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GROWTH AND PHOTOSYNTHETIC RESPONSES OF LONG BEAN (*Vigna unguiculata*) AND MUNG BEAN (*Vigna radiata*) RESPONSE TO FERTILIZATION

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ABSTRACT

Long bean (*Vigna unguiculata*) and mung bean (*Vigna radiata*) response to fertilization was studied through greenhouse experiments conducted at University of Malaya during 2011-2012. Long bean grown with fertilizer obtained the highest total chlorophyll (2.99 mg g^{-1}), chlorophyll *a* (2.25 mg g^{-1}), photosynthesis rate (1.26 µmol m^{-2} s^{-1}) and identical total dry matter (31.54 g $plant^{-1}$). Mung bean grown without fertilizer possessed the highest chlorophyll *b* (0.80 mg g^{-1}**)** and chlorophyll a/b ratio (0.62 mg g^{-1}). Both long bean and mung bean grown without fertilizer produced the highest number of nodules and number of pods per plant. Fertilizer application had no significant response on seeds per pod, 100-seed weight and seed yield per plant for both crops. The results revealed that greater nodulation, higher pods per plant and identical seed yield can be a good indicator to avoid use of chemical fertilizer for both crops production in tropical Malaysia.

Key words: Photosynthetic pigments, nodule, dry matter.

INTRODUCTION

Legumes are able to form symbiotic association with *Rhizobium* (Ferguson *et al*. 2010) which have tremendous ability in fixing N (Samac and Graham, 2007; Varges *et al.* 2000) and their interaction play a significant role in the agricultural crop production (Giller, 2001; Hirsch, 2009). Legumes can be used as an alternative of chemical N fertilizer due to its high potential in improving crop growth, quality and yield of grains and cereals by increasing the availability of N uptake (Ibrahim *et al.* 2012). The threats of malnutrition of protein energy can be reduced by utilizing legumes, enriched sources of protein, which can lead to a balance daily diet as well (Qayyum *et al.* 2012). Nitrogen is the key limiting factor for the production of essential constituent of proteins, enzymes and metabolic processes such as synthesis and transfer of energy, vitamins, nucleic acids and other organic molecules like chlorophyll for photosynthesis (Reynolds, 2005; Hgaza *et al.*2009). Leguminous plants are able to act as a promising alternative to fertilizer in plant productivity in developing countries (Degefu *et al.* 2011). Healthy plants with large amount of chlorophyll are expected to have maximum growth than unhealthy ones (Campbell and Reece, 2005). Nitrogen is required for the synthesis of amino acids, proteins, NADH, ATP, carbohydrates and lipids. Deficiency of N limits production which leads to stunted growth, appears yellowish leaves, decrease leaf area, photosynthetic rate (Bojovic and Markovic, 2009) and suppression of chlorophyll formation. This crucial pigment also plays role as an index of plant growth and production of organic matter (Lahai *et al.* 2003). Most plants possess chlorophyll a and chlorophyll b which are the main photosynthetic pigments (Campbell and Reece, 2005). Chlorophyll content of leaf tissue is a good index of photosynthetic activity (Chowdhury and Kohri, 2003) and timing of fertilizer application (Haboudane *et al.* 2002; Wu *et al.* 2008) of crop.

In agricultural systems, N is known as the imperative factors in plant growth and governs a major constituent of chlorophyll and photosynthetic activity. Potash (K) governs fruit quality improvement, increases disease resistance; prevent lodging and capability of plants for surviving moisture stress. Phosphorus (P) plays a role in development of fruit, root and flower. Uptake of these nutrients may affect qualitatively and quantitatively the growth and yield (Rathore *et al.* 2008) where the growth conditions may affects the biological composition of a plant (Din *et al.* 2011). Ideal conditions such as nutrient availability, soil condition and field cropping history are required for initiation of biological nitrogen fixation which leads to optimum nodulation. Legume plants provide abundant supply of N through nodulation and that uptake by plants from the soil. On the other hand, excess N may inhibit nodules production (Reynolds, 2005) thus application of N fertilizer in legume field is a great concern for successful production of legume crops (Varges *et al*. 2000). Both long bean and sprouted mung bean seeds are very popular vegetables in Malaysia but their scientific information regarding nodulation, photosynthetic responses and seed yield of both legumes are limited under tropical condition. Therefore present study was undertaken to compare nodulation pattern, photosynthetic responses and seed yield of long bean and mung bean grown with or without fertilizer.

MATERIALS AND METHODS

Long bean (*Vigna unguiculata*) and mung bean (*Vigna radiata*) response to fertilization was studied through greenhouse experiments conducted at University of Malaya, Kuala Lumpur (3°7'25"N, 101°39'11"E), Malaysia during 2011-2012. Long bean and mung bean were grown with N-P-K fertilizer at the rate of 2-4-2 g m^{-2} and without fertilizer. Urea (46% N), triple super phosphate (52% P) and muriate of potash (60% K) were used as the sources of N-P-K fertilizer. Each legume plant was tested as an individual experiment and repeated twice. The experiments were conducted under completely randomized design (CRD) with six replications. The soils of the experiment were clay loam having pH 6.14. Plastic pot (0.25 m^2) was used to conduct the experiment and each pot was filled with 5 kg of soils. Seeds were planted in pots as per treatment schedule. Nitrogen fertilizer was applied into each pot at 10 days after emergence (DAE) of each legume while P and K were applied before planting of both crops. Prior to planting and after harvesting of both crops, soils were collected for the estimation of elemental N concentrations (%). Collected soils were dried in an oven at 72^0C for 48 hours. The samples were ground into very fine powdered form using mortar and pestle. Elemental N was analyzed using PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (2400 Series II)

Total chlorophyll content was estimated quantitative following Arnon's method (Arnon 1949) and SPAD leaf chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan). Randomly six fully expanded leaves were selected at vegetative, flowering, pod filling and maturity stages to determine the chlorophyll contents. Approximately 50 mg leaf tissue was homogenized in 10 mL of 80% acetone using mortar and pestle in dark condition. The suspension was decanted through filter funnel (Buchner) using Whatman filter paper No. 1. The residue in the mortar was washed with the solvent and filtered. Chlorophyll absorbance was determined by using 645 nm and 663 nm in Thermo Scientific Genesys 10S UV VIS spectrophotometer. The chlorophyll concentration was calculated using specific absorption coefficients for total chlorophyll, chlorophyll *a* and chlorophyll *b* provided by the following:

Total chlorophyll = 20.2 (Abs645) + 8.02 (Abs663);
Chlorophyll a = 12.7 (Abs663) – 2.69 (Abs645) and
Chlorophyll b = 22.9 (Abs645) – 4.68 (Abs663);

Where, Abs645 and Abs663 = Absorbance at 645 nm and 663 nm wavelength, respectively (Arnon *et al.* 1949; Makeen *et al*. 2007; Wu *et al.* 2008). A chlorophyll meter [SPAD-502, Soil–Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan] was used for measurements (Hgaza *et al* 2009) of six topmost fully expanded leaves per plant at vegetative, flowering, pod filling and maturity stages. A portable Lci-SD (Leaf Chamber/Soil Respiration Analysis System) (Thermoscientific) was used to measure photosynthetic active radiation (PAR) of legume plants at vegetative, flowering, pod filling and maturity stages. The readings were recorded randomly from six fully expanded leaves of selected plants once they were placed at the leaf chamber.

Six individual plants of each crop were excavated at vegetative, flowering, pod filling and maturity stages. Plant parts were oven dried at 72^0C for 48 hours and dry matters of the plant parts were recorded. Number of pods per plant, seeds per pod, 100-seed weight and seed yield per plant were recorded from randomly selected six individual plants in each treatment. Data were subjected to the analysis of variance (ANOVA) to compare the effects of treatments. When significant differences occurred, the means were evaluated using the Least Significant Difference (LSD, $P < 0.05$) method.

RESULTS AND DISCUSSION

The initial status of soil N in the experimental soil was 70.0±4.0 mg kg^{-1}. After harvesting of both crops the soil N status increased significantly, long bean (160.0±4.0 mg kg^{-1}) and mung bean (180.0±4.0 mg kg^{-1}) once grown without fertilizer application while soil N status was also increased a bit lesser when both crops were grown with fertilizer application (Table 1). These results reflected that fertilizer application in both legumes did not show positive response. Nitrogen, K and P are primary nutrients which are deficient from the soil due to uptake by plants for survival. Soil is a massive pool of bacteria (Achakzai, 2007) which prominent in biological nitrogen fixation and they are often naturally maintained in the soil in sufficient quantities where supplementation with fertilizers is not required.

Total chlorophyll, chlorophyll a, chlorophyll b and chlorophyll a/b ratios of both legumes were significantly affected by fertilizer application over time. The highest total chlorophyll was observed in long bean (2.99 mg g^{-1}) and mung bean (2.97 mg g^{-1}) at the onset of flowering and thereafter declined when both crops grown with fertilizer (Fig. 1). The highest chlorophyll *a* (2.25 mg g^{-1}) was recorded in long bean grown with fertilizer. Mung bean grown without fertilizer obtained minimum chlorophyll *a* (1.73 mg g^{-1}) and the highest chlorophyll *b* (0.80 mg g^{-1}) recorded at flowering stage while chlorophyll a/b ratio (0.62 mg g^{-1}) recorded at pod filling stage. In contrast both legume species the incremental rate of chlorophyll with fertilizer was more pronounced compared to the legumes grown without fertilizer. Leaf SPAD values of both legumes were affected significantly over time. Long bean grown with fertilizer showed the highest SPAD value (41.35) at flowering stage. The leaf SPAD values were higher in long bean and mung bean grown with fertilizer than both bean grown without fertilizer (Table 2). Both legumes showed sharp increase of leaf SPAD values at vegetative stage and reached peak at initiation of flowering stage and thereafter declined gradually.

Both legumes possessed the highest chlorophyll content at the initiation of flowering stage and declined thereafter. At later stages, the chlorophyll content decreased and it might be due to source-sink relationship. Crops grown with fertilizer obtained higher chlorophyll content than crops grown without fertilizer as a result of fertilizer application. Many researchers proved that there is a very close link between N and chlorophyll content (Tucker, 2004; Amaliotis *et al*. 2004). The chlorophyll synthesis is depending on mineral nutrition (Daughtry, 2000) where N is responsible for the leaf growth and one of the constituent elements of chlorophyll molecule that contains 4N in tetrapyrolle ring. Nitrogen also acts as prominent element in green leaves and related to chlorophyll content (Haboudane *et al*. 2002) and protein molecules which affects the chloroplast formation (Daughtry, 2000). Phosphorus affects the chlorophyll molecules stability in plants and K plays role indirectly in the chlorophyll synthesis by enhancing the uptake of N, Fe, Mg and SO_4 (Gairola *et al*. 2009). Long bean grown with fertilizer obtained the highest chlorophyll content due to the highest chlorophyll a content. Mung bean grown without fertilizer possessed the least total chlorophyll content due to lowest chlorophyll a content. This is because chlorophyll a content able to capture a limited wavelength only. Mung bean grown without fertilizer needs to produce more chlorophyll b which leads to the highest chlorophyll a/b ratio in order to increase its photosynthetic ability because chlorophyll b can capture a wider range of light (Kumar, 2004). The highest photosynthetic activity was found in long bean grown with fertilizer due to the highest total chlorophyll and chlorophyll a content. Hesketh *et al*. (1981) demonstrated a positive correlation between leaf photosynthesis rate and chlorophyll content.

Photosynthetic rate was influenced significantly by application of fertilizer throughout the crop growing period (Table 2). The highest photosynthetic rates of both legumes were recorded at the onset of flowering and thereafter decreased gradually up to the end of crop growth. Long bean grown with fertilizer showed higher photosynthesis rate (1.26 μmol m^{-2} s^{-1}) than mung bean grown with fertilizer (0.97 μmol m^{-2} s^{-1}). At onset of flowering stage, both crops recorded the highest photosynthetic activities and decreased with increase of plant age. At this stage, higher stomatal conductance in leaves gives rise to diffusion of CO_2 which favors higher photosynthetic rates. The photosynthetic activity decreased at later growth stages as chloroplast lose its integrity, lower stomatal conductance, higher intercellular carbon dioxide concentration (Biswas *et al*. 2001) and decrease in chlorophyll a content because chlorophyll a molecules are vital in photosynthesis energy phase that are necessary before photosynthesis begin. Bojovic and Markovic (2007) also measured the lowest leaf chlorophyll content in plants grown on unfertilized soil. These elements, especially N, involved indirectly in photosynthesis process (Haboudane *et al*. 2002) and photosynthetic capacity due to the activated RUBISCO content (Jia and Gray, 2004), increases the leaf area of plants (Evans, 1989) and helps in increment uptake of nitrate and K ions which contribute to increase in chlorophyll content. Phosphorus influences the photosynthesis activity of crops by modulating directly or indirectly the activated RUBISCO content (Kumar, 2004).

Number of nodules had overriding influence by application of fertilizer for both crops. Long bean and mung bean grown without fertilizer recorded the highest number of nodules (Table 2). Maximum nodule was observed at initiation of flowering and thereafter declined with increase of plant age. Long bean and mung bean grown with or without fertilizer recorded identical total dry matter which showed that fertilizer did not reflect on both crops. Long bean and mung bean grown without fertilizer produced slightly higher number of pods than that of grown with fertilizer (Table 3). Fertilizer application had no significant response on seeds per pod, 100-seed weight and seed yield per plant for both crops (Table 3). Nitrogen is responsible for leaf growth and increased dry matter of plants (Reynolds, 2005), P is responsible for root development while K is responsible for flower and fruit development and increases the dry weight of plants (Arrese-Igor *et al*. 1998). Sharma *et al*. (2000) stated that promoting effect of fertilizer application attributed to increase the dry matter compared to control and reflected in the increase in the yield and yield components (Agamy *et al*. 2012) but in this study results differed and it could be due to available N present in soil and in addition experiment was conducted under pot culture.

Number of nodules was the highest at the onset of flowering for both crops when grown without fertilizer and declined at later stages due to source-sink relationship where carbohydrate from nodules sink into the seed production that helps in pod formation until its maturity. Crops grown with fertilizer obtained less number of nodules than crops grown without fertilizer as a consequence of application of fertilizer. Javaid (2009) also reported that crops grown in unamended soil recorded the highest number of nodules while application of high rates of N fertilizer decreased dry and fresh

Table 1: Nitrogen content in soil before planting and after harvesting of long bean and mung bean

Legumes	Nitrogen (mg kg $^{-1}$)	
	Before sowing	After harvesting
Long bean		
Control	70.5 ± 4.30	134.5 ± 1.15b
Fertilizer	71.0 ± 7.25	160.3 ± 4.20a
Mung bean		
Control	71.0 ± 7.25	93.5 ± 1.25b
Fertilizer	70.5 ± 1.25	184.5 ± 4.15a

Means followed by the same letters are not significantly different for each treatment means
($P < 0.05$) by LSD, ns = non-significant

Table 2: Leaf SPAD value, photosynthesis rate and nodulation of long bean and mung bean grown with or without fertilizer

SPAD value

Treatment	Growth stages			
	Vegetative	Flower initiation	Pod filling	Maturity
Long bean				
Control	33.12 ± 1.05b	38.42 ± 1.66b	32. 02 ± 1.40b	26.70 ± 1.88b
Fertilizer	36.18 ± 1.21a	41.35 ± 1.68a	36.45 ± 1.06a	30.55 ± 1.88a
Mung bean				
Control	26.60 ± 0.99b	34.60 ± 1.14b	31.30 ± 1.08b	26.68 ± 1.09b
Fertilizer	28.88 ± 1.16a	37.08 ± 1.45a	33.32 ± 1.21a	29.07 ± 1.42a

Photosynthesis rate

Treatment	Photosynthesis rate (µmol m $^{-2}$ s $^{-1}$) at growth stages			
	Vegetative	Flower initiation	Pod filling	Maturity
Long bean				
Control	0.34±0.02b	0.91 ± 0.13b	0.48 ± 0.05b	0.38 ± 0.05b
Fertilizer	0.56±0.04a	1.26 ± 0.06a	0.75 ± 0.08a	0.49 ± 0.04a
Mung bean				
Control	0.32 ± 0.02b	0.82 ± 0.06b	0.43 ± 0.03ns	0.33 ± 0.05b
fertilizer	0.39 ± 0.02a	0.97 ± 0.18a	0.45 ± 0.03ns	0.37 ± 0.04a

Number of nodules

Treatment	Vegetative	Flower initiation	Pod filling	Maturity
Long bean				
Control	6.83 ± 1.51a	17.33 ± 2.83a	10.17 ± 1.72a	6.83 ± 1.08a
Fertilizer	6.17 ± 1.01b	10.67 ± 2.32b	6.00 ± 1.31b	5.17 ± 1.11b
Mung bean				
Control	6.00 ± 1.24a	15.50 ± 3.03a	9.50 ± 2.34a	7.00 ± 1.75a
Fertilizer	1.17 ± 0.31b	5.83 ± 2.15b	5.00 ± 1.37b	3.17 ± 1.30b

Means followed by the same letters are not significantly different for each treatment means ($P < 0.05$) by DMRT, ns = non-significant

Table 3: Seed yield attributes, seed yield and dry matter of long bean and mung bean

Treatment	Pods per plant (no.)	Seeds per pod (g plant $^{-1}$)	100-seed weight (g)	Seed yield (g plant $^{-1}$)	Dry matter (g plant $^{-1}$)
Long bean					
Control	5.83 ± 0.27a	13.75 ± 0.52ns	12.68 ± 0.22ns	10.07 ± 0.76ns	32.93 ± 0.24ns
Fertilizer	5.50 ± 0.22b	13.72 ± 0.44ns	12.68 ± 0.19ns	10.21 ± 0.45ns	31.54 ± 0.44ns
Mung bean					
Control	5.80 ± 0.43a	11.55 ± 0.45ns	4.80 ± 0.09ns	3.62 ± 0.09ns	21.78 ± 0.15ns
Fertilizer	5.77 ± 0.17b	11.57 ± 0.49ns	4.78 ± 0.06ns	3.65 ± 0.11ns	20.30 ± 0.12ns

Means followed by the same letters are not significantly different for each treatment means
($P < 0.05$) by DMRT, ns = non-significant

nodules weight (Olson *et al*. 1981; Eriksen and Whitney, 1984). Higher rates of N fertilizer resulted in linear decrease of nodules dry weight as a consequence of regulatory mechanism (Reynolds, 2005; Achakzai, 2007). Our results coincided with previous study that fertilizer application is not needed to maximize nitrogen fixation in soil. Both long bean and mung bean grown without fertilizer obtained the highest number of nodules per plant due to translocation of carbohydrates from nodules to reproductive organ resulting higher pods per plant.

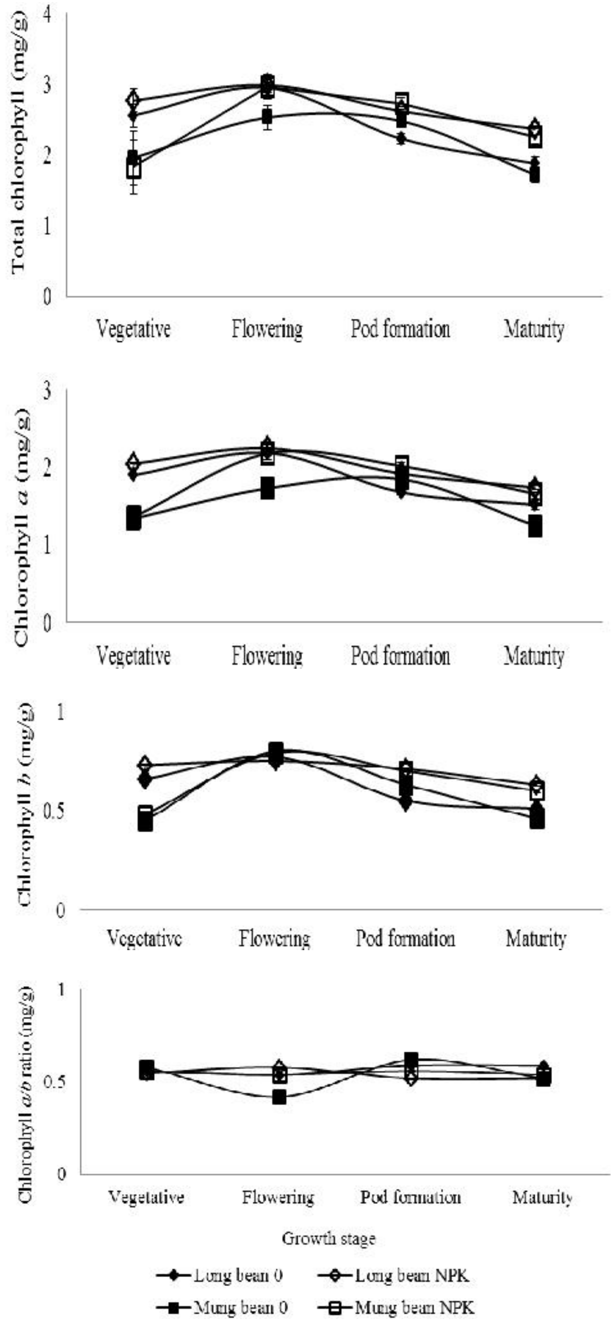

Fig 1. Chlorophyll content of Long bean and Mung bean at different growth stages

Conclusions: Long bean and mung bean grown without fertilizer performed better regards to nodulation which provided addition N into soil compared to crops grown with fertilizer. Crops grown without fertilizer possessed better root nodulation based on nitrogenase reductase activity in nodules whilst crops grown with fertilizer obtained weak root nodulation due to N fertilizer application which interrupts the nitrogen fixation process.

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