species (Cheng, 2018; Cheng et al., 2019). Main criterias for underutilized species to be considered as potential crops for the future include, among others, nutritional dense, economically viable and climate resilient (FAO, 2010).

Besides helping in maintaining global food security, underutilized species with good nutritional profiles can also aid in reducing malnutrition especially in developing countries (Cheng et al., 2019). Dansi et al. (2012) reported that many underutilized species are nutritionally rich, compacted with vitamins and minerals. For example, some underutilized cereals like teff generally contain higher composition of essential amino acids compared to the major cereals like wheat (Cheng et al., 2017). Some underutilized legumes (or pulses), on the other hand, have been reported to have high protein content, such as winged bean and bambara groundnut (*Vigna subterranea*), which can be utilized as protein alternatives to meat (Cheng et al., 2019).

Many underutilized species also can adapt to varying environment conditions such as drought and heat stress, indicating that they can be developed as climate-resilient crops (Chivenge et al., 2015). Besides, some of these species contain important tolerance and resistance genes which can be used to breed varieties that can adapt to the changing climate (Mal and Joshi, 1991; Subbarao et al., 1995; Ojiewo et al., 2019). With the greater utilization of underutilized species, biodiversity can be conserved and this may also help to bring back the 75% loss of genetic crop diversity which occurred during the past century (Chivenge et al., 2015).

2.3 Potential of Underutilized Legumes

In recent years, more research attention has been given to underutilized crops, including a number of species from the Leguminosae (or Fabaceae) family. This attention has been driven by numerous findings on nutritional values of legumes as plant-based sources of dietary proteins and other essential nutrients, especially in countries where animal protein sources are scarce and considered expensive (Chapman, 2015; Henchio et al., 2017). The Leguminosae family is one of largest families of flowering plants, consisting about 650-750 genera and 18000 to 19000 described species (Lewis, 2005; Ekanayake et al., 2000; Salman and Hassan, 2014).

There are a few unique characteristics of legumes that make them suitable to be developed as climate-resilient crops. One of the characteristics is the capability to fix nitrogen naturally and develop root nodules via symbiosis with some root microorganisms (Graham and Vance, 2003; Stuedemann and Franzluebbers, 2007; Mohamad et al., 2010). This characteristic also allows legumes to improve soil health especially by adding nitrogen and organic matter (Andrews and Andrews, 2017). Previous research has reported the benefits of legume cover crops used to increase soil fertility (Gomes et al., 2009; Mazzoncini et al., 2011; Zhou et al., 2012) and to conserve land conservation (Plieninger & Gaertner 2011). Some grain legumes such as peanuts (*Arachis hypogaea*) and soybeans (*Glycine max*) can be categorized as good nitrogen fixers because they can fix up to 113.34 kg of nitrogen per acre (Walley et al., 1996). Additionally, some forage legumes such as alfalfa, sweet clover, true clovers, and vetches are able to fix approximately 113-226 kg of nitrogen per acre (Robert and Idowu, 2015).

Large-scale plantation is known to be one of the major causes of deforestation, leading to issues such as decrease in soil quality, nutrient, and water holding capacity. Utilization of legumes as cover crops can reduce soil erosion and increase soil organic matter content, helping to restore soil fertility (Cunningham and Smith, 1961; Wood and Lass, 2001). Moreover, cover crops have been reported to have the ability to improve soil by increasing biological activities and suppressing weed growth (Fageria et al., 2005; Ehrmann and Ritz., 2014). Legumes as considered as good cover crops mainly because of their natural ability to fix nitrogen (Sahruzaini et al., 2020). A study conducted by Mupangwa et al. (2017) showed that utilization of cover crops significantly reduce yield gap between organic arable farming and conventional farming.

Other than their natural ability to fix nitrogen, some underutilized legumes have been identified as potential crops for the future because of their nutritional status (Ekanayake et al., 2000; Jaenicke., 2012; Chivenge et al., 2015). Legumes are known to have good source of nutrients (such as protein, starch, minerals and vitamins) and important secondary metabolites (such as phenolics, inositol phosphates and oligo-saccharides) (Salman and Hasan, 2014; Frassinetti et al., 2015). Examples of high-protein legumes include, among others, winged bean, bambara groundnut, lentil (*Lens culinaris*), lima bean (*Phaseolus lunatus*), and lablab (*Lablab purpureus*) (Cheng et al., 2019). Table 2.1 shows the important nutrients in the matured seeds of some edible legumes in comparison with soybean, the most economically important legume worldwide (Cheng et al., 2019).

Increased awareness regarding the importance of plant-based proteins in recent years has promoted the greater use of legumes, particularly because they are generally cheaper than cereal grains and can be utilized as alternatives to animal-based proteins (Henchion et al., 2017).

Table 2.1: Comparison of main nutrients (g/100g) in matured seeds of some edible legumes with soybean (Source: Cheng et al., 2019).

Common name	Botanical name	Protein	Carbohydrate	Total monounsaturated fatty acid
Soybean	*Glycine max*	36.49	30.16	4.40
Winged bean	*Psophocarpus tetragonolobus*	29.65	41.71	6.01
Peanut	*Arachis hypogaea*	25.80	16.13	24.43
Lentil	*Lens culinaris*	24.63	63.35	0.19
Lablab	*Lablab purpureus*	23.90	60.74	0.08
Cowpea	*Vigna unguiculata*	23.52	60.03	0.11
Pea	*Pisum sativum*	23.12	61.63	0.62
Pigeon pea	*Cajanus cajan*	21.70	62.78	0.01
Lima bean	*Phaseolus lunatus*	21.46	63.38	0.06
Bambara groundnut	*Vigna subterranea*	18.80	50.20	23.46

Other than macronutrients, some underutilized legumes also contain high vitamins and minerals (Bhat and Karim. 2009). Legumes typically contain high fiber with good quantity of vitamins and minerals such as iron, coppers, magnesium, manganese, zinc, and phosphorous (Polak et al., 2015). Additionally, they possess low glycemic index, usually ranging between 10 and 40, and are good source of linoleic (21% - 53%) and alpha-linolenic acid (4% - 22%) (Messina, 1999; Bouchenak and Lamri-Senhadj, 2013).

Majority of legumes also contain phytochemicals and bioactive compounds, including oligosaccharides, saponins, and phenolic compounds, which play important metabolic roles in humans (Bouchenak and Lamri-Senhadj, 2013). Moreover, they also are important sources of natural antioxidants, as such phenolic compounds (Salas-Lopez, 2018).

Some studies showed that legumes have the capability to improve blood glucose and reduce the risk of non-insulin dependent diabetes (NIDDM) (Becerra-Tomás, 2018). Frequent legume consumption (four or more times weekly compared with less than once a week) has been reported to be associated with 22% and 11% lower risk of coronary heart disease and cardiovascular diseases (Bazzana et al., 2001). The findings from a meta-analysis showed that the consumption of up to half a cup of legumes per day for more than four weeks would significantly reduce fasting blood glucose and insulin levels (Sievenpiper et al., 2009). Similarly, a study on NIDDM demonstrated that substituting 50 g of beans (pinto beans or dark red kidney beans or black beans) with white long grain rice improved the glycemic response (Thompson et al., 2012). Hence, promoting the cultivation of potential underutilized legumes may sustain the demand for protein and healthy diets, and help to achieve several SGDs and food security.

2.4 Winged bean (*Psophocarpus tetragonolobus*)

2.4.1 Morphology and taxonomy

Winged bean is a multipurpose tropical legume from the Leguminosae family (Lepcha et al., 2017). Table 2.2 shows the taxonomy of winged beans. This legume is cultivated commonly in many Asian countries such as Indonesia, Malaysia, Thailand, India, Sri Lanka and Bangladesh and several other countries in the Africa continent (Hymowitz and Boyd. 1977). Winged beans have been utilized as foods for both humans and animals as most of the plant parts are edible, being recognized as ‘a supermarket on a stalk” (National Academy of Sciences. 1975; Lepcha et al., 2017). It has a diploid genome (2n = 2x = 18) with an estimated size of 1.22 Gb (Harder and Smartt, 1992; Vatanparast et al., 2016).

Winged bean is a vining plants that can grow up to 4 m in height (National Academy of Sciences, 1975). Its leaves appear in triplets, bounded to vining stem with bluish-white or purple flower. Winged bean has an extensive root system, with abundant nodules that are larger than those found on the roots of other edible legumes (National Research Council 1975). As a legume, the winged bean can fix atmospheric nitrogen, which consequently increases soil fertility and production of other crops such as rice (Rahman et al., 2014). The winged bean fruit, which contains between 5 and 21 seeds, appears in a pod structure that can reach up to 40 cm (Lepcha et al., 2017). Multiple winged bean parts are shown in Figure 2.2.

Table 2.2. Taxonomy classification of winged bean.

Kingdom	**Plantae**
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Fabales
Family	Fabaceae
Genus	*Psophocarpus*
Species	*tetragonolobus*

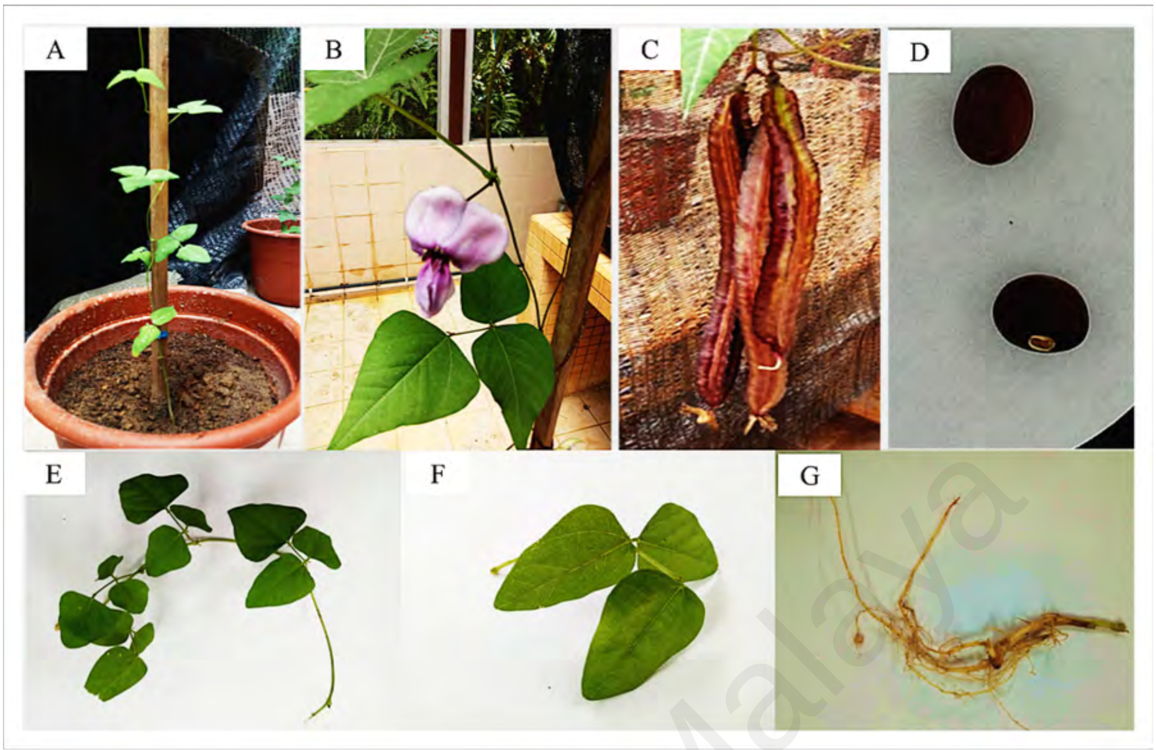


Figure 2.2: Winged bean parts (A) plant; (B) flower; (C) pods; (D) seeds; (E) stem; (F) leaves; and (G) roots.

2.4.2 Nutritional value

Apart from having a high protein content equivalent to the soybean (Table 2.1), its seeds contain a good balance of amino acids, including lysine, which is rare in cereal-based diets (Kadam et al., 1984; Maphosa and Jideani, 2017). Winged bean seeds are also rich in oil, particularly unsaturated oil, which is rich in Vitamin E. Besides, flour made from winged bean seeds has been found suitable as a milk substitute in the treatment of children suffering from kwashiorkor (Cerny and Addy, 1973; National Research Council 1975). Some of the minerals found in this plant have been reported to be higher than soybean, including thiamin, riboflavin, and niacin (Jaffe and Korte. 1976). Table 2.3 shows the summary of the nutrient, minerals, and physio-chemical properties of winged bean seeds (Amoo et al., 2006; Lepcha et al., 2017).

Table 2.3: Nutrient, minerals, and physio-chemical properties of winged bean seeds (Sources: Amoo et al., 2006; Lepcha et al., 2017).

Nutrients/minerals/physio-chemical properties	Winged bean (concentration/ amount)
Moisture (%)	9.22 ± 0.18
Total Ash (%)	4.91 ± 0.01
Fat (%)	17.51 ± 0.35
Crude fiber (%)	12.23 ± 0.13
Crude protein (%)	33.83 ± 0.61
Carbohydrates (%)	22.30 ± 0.82
Magnesium (mg/kg)	2,238.18 ± 0.04
Zinc (mg/kg)	36,476 ± 0.64
Copper (mg/kg)	90.79 ± 0.72
Calcium (mg/kg)	889.86 ± 0.63
Sodium (mg/kg)	1972.34 ± 0.69
Potassium (mg/kg)	4219.30 ± 0.81
Peroxide value (meg/kg)	11.41 ± 0.30
Saponification value (mgKOH/g)	190.34 ± 0.64
Unsaponification matter (g/kg)	16.36 ± 0.64
Acid value (mgKOH/g)	0.71 ± 0.01
Iodine value	144.57 ± 0.53
Refractive Index at 25◦C	1.47 ± 0.01

The cultivation of winged bean requires low external inputs, making it suitable to be grown by farmers in areas that face increasing water scarcity and hot temperatures due to climate change (Massawe et al., 2016). However, the winged bean has not been planted on a large scale in many countries, mainly because of its relatively low and variable yield, and because existing knowledge on its growth patterns remains limited (Wong et al., 2015; Cheng et al., 2017).

2.5 Effects of Light on Plant Growth and Development

Light is an essential energy source for photosynthesis in plants and also one of the most important exogenous factors that determines plant growth and development (Yang et al., 2018; Nyugen et al., 2019). Light intensity and quality have been reported to provide different effects on plant morphological, physiological and biochemical responses, including their nutritional values (Macedo et al., 2011; Nyugen et al., 2019). Moreover, some plants have the ability to develop mechanisms to counter different light intensities to grow and survive (Zhang et al., 2003; Fan et al., 2018). It has been reported that some plant species are tolerant to low light intensity, having a higher elasticity or plasticity to respond to changing environments (Boardman, 1997; Chazdon et al., 1996). It was reported that light intensity is one of the main factors affecting the processes in plants such as germination, leaf proliferation and expansion, photosynthesis, buds and flower initiation, and cell division (Kong et al., 2016; Wu et al., 2018). It is important to note that C3 plants like winged bean and other legumes have higher net photosynthetic rate with good tolerance to light (or shade) stress compared to C4 plants (Su et al., 2014).

Different light intensities have been reported to significantly affect their macronutrients productivity. Lichtenthaler et al. (2007) concluded that carbohydrates were greatly influenced by light intensity because the production of carbohydrates would decrease when photosynthesis is slowed down due to limited amount of light. Most plants can adapt to low light intensity by several ways such as reducing respiration and crop tissue reconstruction (Givnish, 1988) and increasing leaf area and leaf thinning (Givnish, 1988). Additionally, the reduction of light was found to increase the amount of protein in certain plants (Yang et al., 2018). Several studies showed that carotenoids play an important role in protective mechanisms related to photosystem II (Wilson et al., 2006; Wilson et al., 2007).

2.6 Key research areas on improving legumes

Legumes are easy and sustainable crops to grow (Stagnari et al., 2017). Nevertheless, the adoption rates of these crops remain low in many countries, especially those in sub-Saharan Africa (Waldman et al., 2016). More studies are required to be carried out to improve these crops (Cheng et al., 2019). During the past decade, researchers have been investigating their promising nutritional quality, specifically for human health benefits. They are an integral part of many healthy eating diets or patterns, including vegetarian and vegan diets, the Dietary Approaches to Stop Hypertension (DASH) eating plan, and lower-glycemic index (GI) diets (Jenkins et al., 2012; Polak et al., 2015). Studies have shown that legumes with a low GI ranging between 10 and 40 (Jenkins et al., 2012), can play a significant role in the prevention and management of many health conditions, including type 2 diabetes by improving both glycemic and lipid control (Becerra-Tomás et al., 2018), hyperlipidemia by lowering total and LDL cholesterol levels (Bazzano et al., 2011), and hypertension by reducing blood pressure, triglycerides, weight, and waist circumference (Jayalath et al., 2014).

The United Nations declared 2016 as the International Year of Pulses (IYP), aiming to position legumes as one of the primary sources of protein and some other nutrients essential to life and health (Calles, 2016). To accelerate legume research investment, several research organisations have developed the 10-Year Research Strategy, which has been made publicly available and the chapters in the Report are summarized in Figure 2.3 (Cheng et al., 2019).

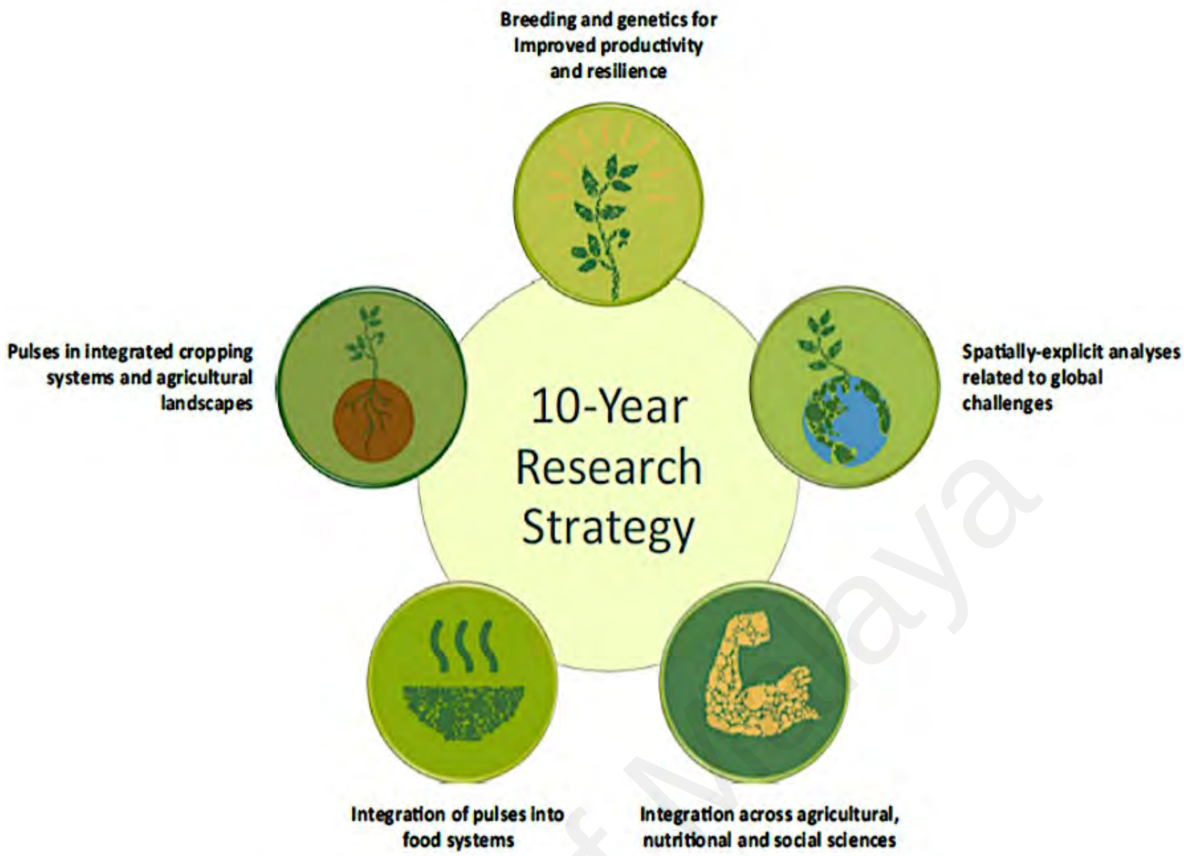


Figure 2.3: 10-Year Research Strategy for Legumes (Source: Cheng et al., 2019).

In the case of winged beans, Lepcha et al., (2017) have highlighted some potential future research for this high-protein legume. For example, the origin of winged bean domestication needs to be further studied to provide more details on the genetic relationship between the cultivated and wild species of this legume. Additionally, the relevant genes or suitable molecular markers for various important traits in winged bean should be identified or developed to assist the breeding programs of this crop. More studies are also required to better understand the relationships between the growth and development of this legume with relevant exogenous factors to develop it to its full potential (Vatanparast et al., 2016; Tanzi et al., 2019) .

CHAPTER 3: MATERIALS AND METHODS

This study was carried out to examine the effects of three different shade levels on the morphological, physiological and biochemical responses of winged bean. The research flowchart is presented in Figure 3.1.

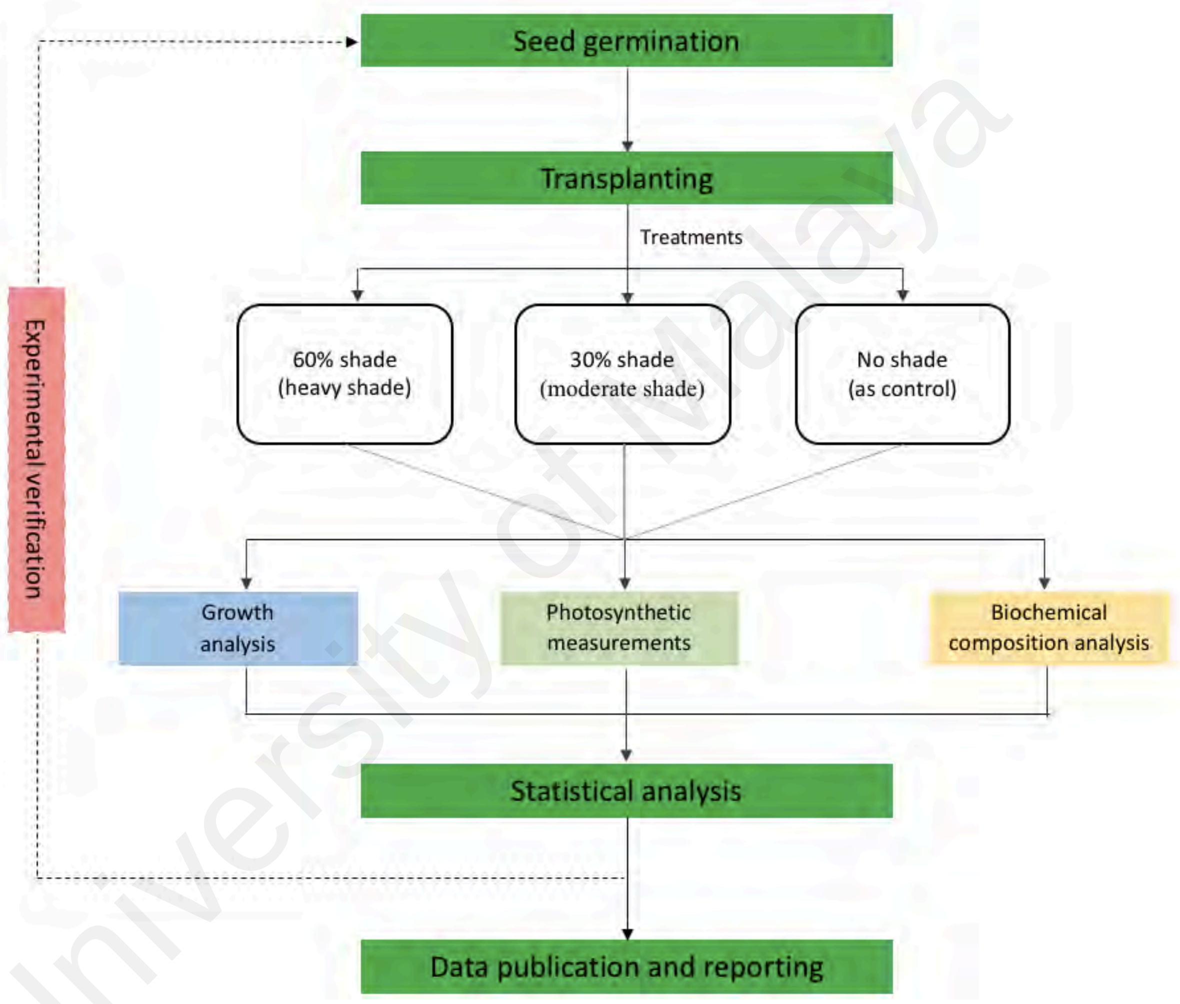


Figure 3.1: Research flow chart.

3.1 Plant Material and Experimental Design

The shade house experiments were conducted using complete randomized design. For 16 weeks, 18 winged bean plants were grown under each shade treatment: (1) without shade (control); (2) 30% black nylon shade net (moderate shade), and (3) 70% black nylon shade net (heavy shade).

Seeds of winged beans (accession S319) from Sri Lanka were used in the present study. A total of 10 sets of germination were initiated, with each set containing 8 seeds placed in a sterilized Petri dish lined with moist filter paper. The process of seed germination was monitored closely and healthy seedlings were transplanted to pots and growed in a shade house in Rimba Ilmu Botanical Garden, University of Malaya (3.130N, 101.660E). For each light treatment, pots were spaced 60 cm and 100 cm between plants and rows respectively (Appendix A). Soils were prepared with a combination of readymade commercial black soil mixed with red soil in ratio 1:1. Light intensities in the shade house were recorded using LICOR Li-250A photometer for seven days continuosly between 10:00 to 14:00 h and soil pH level was recorded randomly 3 days before transplanting. All plants were irrigated daily throughout the growing period. Fertilizers were applied every two weeks and the details are provided in Table 3.1. The average air temperature in the shade house was recorded using thermometer.

Table 3.1: Details of fertilizer application.

Fertilizer	Amount	Timing of fertilizer application
NPK green (15:15:15)	30mg	Every week after transplanting
NPK blue (12:12:7)	40-50mg	Every two week of the first fertilizer application

3.2 Evaluation of Important Parameters

A total of 25 important parameters on plant growth and development were selected to be evaluated in this study. These include six morphological traits, five physiological traits, nine yield-related traits, three assimilation pigments, and also the crude protein content for both winged bean seed and leaf. For each treatment (i.e., shade level), data from seven healthy plants were used to evaluate 25 traits selected in the present study (Raai et al., 2020).

3.2.1 Morphological parameters

Morphological parameters related to winged bean growth, including plant height, number of branches, number of leaves, number of nodes, internode length, and days to flowering, were evaluated in this study.

3.2.1.1 Plant height

Measurement for plant height was taken on the main stem of the winged bean plant, from soil references point to top of nodal region using a measuring tape with an accuracy of ±1 mm from 2nd week to 9th week of experiment at 10:00 to 14:00h

3.2.1.2 Number of nodes, leaves, and branches

Number of nodes and leaves per plant were recorded on weekly basis from the 2nd to 9th weeks. To obtain number of nodes, nodes were counted manually between branches, starting from the lowest branches. On the other hand, number of leaves were counted manually on every branch that grew only on the main stem. Leaves formed at the side stems were not counted.

3.2.1.3 Internode length

Internode length was taken at the 7th internode from the soil reference point using a measuring tape with an accuracy of ±1 mm from 2nd week to 9th week of experiment 10:00 to 14:00h

3.2.1.4 Days to flowering

Days of flowering were recorded based on the number of days between germination to first flowering.

3.2.2 Physiological parameters

Physiological parameters, including *in-situ* chlorophyll, photosynthetic rate, stomatal conductance, internal carbon dioxide (CO2) concentration, and transpiration rate were evaluated in this study. Some images of evaluation of physiological parameters are presented in Appendix B.

3.2.2.1 Relative chlorophyll content

Relative chlorophyll content were measured weekly between the 2nd week and 12th week using SPAD-502 (Minolta, Japan). For each plants, five readings for each plants were randomly taken at time range about 10:00-14:00 h for each treatment and mean of readings for each plants were calculated and used for further analysis

3.2.2.2 Photosynthetic gas exchange measurements

Between the 7th and 8th weeks, gas exchange measurements, including photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate were measured using the LI-6400XT Portable Photosynthesis System (LI-COR, USA). Before any measurements were taken, the LI- 6400XT system was calibrated for about 15 minutes to allow proper parameter settings. The parameters were set-up as: (1) leaf temperature of 30 °C; (2) PAR of 1,000 µmol $m^{-2}s^{-1}$; and (3) adjusted CO_2 flux at 400 µmol s^{-1} inside the chamber. Data taken from three random plants from each shade were selected, and three readings for each trait were recorded from each plant between 10:00 h and 14:00 h.

3.2.3 Yield parameters

A total of nine yield parameters, including number of pods per plant, pod diameter, pod length, pod weight, seeds per pod, seed diameter, hundred-seed weight, yield per plant and harvest index, were recorded in this study. Measurements for pod length, pods per plant, and pod weight were carried out on fresh samples while measurements for the rest of the traits were done after the samples went through the drying process in an oven at 65^0C for 72 h. Some images of evaluation of yield parameters are presented in Appendix C.

3.2.3.1 Number of pods per plant, pod diameter and length

Before the drying process, the number of pods which had matured on the 16th week were recorded for plants from each shade treatment. The broadest part of pods were measured using rope and measuring tape to obtain the pod diameter. Pod length was then measured from one end of the winged bean pod to the other end with a measuring tape.

3.2.3.2 Pod weight

After the drying process, seeds from each pod were weighed with electronic balance and the average weight was calculated. This was followed by measurements of other yield-related traits in the subsections below.

3.2.3.3 Seeds per pod and seed diameter

The number of seeds were recorded for each pod and the average number of seeds per pod was calculated. The length of five random seeds was measured for each pod with a digital calliper and the average length was then calculated.

3.2.3.4 Yield per plant and 100-seed weight

The yield per plant for winged bean grown under different shade treatment was obtained by measuring the total dried seed weight for all seeds. The weight of 100 random seeds were then recorded.

3.2.3.5 Harvest index

Harvest index was calculated as the ratio of harvested seeds mass to the total aboveground plant biomass.

3.2.4 Assimilation pigments and crude protein content

3.2.4.1 Assimilation pigments

Evaluation of assimilation pigments was carried out according to Sumanta et al., (2014) with minor modifications. About 0.5 g of fresh seeds sample was homogenized until it changed into the powder form. Homogenized samples were then mixed with 5 ml of 80% acetone and underwent centrifugation for 10000 rpm for 15 min at 4°C. Supernatant was collected and all the previous steps were then repeated using another 0.5 g fresh sample from the same plant. After that, the two supernatants were mixed together, and 0.50 ml of the sample was diluted with 0.45 ml of solvent. The solution mixture was then analyzed for chlorophyll a, chlorophyll b, and carotenoids in spectrophotometer in 663.2 nm, 646.8nm and 479.0 nm. All analyses for assimilation pigments were made in triplicate. The equation used for the quantification of chlorophyll a, chlorophyll b, and carotenoids as follows: -

Chlorophyll a = $12.25A_{663.2} – 279A_{646.8}$

Chlorophyll b =$21.5A_{646.8} – 5.1A_{663.2}$

Carotenoids = $(1000A_{470} – 1.82C_a –85.02C_b)/198$

3.2.4.2 Crude Protein Content

Crude protein content for both winged bean leaf and seed was measured using the Kjeldahl method with minor modifications (AOAC, 1980) (Appendix D). The overall protein content analysis involved three major steps: digestion, distillation, and neutralization. All analyses for crude protein content were made in triplicate.

3.2.4.2.1 Digestion

Approximately 0.2 g of samples were homogenized, and placed into digestion flask with 20 ml of 95% concentrated sulphuric acid (H_2SO_4), 1 g cupric sulphate ($CuSO_4.5H_2O$), and 1 g potassium sulphate (K_2SO_4). The mixture was heated up for 90min until colour changes were observed. After that, the digested sample was left to cool down at room temperature before it was diluted with 50 ml of distilled water.

3.2.4.2.2 Distillation

A total of 100 ml of 4% (w/w) or 0.647 M boric acid (H_3BO_3) was placed into a new flask which was utilized as the receiving flask. The digestion flask was connected to this receiving flask with a distillation unit. A drop of methyl red was added to the H3BO3 to indicate the pH change during the distillation process. After that, 50 ml of 35% (w/w) or 8.75 M sodium hydroxide (NaOH) was added to the solution in the digestion flask. The solution in the digestion flask was then heated.

3.2.4.2.3 Neutralisation

The solution in the receiving flask was titrated with 0.25 M hydrochloric acid (HCl). The volume of acid needed to reach the end point was recorded. The amount of H+ ions required to reach the end point and the nitrogen concentration of the sample were determined by using the following equation: -

%N = (x mol / 1000ml) x (vs - vb ml / M g) x (14g/mol) x 100

Where

x is the mole of HCl used for the titration

vs is the titration volume of the sample

vb is the titration volume of the blank

M is the mass of the sample

14g/mol is the atomic weight of nitrogen

Finally, the protein content of the sample was determined by:

%Protein = F x %N

(where F is the conversion factor in which we use 6.25)

3.3 Statistical analysis

The recorded data were subjected to descriptive statistics and one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test, and the Pearson's correlation technique was used to examine the relationships among variables. Simple linear regression was also used examine the highly significant pairwise correlations. All analyses were performed using the SPSS Statistics Software (Version 25).

CHAPTER 4: RESULTS

This study was conducted to examine the effects of three different shade levels (non-shade, moderate shade and heavy shade) on 25 traits important associated with the growth and development of winged bean (Raai et al., 2020). Tables of statistical analyses, including ANOVA tables, are provided in Appendix E.

4.1 Growth Performance

The data analysis for morphological parameters are shown in Table 4.1. There were significant differences ($P<0.05$) in plant height, number of leaves per plant and day to 50% flowering for winged bean plants grown under different shade treatments. Based on the results, winged bean plants revealed greater growth responses towards non-shaded condition (Table 4.1). Non-shaded plants had higher plant height, days to flowering, number of branches, number of leaves, and internode length ($P<0.05$) than the shaded plants grown under both 30% and 60% black nylon shades.

Table 4.1: Morphological parameters analysis between winged bean plants grown under different shading regimes. [Data are means of treatments, N = 3; Rep = 7; Means with different letters on top of each standard error of means are significantly different at p<0.05].

Trait	Non-shaded plants	Moderately shaded plants	Heavily-shaded plants
Plant height	374.9 ± 15.61 ^{a}	333.0 ± 3.83 ^{b}	290.2 ± 3.93 ^{c}
Number of branches	47.9 ± 3.10 ^{a}	35.7 ± 0.81 ^{b}	33.6 ± 2.44 ^{b}
Number of leaves	104.0 ± 7.06 ^{a}	90.9 ± 1.69 ^{b}	76.0 ± 2.67 ^{c}
Number of nodes	16.6 ± 0.94^{a}	15.4 ± 0.78 ^{ab}	13.6 ± 0.38 ^{b}
Internode length (cm)	14.3 ± 0.13 ^{a}	12.0 ± 0.45 ^{b}	10.9 ± 0.46 ^{b}
Days to flowering	48.9 ± 2.28 ^{a}	63.9 ± 0.34 ^{b}	67.1 ± 0.56 ^{c}

4.2 Analysis of Photosynthetic Gas Exchange

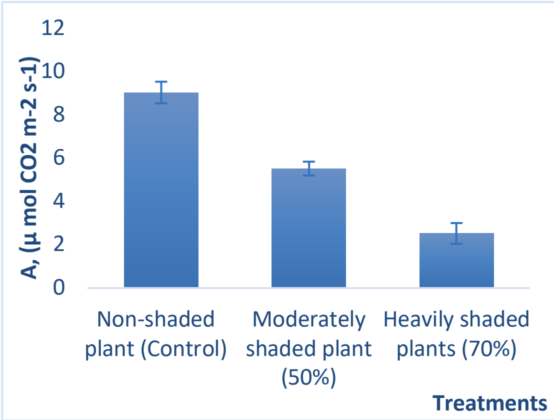
Table 4.2 shows the data analysed for five physiological parameters. As expected, the non-shaded plants showed higher physiological responses than all the shaded plants, recording greater values for stomatal conductance, photosynthetic, transpiration rate and in-situ chlorophyll ($P<0.05$). There was, however, no statistically significant different for internal CO_2 concentration ($P>0.05$) among all the shaded and non-shaded plants (Table 4.2).

Table 4.2: Physiological parameters analysis between winged bean plants grown under different shade levels. [Data are means of treatments, N = 3; Rep = 7; Means with different letters on top of each standard error of means are significantly different at p< 0.05].

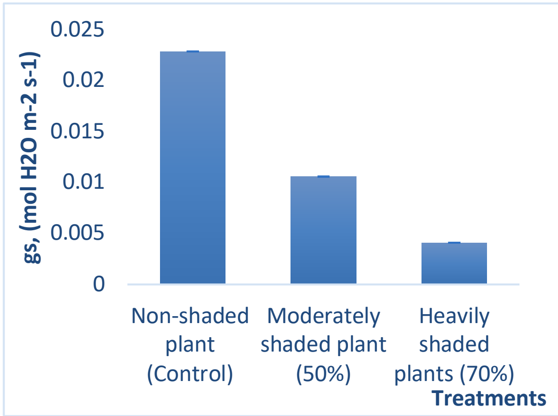
Trait	Non-shaded plants	Moderately shaded plants	Heavily-shaded plants
A, (μ mol CO_2 m^{-2} s^{-1})	9.05 ± 0.50^{a}	5.53 ± 0.32^{b}	2.53 ± 0.48^{c}
g_s, (mol H_2O m^{-2} s^{-1})	0.0229 ± 0.00^{a}	0.0106 ± 0.00^{b}	0.0041 ± 0.00^{c}
Ci, (μ mol CO_2 mol^{-1})	593.62 ± 341.86^{a}	307.96 ± 91.81^{a}	720.50 ± 106.13^{a}
E, (m mol H_2O m^{-2} s^{-1})	0.95 ± 0.01^{a}	0.53 ± 0.08^{b}	0.20 ± 0.02^{c}
Chl (μmol per m^{-2})	36.17 ± 0.65^{a}	28.18 ± 2.18^{ab}	25.03 ± 2.00^{b}

Net photosynthesis rate, A; stomata conductance, gs; intercellular CO_2, Ci; transpiration rate, E

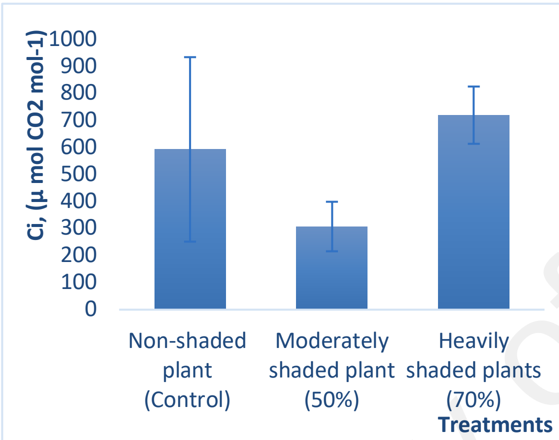
Figure 4.1 shows the effects of shading treatments on shading treatments on net photosynthesis rate; stomata conductance; intercellular CO_2 concentration, and transpiration rate.



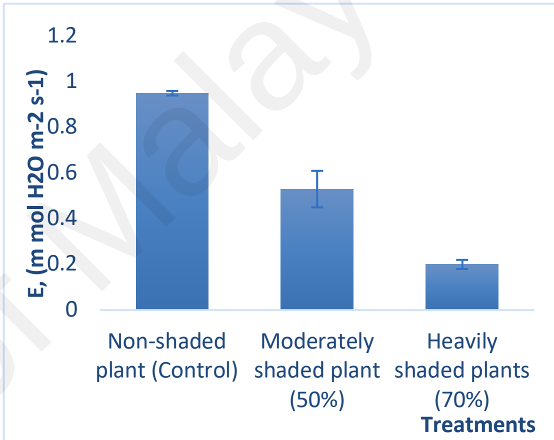
A)



B)



C)



D)

Figure 4.1: Effects of shading treatments on (A) net photosynthesis rate, A; (B) stomata conductance, gs; (C) intercellular CO_2 concentration, Ci; and (D) transpiration rate, E. Bars represented standard error of differences between means.

4.3 Evaluation of Yield and Yield-related Traits

The results for yield-related traits are shown in Table 4.3. The non shaded-plants generally demonstrated superior growth rates compared to the shaded plants. Nevertheless, moderately shaded plants recorded 28.9 g for yield per plant, which significantly differed ($P<0.05$) from the non-shaded (23.8 g) and heavily shaded (21.4 g) plants (Table 4.3). Additionally, moderately shaded plants also recorded higher number per pod, pod length, number of seeds per pod and 100-seed weight (Table 4.3). Only the pod diameter trait did not mark a significant difference (P>0.05) between plants grown in three different shading regimes. Hinged on the nine yield parameters analyzed, winged bean plants yielded the most under moderately shaded condition (i.e., with 30% shade) but did not perform well under heavily shaded condition (i.e., with 60% shade). Except for pod diameter, all other yield related traits for heavily shaded plants were significantly lower (P<0.05) than the moderately shaded plants (Table 4.3).

Table 4.3: Yield parameters analysis between winged bean plants grown under different shade levels. [Data are means of treatments, N = 3; Rep = 7; Means with different letters on top of each standard error of means are significantly different at p< 0.05].

Traits	Non-shaded plants	Moderately shaded plants	Heavily-shaded plants
Number of pods	7.7 ± 0.36^{b}	10.3 ± 0.57^{a}	7.6 ± 0.53^{b}
Pod diameter (cm)	9.4 ± 0.88^{a}	9.9 ± 0.91^{a}	8.0 ± 0.61^{a}
Pod length (cm)	18.5 ± 1.70^{b}	26.9 ± 2.46^{a}	17.1 ± 2.36^{b}
Pod weight (g)	6.3 ± 0.79^{ab}	7.5 ± 0.43^{a}	5.3 ± 0.97^{b}
Seeds per pod	10.6 ± 1.34^{b}	13.5 ± 0.63^{a}	8.9 ± 1.41^{b}
Seed diameter	0.8 ± 0.11^{ab}	1.0 ± 0.03^{a}	0.7 ± 0.08^{b}
Hundred-seed weight	20.8 ± 0.05^{b}	25.0 ± 0.05^{a}	19.6 ± 0.08^{b}
Yield per plant (g)	23.8 ± 1.06^{b}	28.9 ± 1.47^{a}	21.4 ± 1.48^{b}
Harvest index	0.2 ± 0.03^{ab}	0.3 ± 0.02^{a}	0.2 ± 0.02^{b}

4.4 Analysis of Assimilation Pigments and Protein Content

For the three studied assimilation pigments, chlorophyll A, chlorophyll B and carotenoids, there were no statistically significant differences (P>0.05) between plants grown under all shade levels (Table 4.4). Nevertheless, shading was seen to affect the protein content on winged bean, both on its leaves and seeds (Table 4.4; Figure 4.2).

Moderately shaded plants recorded the highest protein content in the leaves, which was was higher than both non-shaded plants and heavily shaded plants (Table 4.4). Furthermore, moderately shaded plants also had the highest protein content in the seeds, which was similar to the non-shaded plants but higher than the heavily shaded plants (Table 4.4).

Table 4.4: Protein and assimilation parameters analysis between group of plants subjected to different shade levels. [Data are means of treatments, N = 3; Rep = 7; Means with different letters on top of each standard error of means are significantly different at p< 0.05].

Traits		Non-shaded plants	Moderately shaded plants	Heavily-shaded plants
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	ProteinL (%)	15.8 ± 0.28 [b]	17.6 ± 0.30 [a]	14.6 ± 0.25 [c]
content	ProteinS (%)	31.7 ± 3.88 [ab]	35.4 ± 1.04 [a]	23.2 ± 1.86 [b]

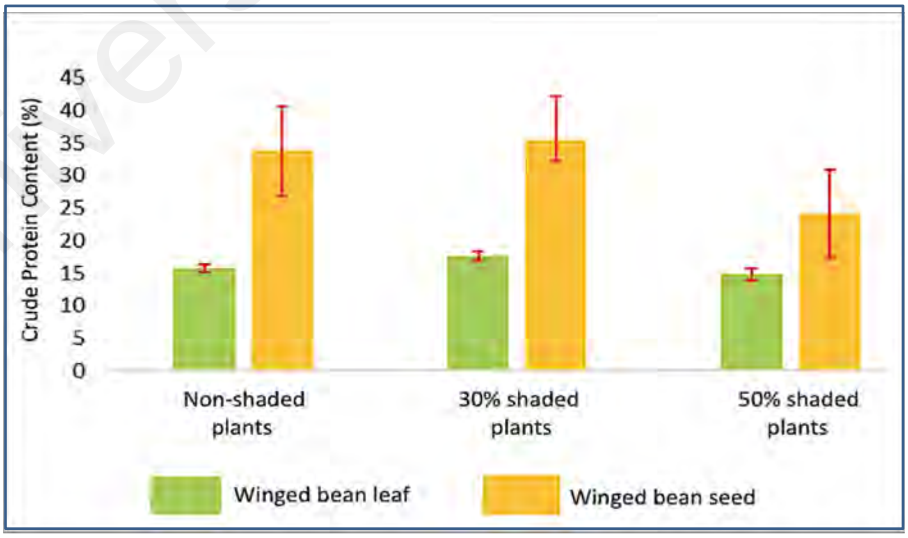


Figure 4.2: Crude protein content in winged bean leaves and seeds under different shading treatments [Data are means of treatments, N = 3; Rep = 7; Means with different letters on top of each standard error of means are significantly different at p< 0.05].

4.5 Correlation and Regression Analysis

Table 4.5 shows the Pearson's correlation coefficients between studied parameters. Pearson correlation analysis showed a strong negative correlation between plant height and days to flowering (r=-0.85**), suggesting that winged bean plants with rapid growth require shorter period to reach the flowering stage. On other hand, positive correlations were found between seed diameter and both protein content for leaf (r=0.71**) and seed (r=0.56**), indicating that the bigger the winged bean seed, the higher the protein content of the winged bean plant. Regression analysis showed that A had significantly increased plant height (r^2=0.6449*) and number of leaves (r^2=0.6012*) of winged bean (Figure 4.3), displaying the strength of source and sink relationship with the increasing of photosynthetis rate.

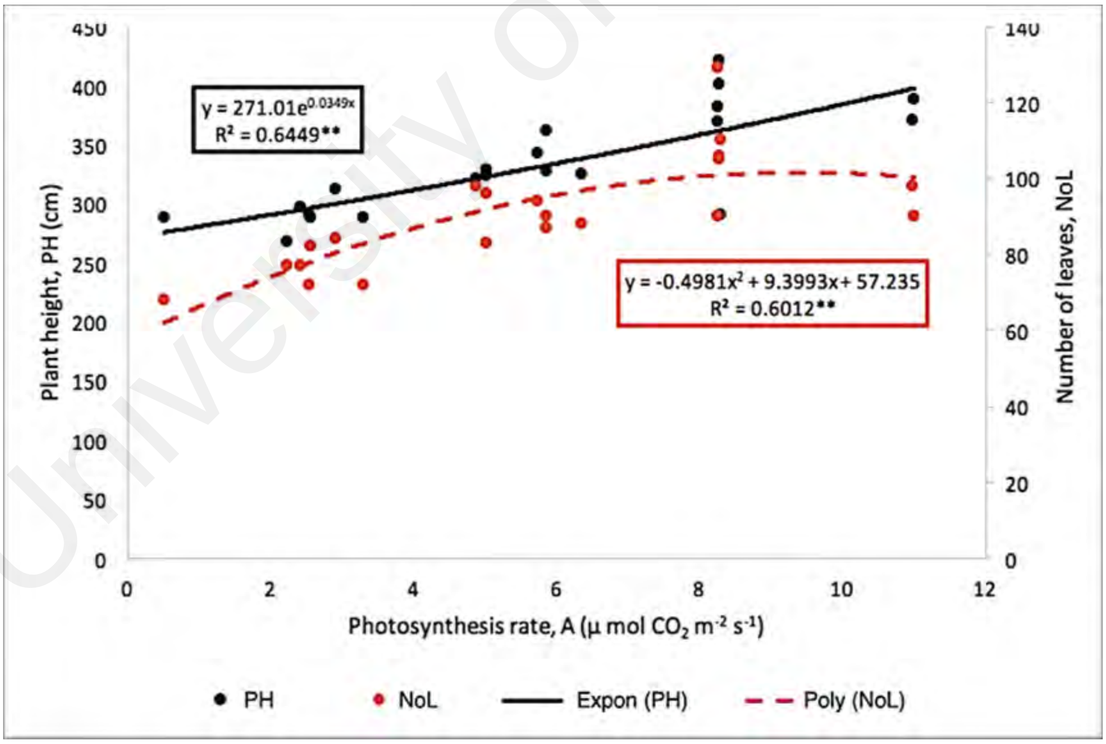


Figure 4.3: Relationship between plant height, number of leaves and photosynthesis rate of winged bean plants during the 8th week of observation [significant at p ≤ 0.01, n = 7].**

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Table 4.5: Correlation between morphological, physiological, yield parameters, protein content and assimilation of winged bean.

	PH	NoB	NoL	NoN	InL	DTF	A	Gs	Ci	E	Chl	PodN	PoD	PodL	Pod W	NoS	SD	HSW	YPP	HI	ProL	ProS	ChlA	ChlB	Car
PH	1.00																								
NoB	0.49*	1.00																							
NoL	0.65**	0.54*	1.00																						
NoN	0.52*	0.40	0.51*	1.00																					
InL	0.60**	0.64**	0.65**	0.28	1.00																				
DTF	-0.85**	-0.59**	-0.76**	-0.61**	-0.73**	1.00																			
A	0.80**	0.73**	0.71**	0.61**	0.67**	-0.91**	1.00																		
Gs	0.72**	0.71**	0.76**	0.57**	0.73*	-0.89**	0.85**	1.00																	
Ci	-0.18	-0.42	-0.32	-0.16	-0.43	0.33	-0.18	-0.65**	1.00																
E	0.68**	0.65**	0.75**	0.55**	0.70**	-0.85**	0.79**	0.99**	-0.70**	1.00															
Chl	0.45*	0.43*	.529*	8.00	0.74**	-0.53*	0.54*	0.66**	-0.45*	0.66**	1.00														
PodN	0.21	0.10	0.24	0.27	0.21	-0.24	0.27	0.08	0.26	0.13	0.04	1.00													
PodD	0.20	0.30	0.03	0.35	-0.16	-0.20	0.37	0.22	0.06	0.22	-0.12	0.15	1.00												
PodL	0.36	0.52*	0.32	0.34	0.28	-0.53*	0.56**	0.57**	-0.33	0.53*	0.21	-0.12	0.54*	1.00											
Pod W	0.06	-0.05	0.04	-0.02	0.27	-0.25	0.17	0.14	0.00	0.19	0.11	.436*	0.00	-0.21	1.00										
NoS	0.15	-0.09	-0.01	0.10	-0.02	-0.24	0.13	0.18	-0.13	0.19	-0.10	-0.08	0.36	0.455*	0.14	1.00									
SD	-0.12	-0.21	0.04	0.06	-0.22	0.14	-0.07	-0.26	0.43	-0.21	-0.39	0.72**	0.21	-0.14	0.29	0.10	1.00								
HSW	0.01	-0.01	-0.07	-0.20	0.00	0.03	-0.03	0.05	-0.17	0.08	0.39	-0.02	-0.13	-0.08	0.11	-0.26	-0.23	1.00							
YPP	-0.17	-0.527*	-0.18	-0.29	-0.12	0.22	-0.24	-0.30	0.30	-0.24	0.20	0.25	-0.24	-0.37	0.43	-0.17	0.28	0.62**	1.00						
HI	0.26	-0.17	0.25	-0.10	0.07	-0.16	0.14	0.05	0.19	0.11	0.23	0.58**	-0.12	-0.25	0.39	0.05	.482*	0.38	0.62**	1.00					
ProteinL	0.28	-0.10	0.33	0.31	0.16	-0.30	0.24	0.15	0.18	0.22	0.03	0.85**	0.02	-0.14	.491*	0.11	0.71**	-0.07	0.34	0.72**	1.00				
ProteinS	0.31	0.06	0.59**	0.30	0.15	-0.30	0.31	0.28	0.03	0.32	0.13	0.48*	0.02	-0.12	0.28	0.00	0.56**	-0.05	0.16	0.57**	0.67**	1.00			
ChlA	-0.16	-0.22	-0.25	-0.30	-0.04	0.21	-0.21	-0.28	0.25	-0.30	0.08	-0.02	-0.25	-0.21	-0.27	-0.18	-0.31	0.25	0.11	-0.11	-0.17	-0.36	1.00		
ChlB	0.00	0.09	0.08	-0.04	0.32	-0.09	0.11	0.14	-0.13	0.16	.468*	0.09	0.12	0.14	-0.04	0.37	-0.13	-0.14	-0.10	0.00	-0.09	-0.16	0.25	1.00	
CAR	-0.49*	-0.72**	-0.42	-0.33	-0.35	0.48*	-0.60**	-0.48*	0.13	-0.40	-0.10	-0.13	-0.19	-0.42	0.13	-0.05	0.02	0.10	0.50*	0.04	0.04	-0.14	0.25	0.01	1.00

CHAPTER 5: DISCUSSION

The effects of different intensities of shades towards the growth, development and biochemical composition of winged bean were examined in the present study. Light availability is widely known as one of the crucial exogenous factors that can affect plant growth and development, along with several other factors such as temperature and water availability (Shao et al., 2014). To date, the existing knowledge on the growth and development patterns of winged bean under different environmental conditions is still limited.

According to Herath and Ormrod (1979), winged bean is sensitive to photoperiod with a 12-h critical day length. In the current study, winged bean plants were grown in a shade house under a controlled environment with similar temperature and water availability. This allows for a more accurate comparison between plants grown under different shading shade treatments. Overall, approximately 80% of the studied variables showed significant responses to at least two shading treatments (Table 4.1–4.4).

5.1 Growth and Development of Winged Bean under Different Shading Regimes

Generally, non-shaded plants displayed superior morphological traits like plant height, number of leaves, and internode length than the moderately shaded and heavily 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 and Sadras, 2014) and soybean (Iqbal et al., 2019).

The non-shaded plants took a shorter period (approximately 49 days) to flower than the moderately (approximately 64 days) and heavily fully shaded (approximately 67 days) plants (Table 4.1), supporting the preliminary conclusion made by Herath and Ormrod (1979) that flowering in winged bean could be delayed by low light intensity. It has also been suggested in some other studies that low light intensity (<30% solar radiation) may result in delayed flower initiation or incomplete flower development in plants (Kiniry et al., 2004). Nonetheless, a recent study conducted by Khalid et al. (2019) on soybean reported that light shading can promote the overall growth of soybean.

The rate of photosynthesis in on non-shaded plants was almost two and four times higher than moderately shaded and heavily shaded plants, respectively (Table 4.2). 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). This notion, however, can only apply to cases where other major limiting factors, including carbon dioxide concentration and temperature, are negligible (Kaiser et al., 2014). To obtain reliable results, the experimental site in the current study was carefully set-up to ensure that all three different shading regimes retain the same temperature profile throughout the two growing seasons. Although non-shaded plants recorded higher photosynthetic rate, stomatal conductance, and transpiration rate than the moderately shaded plants and heavily shaded plants, their intercellular CO_2 concentration did not differ significantly (Table 4.2).

A multitude of plants from various species has shown to have effective physiological processes when grown under direct or full sunlight (Zhu et al., 2017). By and large, photosynthesis is one of the major yield-contributing factors in plants. The regression analysis in the present study showed that photosynthetic rate had significantly increased the plant height (r =0.6449**) and number of leaves (r =0.6012**) of winged bean (Figure 4.3), 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 like neorotropical species (Rijkers et al., 2000) and dipterocarp species (Kenzo et al., 2006). The days to flowering trait, on the other hand, had significant negative correlation coefficient with photosynthetic rate (r = -0.91**; Table 4.5) and stomatal conductance (r =-0.89**; Table 4.5). This indicates that maintaining high photosynthesis rate and stomata conductance are important in accelerating sink strength in promoting early senescence.

All yield-related traits in this study, excluding pod diameter, were found to be responsive to different shading intensities (Table 4.3). It has been reported that crop productivity is directly associated with the availability of light (Kiniry et al., 2004), and low light availability will most likely decrease the yield components of a crop (Maddonni and Otegui, 2004). Some studies demonstrated that the efficiency of nitrogen assimilation can mark weighty effects on the productivity of certain plants, whereby nitrogen deprivation may cause several metabolic deficiencies, such as carboxylation efficiency, in these plants (Mattson et al., 1991; Delgado et al., 1994).

Interestingly, the moderately shaded plants recorded the highest values for all yield components, having significantly greater number of pods per plant, pod length, number of seeds per pod, 100-seed weight, and yield per plant than both non-shaded and heavily shaded plants (Table 4.3). 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 (Khalid et al., 2019).

The heavily shaded plants, on the other hand, exhibited the lowest yields with the shortest plant stature (Table 4.1 and Table 4.3). 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., 2019). Nevertheless, different plant species have their own optimal light intensity ranges to grow, which impact their morphology, physiology, and also secondary metabolite production (Pan and Guo, 2016).

5.2 Biochemical Responses of Winged Bean under Different Shading Regimes

Previous studies on some other plants such as mahogany (*Swietenia macrophylla King*), tonka bean (*Dipteryx odorata* Aubl. Willd), and some trees (*Acer pseudoplatanus, Fagus sylvatica, Tilia cordata*, and *Abies alba*) showed that the concentrations of assimilation pigments were significantly affected by the changes in light availability (Lichtenthaler et al., 2007). In the present study, however, the chlorophyll A, chlorophyll B, and caratenoid contents did not significantly affected by the shade treatments differ ($P>0.05$) among plants grown under different shading levels (Table 4.4). These results suggested that winged bean is capable of adjusting their photosynthetic presentation to accommodate different levels of light. Several studies on algae species like 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.

Generally, plants adapt to different environments by developing appropropriate strategies such as producing larger and thinner leaves that have higher chlorophyll content (Taiz and Zeiger, 2002). The synthesis and degradation of photosynthetic pigments may be associated with acclimation to different environments. Chlorophyll is often synthesized and photo-oxidized in the presence of light. Nevertheless, excess light can cause greater degradation and consequently decrease chlorophyll levels (Gonçalves et al., 2005). On the other hand, under limiting light, plants set into motion a series of compensatory mechanisms, such as a substantial increase in photosynthetic pigments. This response allows the plant to maintain a photosynthetic antennae sufficient to capture the required light energy (Czeczuga, 1987) considering that highly pigmented leaves show a higher light absorption efficiency per leaf, which may allow the plant to achieve a carbon balance under light-deficit conditions (Dai et al., 2009).

Winged bean has recently been hailed as one of the potential crops for the future, owing to its ability to strive in extreme environments and exceptionally, its high protein content (Cheng et al., 2019). The average crude protein contents in both winged bean leaf and seed were found to be close between the non-shaded and moderately shaded plants (Table 4.4; Figure 4.2). 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.

Being a nitrogen-fixing plant, winged bean has a symbiotic relationship with the motile bacteria rhizobia which help to replenish the soil (Somasegaran and Hoben, 2012). Monoculture oil tree plantation has long been a prime issue in the tropical regions (Putz et al., 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 this study recorded only about 70% of the protein in winged bean seeds produced by those under full sunlight shading. This implies that winged bean is not a suitable cash crop for plantations with a high percentage of canopy cover.

CHAPTER 6: CONCLUSIONS

6.1 Conclusions

The outcomes of the present study, for the most part, demonstrated that winged bean grows well and yields better under full sunlight shading condition. This study provides valuable information for improving its agronomic traits under optimal light requirements. Winged bean is a potential crop for the future, owing to its ability to strive in extreme environments and exceptionally, its high protein content. The average crude protein contents in both winged bean leaf and seed were found to be close between plants grown under non-shaded and moderately shaded conditions. 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.

6.2 Future Recommendations

Further research is recommended to assess the crop's response to other important exogenous factors such as temperature and water availability. The agronomic traits of winged bean should be improved, along with its resistance to common diseases and its tolerance to extreme environments. It is also important to promote the utilization of winged bean and its products. The development of winged bean and other underutilized protein-packed legumes which are hardy and beneficial to the environment is one of the momentous solutions to 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. Promoting the utilization of these crops will help achieve several Sustainable Development Goals, especially the first three which include no poverty, zero hunger, and good health and well-being.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Publications

1. Cheng, A., **Raai, M. N.,** Mohd Zain, N. A., Massawe F., Singh, A., & Wan-Mohtar, W. A. Q. I., (2019). In search of alternative proteins: Unlocking the potential of underutilized tropical legumes. *Food Security, 11*, 1205–1215.

2. **Raai, M.N.,** Mohd Zain, N. A., Osman N., Rejab, N. A., Sahruzaini, N. A., & Cheng, A. (2020). Effects of shading on the growth, development, and yield of winged bean (*Psophocarpus tetragonolobus*). *Ciência Rural*, *50*(2), Article#e20190570.

Conference Proceedings

1. **Raai, M.N.**, Mohd Zain, N.A., & Cheng, A. (2018). The Potential of underutilized legumes as alternative proteins to soybean and meat. *The 23rd Biological Sciences Graduate Congress*. 18-20 December 2018, Bangkok, Thailand.