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# EFFECT OF LEGUMINOUS COVER CROP (*Calopogonium mucunoides* DESV.) ON LEAF N, CHLOROPHYLL CONTENT AND GAS EXCHANGE RATE OF BLACK PEPPER (*Piper nigrum* L.)

**Sulok K.M.T.[1], Zainudin S.R.[2], Jarroop Z.[1], Shang C.Y.[1] and Lanying F.[3]**
[1]Research and Development Division, Malaysian Pepper Board,
93916 Kuching, Sarawak, Malaysia
[2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak,
94300 Kota Samarahan, Malaysia
[3]Soil Management Branch, Department of Agriculture Sarawak,
Badruddin Road, 93400 Kuching, Sarawak, Malaysia
Tel: +6019-3301637; Email: kevinmuyang@mpb.gov.my

## ABSTRACT

Planting black pepper in a leguminous cover crop establishment can contribute to a healthier environment apart from being able to reduce the cost of N fertilizer application. The use of leguminous cover crops including *Calopogonium mucunoides* have known to bring benefits such as fixing nitrogen and managing soil moisture. This study was designed to investigate the effect of *C. mucunoides* application on leaf total nitrogen (N) as well as to examine the role of N in affecting the chlorophyll content and gas exchange rate of black pepper. The field experiment conducted was a randomized complete block design (RCBD) with two treatments replicated nine times. Treatments were control (C0) which was *Piper nigrum* vines without application of *C. mucunoides* cover crop and Calopo cover crop (C1), *Piper nigrum* vines with application of *C. mucunoides* cover crop. Soil volumetric water content (VWC) was significantly ($p < 0.05$) higher in a *C. mucunoides* establishment than that of the control. Soil total organic carbon (TOC), soil total nitrogen (N) and soil available phosphorus (P) were significantly ($p < 0.05$) higher in a *C. mucunoides* establishment. Leaf total N, photosynthetic rates ($A$), stomatal conductance ($gs$), and transpiration ($E$) of the *C. mucunoides* treatment were significantly ($p < 0.05$) higher than that of the control. Furthermore, strong relationships have been developed between leaf total N – leaf chlorophyll content as well as $A$ – leaf chlorophyll content. Consequently, higher rates of photosynthesis under conditions of high nitrogen are often attributed to increase in chlorophyll content. Therefore, incorporating *C. mucunoides* in black pepper vineyards can increase the photosynthetic rate of *Piper nigrum* by improving few soil properties.

Key words: *Piper nigrum*, *Calopogonium mucunoides*, chlorophyll, photosynthesis, leaf total N

## INTRODUCTION

*Piper nigrum* Linn. is one of the most common condiments and features prominently in most of the gastronomical cuisines worldwide. Being known as a tropical climbing vine and a member of the family Piperaceae, the genus piper has more than 1000 species but the most economically important species is *P. nigrum* (Shanmugapriya et al. 2012). Pepper cultivation in the Sarawak state of Malaysia dates back to 1856 but more extensive planting started in the 1900s. Overall, the state has produced 10,588 tonnes of pepper valued at RM245 million in the year 2012 thus highlighting the importance of pepper as one of the important cash crops supporting the livelihood of about 67,000 rural dwellers in upland areas of Sarawak (Malaysian Pepper Board 2013). Currently, Malaysia ranks fifth after Vietnam, India, Indonesia and Brazil in terms of pepper production.

Calopo (*Calopogonium mucunoides* Desv.) is indigenous to tropical America and the West Indies but it is wide-spread in the tropics of Asia and Africa through introduction in the early 1900s. Pizarro (2002) reported that it is the most popular legume amongst Brazilian farmers and it is the legume seed produced in the greatest volume in Brazil. As a legume, the plant helps to improve soil fertility with its nitrogen-fixing bacteria found in the root nodules.

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The documentation on *Calopogonium mucunoides* usage in black pepper vineyards in Sarawak is still lacking, which needs attention if cultivation of leguminous cover crops were to be undertaken seriously. Therefore, this study was conducted with the following objectives of to investigate the leaf total N and chlorophyll content of *P. nigrum* grown in a *C. mucunoides* establishment, to investigate the gas exchange rate of *P. nigrum* grown in a *C. mucunoides* establishment, and to examine the role of soil properties in influencing leaf total N, chlorophyll content and gas exchange rate of *P. nigrum*.

## MATERIALS AND METHODS

The experiment was a field study and conducted in a 12 m x 12 m plot near Kampung Jagoi, Duyoh, Bau, Sarawak, Malaysia. The climate is tropical, moderately hot with average annual rainfall around 3775 mm. The soil series at the study site was Bemang Series of the Alluvial group with fine loamy texture derived from accreting riverine alluvium. The soil profile generally has a friable consistence throughout and is well to moderately well drained (Paramanathan 2000). The crops involved in this study were *P. nigrum* and *C. mucunoides*. *P. nigrum* var. Kuching cuttings were rooted in a sand bed. After 4 weeks, the pepper cuttings were then selected and transplanted to the planting site during the first month of study. The pepper planting practices introduced by the Department of Agriculture Sarawak were used in this study (Paulus & Wong 1999).

In order to break the dormancy of the Calopo cover crop seeds, scarification with concentrated sulphuric acid for 30 min was done. After scarification, the seeds were germinated at a rate of 86 % on moist cotton for 3 days. The seeds were not inoculated with introduced rhizobia but instead were let to inoculate with the native rhizobia in the soil. The seeds were then transplanted during the first month of study to the planting site by placing 3 to 4 seeds into a 2.0 cm deep hole. The holes were covered up after seed placement. Calopo seeds were planted in holes placed 20 cm apart within each row. These Calopo rows were established about 1 m away from the rows of pepper vines. The study was conducted from the month of August 2013 until June 2014.

### Experimental design and treatments

The experiment was a randomized complete block design (RCBD) with two treatments replicated nine times. Treatments were control (C0) which was *P. nigrum* plot without application of *C. mucunoides* cover crop, and Calopo cover crop (C1), *P. nigrum* plot with application of *C. mucunoides* cover crop. Uniform pepper vines in terms of height and maturity were carefully selected to avoid erroneous result due to the nature of most part of the terrain being flat to slightly undulating.

### Selected soil properties determination

At 10 months after the initiation of treatment, the soil was analyzed for its water content and texture. Soil moisture content was measured by using soil moisture sensor equipment (WS SMEC 300, Spectrum Technologies, USA) to obtain the soil volumetric water content (VWC) (% volume).

To further understand the fertility status of the soil, samples were collected with an Edelman auger at a depth of 0 – 15 cm and analyzed for its total organic carbon (TOC), total nitrogen (N) and available phosphorus (P). The sieved soil samples were analyzed using the Loss on Ignition method for TOC, Kjeldahl method for total N and Molybdenum Blue method for available P at the Sarawak Plantation Chemistry Laboratory Kuching, Sarawak.

### *Piper nigrum* leaf total nitrogen, chlorophyll and gas exchange rate measurement

At the end of the study, fresh leaf samples were collected for the purpose of this experiment. The samples were washed clean with distilled water, oven-dried at $60^{o}$C and grounded. A kitchen blender (Takada Food Blender, ISB-035) was used to ground the dried leaf samples. Approximately 0.1 g of grounded leaf samples were analyzed for total N at Sarawak Plantation Chemistry Laboratory

Kuching, Sarawak. The total N for black pepper leaves was determined using the micro-Kjeldahl method (Tan 1995). Value is recorded as % N for plant tissue on a dry weight basis.

The relative chlorophyll content of leaves was determined at the end of the 10-month period by using chlorophyll meter (SPAD-502, Minolta, Japan). Readings were recorded when mature fully expanded leaves with the same orientation and the same layer in the crown (middle bottom) were still attached to the tree.

Gas exchange measurement was determined after 10 months according to the method by DiCristina and Germino (2006), carried out on young fully expanded leaves with the same orientation and the same layer in the crown (middle bottom). Replicate of nine leaves were used for this purpose. Measurements of net photosynthesis (*A*) (µmol $CO_2$ $m^{-2}s^{-1}$) on an area basis, leaf stomatal conductance (*g*s) (mol $H_2O$ $m^{-2}s^{-1}$), and transpiration rate (*E*) (mmol $H_2O$ $m^{-2}s^{-1}$) of nine different leaves per treatment were monitored using a LICOR LI-6400 XT (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set as close to outside PAR at 1500 µmol $m^{-2}s^{-1}$. The $CO_2$ flow into the chamber was maintained at a concentration of 400 µmol $mol^{-1}$. The humidity flow into the chamber was fixed at 500 µmol $s^{-1}$. Statistical assessment was done on gas exchange parameters at between 1100 to 1200 h, which was presumed to be the diurnal period when photosynthetic rates would be maximal.

### Statistical analysis

Data was analyzed statistically by independent and paired t-test to detect treatment effect. The statistical software used was the SPSS software (version 15). The relationship of *P. nigrum* leaf chlorophyll content – leaf total N and A – leaf chlorophyll content were correlated using regression of best fit.

## RESULTS AND DISCUSSION

### Soil properties

The results indicated that soil VWC in C1 was 40 % higher than that of C0 (Table 1). This result concurs with a report by Hoorman (2009) which mentioned an increase in stored soil moisture was seen in cover crop treatments compared to standard no-till and conventional till treatments after the first major rainfall event after planting. Hoorman (2009) added that deep rooted cover crop such as *C. mucunoides* can improve rooting depth to attain subsoil moisture and water content is conserved by mulching the topsoil as soil compaction decreases and soil quality improves with time. Research by Jasa (2011) has shown that while a cover crop uses some soil moisture as it grows, it tends to use less water than is lost to evaporation from a bare soil surface.

Total organic carbon (TOC) increased significantly in a *C. mucunoides* establishment (Table 1). This may be due to addition of organic matter from cover crop biomass decomposition process. A similar study done by Ngome et al. (2011) observed that cover crop are known to increase soil organic carbon content by expanding biomass production for restoration and maintenance of soil productivity.

The application of *C. mucunoides* has increased both total N and available P in the soil (Table 1). The increased in soil total N indicates that it is most probably due to the capture of atmospheric $N_2$ by nitrogen-fixing bacteria found in the root nodules of *C. mucunoides* into the soil. When a legume cover crop is incorporated into the soil, a substantial amount of nitrogen is usually mineralized, converted from organic to plant-available forms within a few weeks (Ngome et al. 2011). Meanwhile, the increased in soil available P could be partly due to the presence of beneficial fungi known as mycorrhizae housed by the roots of leguminous cover crops which accumulates P. The filaments (hyphae) of these fungi effectively extend the root systems and help the cover crop tap more soil P (Ngome et al. 2011). Parameters such as soil nitrogen (N), total organic carbon (TOC) and available soil phosphorus (P) are major indicators of the productivity and sustainability of an agricultural production system (Kifuko et al. 2007).

Table 1: Effect of *C. mucunoides* on VWC, TOC, Total N and Available P in the soil

| **Treatment** | **VWC (%)** | **TOC (%)** | **Total N (%)** | **Available P (ppm)** |
|---|---|---|---|---|
| **C0** | 19.81 + 0.90[a] | 2.57 ± 0.09[a] | 0.26 ± 0.02[a] | 4.78 ± 0.67[a] |
| **C1** | 32.89 + 0.73[b] | 3.82 ± 0.11[b] | 0.44 ± 0.03[b] | 7.11 ± 1.05[b] |

Note: Means with different alphabets within column indicate significant difference between treatments by independent t-test at $p < 0.05$. Treatments are C0 – control and C1 – Calopo cover crop (mean ± S.D., n = 7).

### *P. nigrum* leaf total nitrogen, chlorophyll and gas exchange rate

Treatment C1 had a significantly higher leaf total N than in C0 could be due to higher availability of N in the soil (Table 2). Both field and laboratory investigations by Cechin and Fumis (2004) have demonstrated that increasing supply of N availability in soil may result in higher leaf N content. Furthermore, Tucker (2004) hypothesized that total N content in leaves depend on the N content in the soil.

Chlorophyll (SPAD) content had increased by 17 % in the *C. mucunoides* treatment when compared to the control and this result can be attributed to higher N concentration at the leaf cellular level (Table 2). Cabrera (2004) and Sulok et al. (2012a) observed that plants with higher nitrogen content were tend to have darker green leaves.

Photosynthesis rate (*A*) had increased by 27 % in the C1 treatment suggesting that *C. mucunoides* application increased the gas exchange rate of *P. nigrum*. In a similar study by Lawlor (2002) mentioned that the photosynthesis process that leads to increase in reproductive growth and yield is totally dependent upon the adequate supply of nitrogen and soil moisture. Hak et al. (1993) reported that up to 75% of leaf nitrogen is found in the chloroplasts, most of it invested in ribulose-biphosphate carboxylase alone. The process of photosynthesis takes place in the chloroplasts, specifically using chlorophyll, the green pigment involved in photosynthesis.

Leaf stomatal conductance (*gs*) had increased by 44 % in the *C. Mucunoides* treatment when compared to that of the control (Table 2). The result was parallel to that of Sulok et al. (2012b) where plant subjected to higher soil VWC tends to open its stomata. Table 2 also shows that transpiration rates (*E*) in the *C. mucunoides* treatment had increased by 55 % of the control value. Result for leaf transpiration rates (*E*) rate concurs to that of Ashizawa et al. (2003) in which they concluded that *E* rate was progressively increased under conditions of sufficient soil moisture. Additionally, Hoorman (2009) reported that due to the stored soil moisture in some cover crop treatments, the soil is able to provide adequate supply of water to plants thus negating the effects of lower water availability that inhibits stomatal openings that may led to lower *E* and *A* rates. Furthermore, Singh and Singh (2004) concluded that with more available soil water, plants are able to improve its nutrient uptake.

Table 2: Effect of *C. mucunoides* on leaf total N, Chlorophyll (SPAD) and selected gas exchange rate of *P. nigrum*

| Treatment | Leaf Total N (%) | Chlorophyll (SPAD) | *A* (Net Photosynthesis Rate ) ($\mu$mol $CO_2$ $m^{-2}s^{-1}$) | $g_s$ (Leaf Stomatal Conductance) (mol $H_2O$ $m^{-2}s^{-1}$) | *E* (Transpiration rate) (mmol $H_2O$ $m^{-2}s^{-1}$) |
|---|---|---|---|---|---|
| **C0** | 1.63 $\pm$ 0.06$^a$ | 33.87 $\pm$ 3.82$^a$ | 10.47 $\pm$ 0.65$^a$ | 0.09 $\pm$ 0.01$^a$ | 1.55 $\pm$ 0.1$^a$ |
| **C1** | 2.43 $\pm$ 0.12$^b$ | 40.60 $\pm$ 2.28$^b$ | 14.4 $\pm$ 1.4$^b$ | 0.16 $\pm$ 0.01$^b$ | 3.41 $\pm$ 0.17$^b$ |

Note: Means with different alphabets within column indicate significant difference between treatments by independent t-test at $p < 0.05$. Treatments are C0 – control and C1 – Calopo cover crop (mean $\pm$ S.D., n = 9).

Figure 1 shows the relationship between chlorophyll (SPAD) and leaf total N of *P. nigrum*. The strong relationship between chlorophyll and leaf total N regardless of treatments shows a polynomial cubic regression line of zero intercept with $r^2$ = 0.88 indicating that higher N content in the leaf increased chlorophyll in plants. Previous report by Tucker (2004) and Daughtry et al. (2000) revealed that because N is a structural element of chlorophyll, thereby it affects formation of chloroplasts and accumulation of chlorophyll in them.

Similarly, the relationship between leaf photosynthesis rate (*A*) and chlorophyll (SPAD) of *P. nigrum* exposed to different treatments were highly correlated, $r^2$ = 0.91 (Figure 2). The relationship between the two regardless of treatments was best described by a polynomial cubic regression line of zero intercept which explains a value of around 91 % of the variation in leaf photosynthesis. This consequence showed close relations between the two in which leaf *A* rate increased with increasing chlorophyll (SPAD) content. Ndukwe et al. (2011) revealed that the concentration of chlorophyll affects the rate of photosynthesis as they absorb the light energy without which the reactions cannot proceed. Consequently, higher rates of photosynthesis under conditions of higher nitrogen and soil water availability are often attributed to the formation of chlorophyll photosynthetic pigments and Rubisco activity (Toth et al. 2002).

## CONCLUSION

The establishment of the C1 plot using *C. mucunoides* as cover crop responded better in terms of its selected soil properties as well as leaf total N, chlorophyll content and gas exchange rate of *P. nigrum*. In the C1 treatment, the application of *C. mucunoides* affects the selected soil properties considerably by showing comparatively higher soil VWC, soil TOC, soil total N and soil available P. Leaf total N, chlorophyll content, photosynthetic rates (*A*), stomatal conductance ($g_s$), and transpiration rates (*E*) of *P. nigrum* grown in the Calopo cover crop treatment were significantly higher than the control. Furthermore, it was found that leaf total N and photosynthetic rate (*A*) was significantly correlated to chlorophyll content of *P. nigrum*. It can be said that the use of leguminous cover crops such as *C. mucunoides* can bring benefits such as fixing nitrogen and managing soil moisture. Therefore, incorporating *C. mucunoides* in black pepper vineyards can increase the photosynthetic rate of *P. nigrum* by improving soil VWC, soil TOC, soil total N and soil available P.

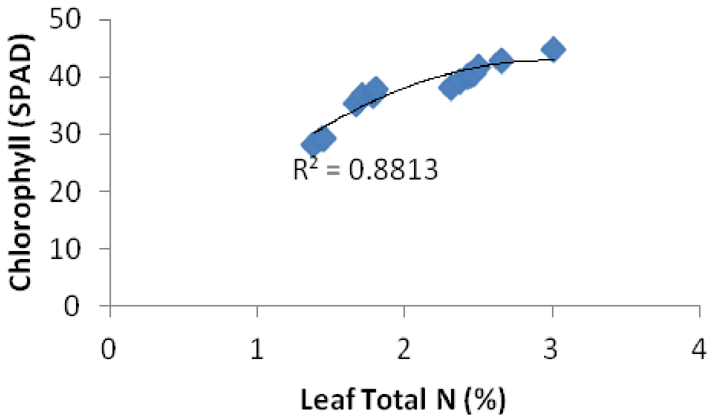


Figure 1: Relationship between chlorophyll (SPAD) and leaf total N of *P. nigrum* subjected to different treatments. Values are means ± s.e. of nine leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

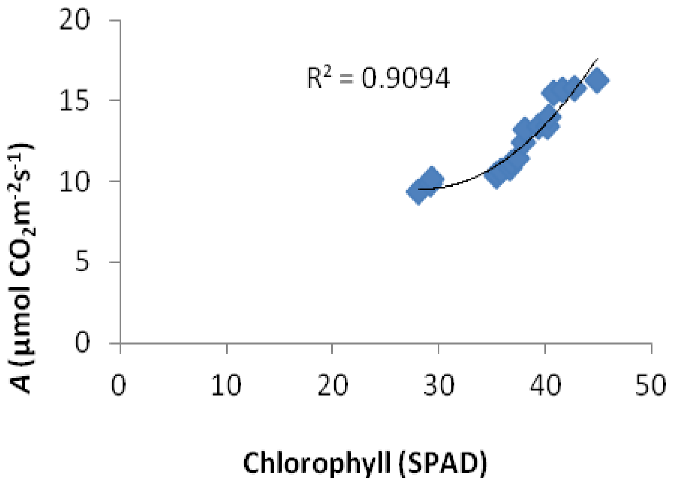


Figure 2: Relationship between leaf *A* rate and chlorophyll (SPAD) of *P. nigrum* subjected to different treatments. Values are means ± s.e. of nine leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

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