

Leaf growth and photosynthetic capacity as affected by leaf position, plant nutritional status and growth stage in *Dioscorea alata* L.

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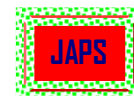
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Key words

Fertilization, growth stage, leaf position; SPAD-502 chlorophyll meter, photosynthetic capacity

1 SUMMARY:

Because nitrogen (N) deficiency can significantly decrease yam (*Dioscorea spp*) tuber yield, it is necessary to monitor and ensure adequate N availability. This study was undertaken (i) to identify optimal leaf position on the vine for the prediction of nitrogen response using the SPAD-502 Chlorophyll meter and (ii) to assess the effect of plant growth stage and its nutritional status on leaf photosynthetic capacity. The photosynthetic capacity of *D. alata* cv TDa 95/00010 leaves was estimated through the specific leaf nitrogen, leaf stomatal conductance and leaf carbon content under field conditions. Individual leaves from the apex to the base on the vine were monitored during vegetative growth stage and during tuber bulking phase under fertilization and non-fertilization conditions. Measurement of leaf N content, leaf expansion rate and SPAD readings was additionally conducted on individual leaves along the vine. The leaf area and SPAD readings increased with plant age while leaf N content decreased. Results indicated that leaves at different positions on the vine differed in photosynthetic capacity. Both young (below 4th position) and older (above 20th position) leaves had lower photosynthetic capacity than the intermediate mature leaves. The yam leaves reached their maximum photosynthetic capacity at between the 4th to the 7th position indicating the optimal leaf position for the prediction of nitrogen response using the SPAD-502 Chlorophyll meter. The photosynthetic performance of mature leaves was related to the plant growth stage and its nutritional status, being higher under fertilization and at tuber bulking phase. By contrast, nitrogen was remobilized for older leaves at this time under non-fertilisation conditions. The results suggest that tuber bulking phase is a critical yield-formation period in which changes in photosynthetic capacity of leaves due to nitrogen deficiency could compromise the yield.



2 INTRODUCTION

Nitrogen (N) is the most important mineral nutrient limiting the growth and the yield of non-leguminous crop plants that require relatively large quantities of this element, such as yam (*Dioscorea spp*) (Aduayi, 1979; Aduayi & Okpon, 1980). Since N deficiency can significantly decrease yam tuber yield (Aduayi, 1979), monitoring N available to ensure adequate availability to plants is necessary. The prediction of plant response to N has traditionally been mainly based on foliar diagnostic technique. This old technique requires destructive sampling of leaves and is time consuming (Samsone *et al.*, 2007). A tool for rapid and non-destructive plant analysis that aroused interest recently, is the hand-held chlorophyll meter SPAD-502 (Minolta – Japan), which evaluates leaf nitrogen status from measurements of chlorophyll content (Vos & Bom, 1993). The SPAD-502 chlorophyll meter has been already used to foliar N assessment in many other crops including potato and maize (Wu *et al.*, 1998; Chang & Robinson, 2003; Gianquinto *et al.*, 2003; Lopez-Bellido *et al.*, 2004; Wang *et al.*, 2004; Fontes & de Araujo, 2006; Scharft *et al.*, 2006).

Unfortunately, there is no information about the SPAD – 502 chlorophyll meter usage in yam crop. The little data available on this topic (Sotomayor-Ramirez, 2003) does not provide information on the effect of leaf position and age or the plant growth stage and fertilization level could affect the SPAD readings. No relationship between SPAD readings and leaf N content has yet been established in yam. As yam is a creeping plant,

it is absolutely necessary to determine at least the right leaf position on the vine in order to obtain accurate assessment of plant N status with regard to the plant growth stages.

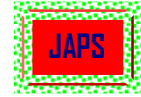
As the photosynthesis rate of the entire crop canopy depends on the photosynthesis of individual leaves; a sufficient supply of N must be available during the development of each leaf (Below, 2001). Leaf photosynthesis can also be influenced by many plant factors such as leaf position and age, sink effects, mutual shading, as well as environmental factors such as light, temperature, nutrition and water availability (Constable & Rawson, 1980; Bhagsari, 1988; Lieth & Pasian, 1990; Rodriguez-Montero, 1997; Aighewi & Ekanayake, 2004). According to these authors, the photosynthetic efficiency of the leaf decreases with age. Thus, photosynthetic efficiencies of individual leaves over their life span may vary with growth stage and nutritional status. There is therefore need to investigate and clarify these differences in yam.

This study aimed to identify the optimal leaf position on the vine for the prediction of nitrogen response using the SPAD-502 Chlorophyll meter and to assess the effect of leaf position and age on growth and photosynthetic capacity of leaves at different growth stages under fertilized and non-fertilized growth conditions. Information obtained in this study might be useful for the SPAD-502 chlorophyll meter calibration and for nitrogen fertilizer recommendations in yam.

3 MATERIALS AND METHODS

3.1 Planting material, site characteristics and cultural techniques: The experiment was conducted during the yam growing season from May to December 2007 in central Côte d'Ivoire at the field station of the Swiss Center for Scientific Research based in Bringakro (N 6°40' W 5°09'). The region is characterized by a transitional equatorial climate zone at the interface between a

moist semi-deciduous forest and a shrub savannah. An improved cultivar of *D. alata*, TDa 95/00010, selected by the International Institute of Tropical Agriculture, was grown in a mound on the savannah shrub side at a density of 1 plant m⁻². Soil characteristics on the first 0 – 20 cm soil layer were as follows: 5.3 g kg⁻¹ of clay content; the pH



measured in water was 5.2 and the average total C and N content were 5.7 and 0.7 g kg⁻¹, respectively.

The experiment was conducted in four replicates per treatment, and the plots were randomly assigned to the field. Each plot had 28 plants, the plot size being 4m x 7m. Treatment included no fertilizers (N0) and the dose of 160 kg N ha⁻¹, 180 kg K ha⁻¹, 10 kg P ha⁻¹ and 110 kg Ca ha⁻¹ (N1) were applied in two equal splits at the maximum growth of the aboveground organs (90 days after planting (DAP)) and during tuber bulking (130 DAP). This dose was based on nutrient exportation per tone of fresh tuber per hectare obtained by Diby (2005).

Head mother setts were used for planting. Setts were soaked in solutions of 600g DIAZINON® L⁻¹ (insecticide), 240g OXAMYL® L⁻¹ (nematicide) and 500 g of MANCOZEB® 80% (fungicide) for 15 to 30 minutes and air-dried the day before planting. During the growth cycle, the plots were kept weed-free through monthly manual weeding. Rain was available throughout the experimental period, with the recorded rainfall during growth period totalling to 1010 mm.

3.2 Data recording: A vine from two healthy plants with vigorous growth exposed to the sunlight was selected per plot at each sampling date. Measurements were carried out by counting the number of leaf node starting from the apical youngest open leaf (position 1) to the oldest one at the base of the vine. Data was collected during the vegetative growth stage (at 75 and 100 DAP) and at tuber bulking phase (130 and 160 DAP). For individual leaves, stomatal conductance was measured using the porometer AP4 (www.delta-t.co.uk; Delta-T, United Kingdom). At the same time and on the same individual leaves, two to six successive readings (depending on the leaf area) in SPAD units were taken using a SPAD (Minolta SPAD-502 chlorophyll meter) across the whole leaf area. The mean unit SPAD value (an index from 1 to 100) of the measurement per leaf area was

calculated using an internally programmed function of the instrument. Leaf stomatal conductance was measured before SPAD reading measurements to avoid stomata closure due to leaf manipulation. These measurements were done between 8 and 11 am at each sampling date. The mean value of leaf stomatal conductance and SPAD readings for all leaves of the vine exposed to the sun rays were also determined at each sampling date.

Immediately after measuring leaf stomatal conductance and taking SPAD readings, the lamina of leaves were harvested and their areas measured in the laboratory. In addition, single leaf samples were oven dried at 70°C, weighed, ground (separately for each leaf position), and leaf N and C content determined using CN analyser. The CN analyser indicated the leaf N and C content per unit dry weight. Multiplication of the N content by specific leaf weight (g m⁻²) for each leaf position resulted in the estimates of specific leaf nitrogen (Shiraiwa & Sinclair, 1993). The mean value of leaf C and N content and the specific leaf nitrogen for all leaves of the vine exposed to the sun rays were also determined at each sampling date.

In this study, the leaf nitrogen content expressed per unit leaf area (specific leaf nitrogen; SLN), the leaf stomatal conductance and leaf carbon content of individual leaves on the vine were used as indicators for individual leaf photosynthetic capacity.

3.3 Statistics and data analysis: Analysis of variance was performed using the general linear model in SAS *version 9.1* to test the effect of sampling date and fertilization on all parameters measured. Means were compared with the least significant difference (LSD) at P<0.05. To determine the mean value for all leaves on the vine, all data were average of multiple replicated measurements and the standard deviations were determined. Graphs were plotted using Origin 6.0 software (OriginLab, CO, USA).

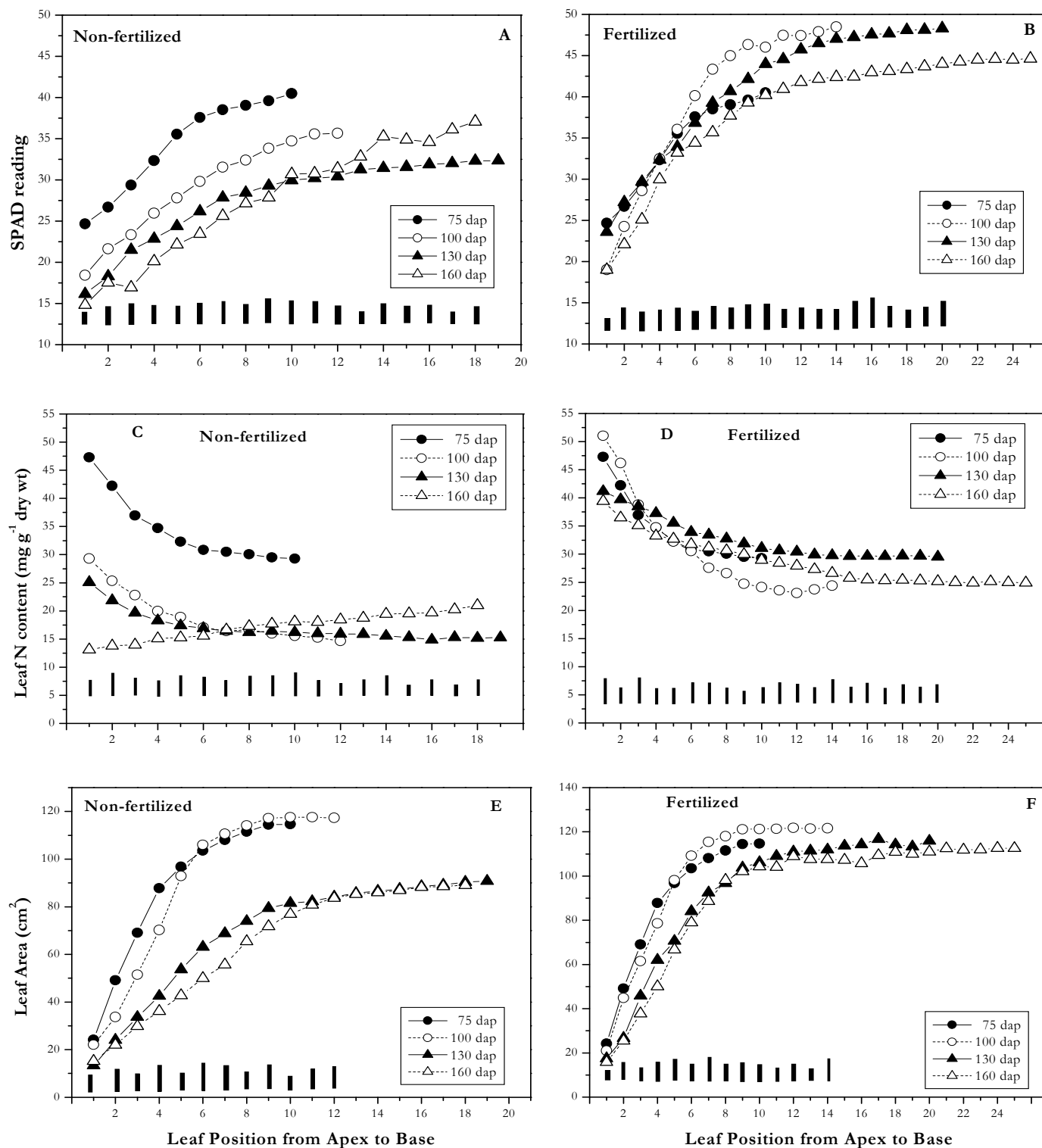


Figure 1: Effect of leaf position and growth stage on SPAD readings (A, B), leaf nitrogen content (C, D) and on leaf area (E, F) under fertilized and non-fertilized growth conditions. Bars are LSD at P<0.05 for comparison between sampling dates.

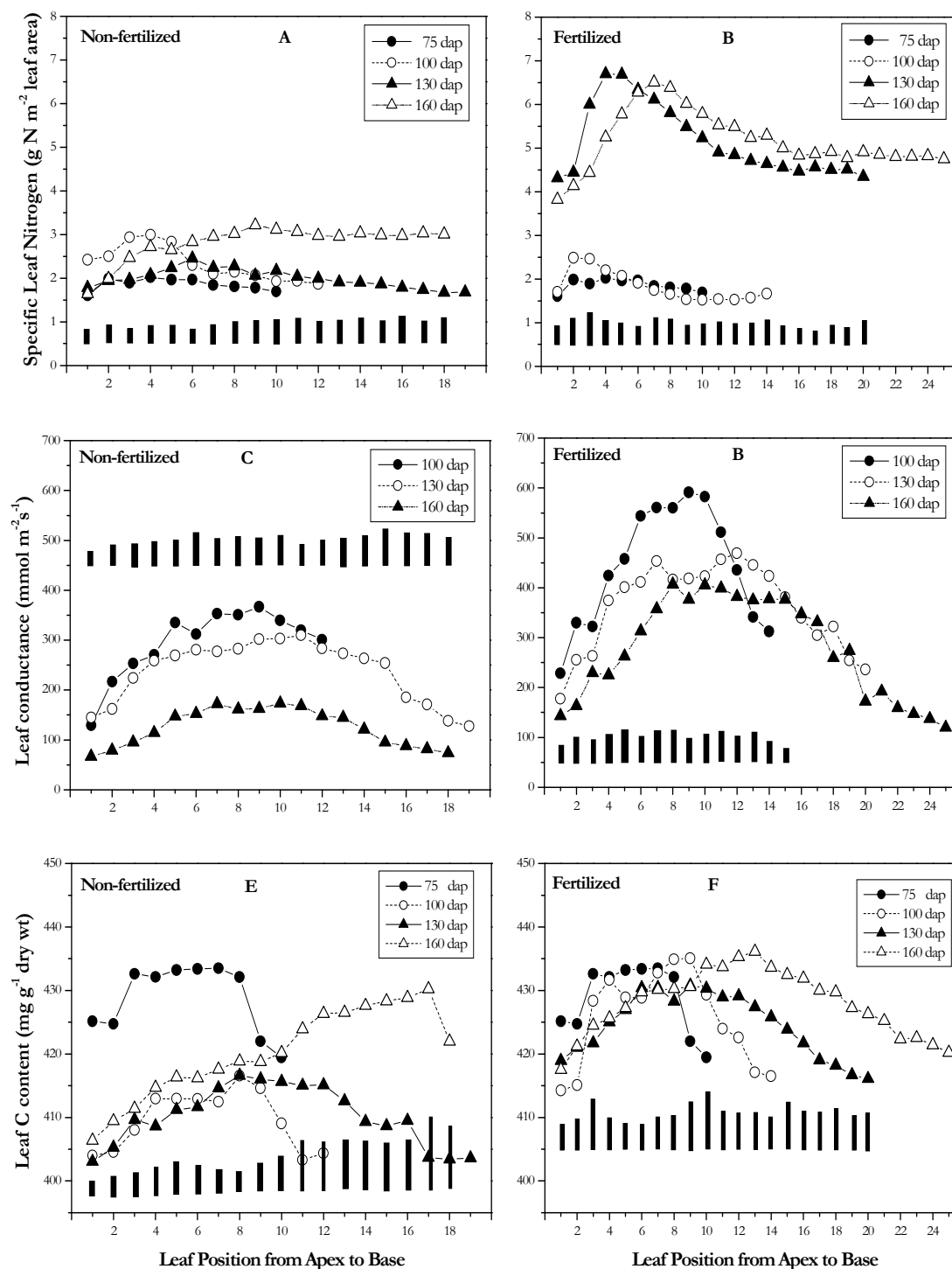
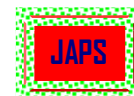


Figure 2: Effect of leaf position and growth stage on specific leaf nitrogen (A, B), leaf stomatal conductance (C, D) and on leaf carbon content (E, F) under fertilized and non-fertilized growth conditions. Bars are LSD at P<0.05 for comparison between sampling dates.



4 RESULTS

SPAD readings, leaf N content and leaf expansion rates were strongly affected by leaf position and fertilization level (Figure 1). The distribution pattern of these parameters along the vine had basically two main phases. SPAD readings (Figure 1 A, B) and leaf area (Figure 1 E, F) increased rapidly with leaf position until above position 8 and then tended to stabilise. The reverse trend was observed with leaf N content (Figure 1 C, D). The maximum value of leaf N content was observed in the youngest leaf at each sampling date and it varied between 40 and 55 mg N g⁻¹ under fertilization. These values were significantly higher than 12 – 30 mg N g⁻¹ observed under non fertilization conditions.

SPAD readings, leaf N content and leaf expansion rate varied significantly with the sampling date for all leaves on the vine under non fertilization but not under fertilization. SPAD reading decreased significantly from 75 to 100 DAP but afterwards no significant difference was observed between sampling dates (Figure 1A). Leaf N content decreased significantly from 75 to 100 DAP and then remained constant until 130 DAP. Thereafter, the leaf N content increased from the apex towards the base (Figure 1C). For leaf expansion rate, no significant difference was observed between 75 and 100 DAP. During the vegetative growth phase (75 and 100 DAP), the leaf area reached the maximum of 120 cm² in both fertilized and non-fertilized plots. While this maximum of 120 cm² remained fairly constant until the tuber bulking phase (at 130 and 160 DAP) under fertilization (Figure 1F), it decreased significantly to 80 cm² at 130 DAP and then remained constant up to 160 DAP under non-fertilization (Figure 1E).

The mean values of SPAD readings for all leaves on the vine and the corresponding values for leaf N content were observed at similar position on the portion from position 4 to 9, independent of fertilization (Table 1). These values were significantly higher under fertilization. At positions 5, 6, 7 and 8, the mean values of SPAD readings were 34.4, 29.2, 27.8 and 27.7, respectively. These values corresponded to 34.4, 19, 17 and 17.3 mg N g⁻¹ of leaf N content, respectively under non-fertilization at 75, 100, 130 and 160 DAP, respectively. Under fertilization, the mean value of

SPAD readings were 34.4, 39.4, 41 and 38.6 and the corresponding values for leaf N content were 34.4, 19, 17 and 17.3 mg N g⁻¹, respectively.

The specific leaf nitrogen (SLN) was not significantly affected by leaf position and sampling date under non-fertilization except at 160 DAP (Figure 2A). By contrast, the SLN which was similar between 75 and 100 DAP, increased significantly at 130 DAP and then remained constant up to 160 DAP under fertilization (Figure 2B). Furthermore, it was significantly affected by leaf position. Indeed, the SLN increased sharply from apical position to reach a peak at position 4 (at 130 DAP) or 7 (at 160 DAP) and then declined rapidly until position 15 at both 130 and 160 DAP. Afterwards, it remained fairly constant until the base of the vine. The table 2 indicates that at leaves at positions <4 and >18 had lower SLN than the mean value for all leaves on the vine at 130 DAP. These positions changed to <5 and >20 at 160 DAP.

In contrast to the SLN, the leaf stomatal conductance and leaf carbon content were significantly affected by the leaf position, independent of fertilization (Figure 2C, D). The pattern of both leaf stomatal conductance and leaf carbon content along the vine was characterized by three main phases. An increase phase from the apex following by a stable phase and then a decline phase was observed until the base of the vine. The effect of sampling date on leaf stomatal conductance differed between fertilization levels. Under non-fertilization, the leaf stomatal conductance was similar between 100 and 130 DAP but decreased significantly at 160 DAP (Figure 2C). By contrast, it decreased significantly from 100 to 130 DAP and then remained constant up to 160 DAP under fertilization (Figure 2D). Before position 8, the leaf carbon content was not significantly different between sampling dates, regardless of fertilization except at 75 DAP under non-fertilization (Figure 2E, F). Afterwards, the leaf carbon content increased significantly over time. The table 2 shows that leaves at positions <4 and >15 had lower stomatal conductance and lower carbon content than the mean value of these parameters for all leaves on the vine at 130 DAP. These positions changed to <5 and >19 at 160 DAP.

Table1: Corresponding position of the mean value of leaf N content, SPAD reading and leaf area for all leaves of the vine under fertilization and non-fertilization

DAP	Variables measured	Non fertilized		Fertilized	
		Mean value (\pm std)	Leaf Position	Mean value (\pm std)	Leaf Position
75	Leaf N content (mg g ⁻¹ dry wt)	34.36 \pm 0.46	4	33.26 \pm 0.76	4
	SPAD reading	34.40 \pm 0.60	5	35.10 \pm 0.30	5
	Leaf area (cm ²)	87.92 \pm 1.21	4	85.72 \pm 2.11	4
100	Leaf N content (mg g ⁻¹ dry wt)	19.00 \pm 1.41	5	30.82 \pm 1.16	5/6
	SPAD reading	29.20 \pm 0.34	6	39.40 \pm 0.41	6
	Leaf area (cm ²)	89.23 \pm 0.89	4/5	98.23 \pm 0.96	5
130	Leaf N content (mg g ⁻¹ dry wt)	17.04 \pm 0.29	5/6	32.70 \pm 0.92	8
	SPAD reading	27.80 \pm 0.26	7	41.00 \pm 0.32	8/9
	Leaf area (cm ²)	69.43 \pm 1.57	7	91.87 \pm 2.43	7
160	Leaf N content (mg g ⁻¹ dry wt)	17.32 \pm 0.82	8	28.70 \pm 0.69	10
	SPAD reading	27.70 \pm 1.54	8/9	38.60 \pm 0.42	8/9
	Leaf area (cm ²)	64.12 \pm 2.92	8	92.49 \pm 1.56	8

* Leaf position was numbered from the apex to the base on the vine. Data were the mean value \pm std.

Table 2: Corresponding position of the mean value of SLN, leaf net carbon content and leaf stomatal conductance for all leaves of the vine under fertilization and non-fertilization

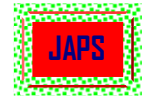
DAP	Variables measured	Non fertilized		Fertilized	
		Mean value (\pm std)	Leaf of Position*	Mean value (\pm std)	Leaf of Position*
75	SLN (g N m ² leaf area)	1.76 \pm 0.09	3/4	1.86 \pm 0.05	3/4
	Leaf net C content (mg g ⁻¹ dry wt)	429.13 \pm 1.99	4	428.83 \pm 2.32	4
100	SLN (g N m ² leaf area)	2.34 \pm 0.10	4	1.83 \pm 0.05	4/3
	Leaf net C content (mg g ⁻¹ dry wt)	409.65 \pm 3.39	3 - 10	425.68 \pm 2.66	4 - 10
	Leaf conductance (mmol m ⁻² s ⁻¹)	295.67 \pm 19.51	4/5	442.96 \pm 16.13	4/5
130	SLN (g N m ² leaf area)	1.98 \pm 0.05	4 - 17	5.15 \pm 0.11	4 - 18
	Leaf net C content (mg g ⁻¹ dry wt)	410.18 \pm 1.44	4 - 14	424.55 \pm 2.97	4 - 15
	Leaf conductance (mmol m ⁻² s ⁻¹)	237.32 \pm 8.12	4 - 15	361.34 \pm 10.15	4 - 15
160	SLN (g N m ² leaf area)	2.79 \pm 0.05	12	5.17 \pm 0.10	5 - 20
	Leaf C content (mg g ⁻¹ dry wt)	420.21 \pm 5.00	10	427.97 \pm 4.25	5 - 19
	Leaf conductance (mmol m ⁻² s ⁻¹)	124.69 \pm 8.74	4 - 14	277.24 \pm 13.08	5 - 19

* Leaf position was numbered from the apex to the base on the vine. Data were the mean value

5 DISCUSSION

Leaf area determines light interception, plant CO₂ fixation and photosynthesis (Liu & Stutzel, 2002), which influences dry matter production of plants. As leaf grows, a rapid growth phase is observed probably due to cell division whilst; a slow growth phase, observed after position 8, reflects cell expansion (Shenxi & Xianshi, 2003). Due to faster cell division and greater number of cells, a rapid growth phase may require N consumption to

support these intensive activities. An important determinant of leaf growth is the availability of nitrogen because the basic composition of leaves requires minimum nitrogen content (Sinclair, 1990). Leaf enzymes, especially those associated with the photosynthetic apparatus, seemingly impose such a minimum N requirement per unit of leaf mass (Sinclair, 1990). Lugg and Sinclair (1981) found that newly-formed soybean leaves contained 50 to 60



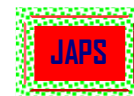
mg N g⁻¹; sorghum young leaves contained about 50 mg N g⁻¹ (Charles-Edwards, 1987) while young wheat leaves had between 40 and 50 mg N g⁻¹ (Karlen & Whitney, 1980). These values are similar to those determined in this study for *D. alata* ranging from 40 to 55 mg N g⁻¹ when fertilized, but were higher than our findings under non-fertilization conditions. The increase in leaf N content from the apex towards the base of the vine observed during tuber bulking phase under non-fertilization may suggest that N was remobilized to the leaves at the base of the vine or to the tuber (Aduayi, 1979). Similar result has been reported in *D. esculenta* (O'sullivan, 2008).

As leaf expansion rate increased with leaf position and age, biochemical changes in the production of fully developed chloroplasts, including synthesis of a variety of molecules and the total number of chloroplasts also increased (Constable & Rawson, 1980; Lieth & Pasian, 1990). Thus, SPAD reading increased sharply, since chlorophyll pigments are packaged in the chloroplasts. SPAD is used for rapid and non-destructive estimation of chlorophyll content in leaves. As several authors have shown, a positive relationship between chlorophyll and N contents in the leaves (Sexton & Carrol, 2002; Chang & Robinson, 2003; Wang *et al.*, 2004), chlorophyll contents can be used as an alternative estimation of plant N status (Chapman & Barreto, 1997). The high content of anthocyanine in the younger leaves, a characteristic of the yam cultivar used in this study, may also account for lower SPAD reading for younger leaves as it masked their greenness. As leaves grew over time, the anthocyanine content in the leaves decreased, resulting in increased SPAD reading, although leaf N content decreased. The slow growth phase observed at positions >8 seems to indicate that leaves had reached their full expansion as they accumulated little N (Below, 2001), and thus their chlorophyll content also tended to be fairly constant. This resulted in little change in SPAD reading values. As leaf N content has been found to give a better indication of the nitrogen nutrition of yam (Aduayi & Okpon, 1980), leaves from position 4 to 9 might be suitable for rapid N status assessment using the SPAD chlorophyll meter. Fertilization caused an increase in leaf N content and thus SPAD readings increased as already reported in previous studies (Chang & Robinson, 2003; Fontes & de Araujo, 2006; Lopez-

Bellido *et al.*, 2004; Scharft *et al.*, 2006). The mean value of SPAD readings ranged from 27.7 to 34.4 and from 34.4 to 41.0 under non-fertilization and fertilization, respectively. These values were higher than those reported by Sotomayor-Ramirez (2003) for *D. alata* cv Diamante ranging from 18.7 to 27. This difference may be due to difference between cultivars as varieties or cultivar characteristics affect SPAD readings (Chang & Robinson, 2003; Lopez-Bellido *et al.*, 2004).

In general, photosynthetic rates have mostly been measured on leaves when they are performing at maximum rates, rather than at average performance in the field, and have not been related to their age and position in the canopy or to the period of yield formation. Our results showed that leaves on yam vine performed differently for photosynthesis depending on the above factors. Thus, the vine could be divided into three portions. The first portion consists of the youngest leaves from position 1 to 4th with lower SLN, leaf C content and stomatal conductance which increases sharply with position and age. Young expanding leaves are characterized by low efficiency of photochemistry and photosynthesis, low capacity for both electron transports through photo-system II, low CO₂ fixation, high capacity for non-radiative thermal dissipation and high respiration rate (Greer & Halligan, 2001). The increase in photosynthetic capacity with leaf position on this portion may be explained by high N content of young leaves that have to build up all the necessary leaves components. According to Rodriguez-Montero (1997), the increase in photosynthetic capacity phase occurred during the first 10 days after leaf appearance in yam.

The second portion consists of mature leaves between positions 5 – 10th and 5 – 20th during the vegetative growth phase and at tuber bulking phase, respectively. The higher and stable stomatal conductance and carbon content observed for these leaves may indicate higher photosynthetic capacity on this portion. Körner *et al.* (1979) reported that C3-plant species with high maximum values of leaf stomatal conductance equally tend to show high photosynthetic capacity. Thus, leaves on this portion were probably the most productive leaves of the vine, with all resources focused on photosynthesis as the leaf C content was higher. Rodriguez-Montero (1997) reported that the most productive period of the yam leaf occurs between



10 to 30 days after leaf appearance. Diby (2005) working on the same cultivar as used in this study, has shown that both earliest emerging leaves (40 DAP) and later emerging leaves (90 DAP) took about 23 days to fully expand, and then remained active for approximately three and two months, respectively. The maximum photosynthetic capacity of individual leaves generally occurred in concert with leaves reaching full expansion (Shenxi & Xianshi, 2003; Aighewi & Ekanayake, 2004). Our data shows that in yam, leaves reached their maximum photosynthetic capacity at between positions 4 and 7 while the leaf area was increasing. The gradual decline in the SLN on the portion after position 4 or 7 was probably related to the remobilization of N to leaves at the base of the vine or to the tuber.

The third portion consists of older leaves at position >10 during the vegetative growth phase or positions >20 at tuber bulking phase. Leaves on this last portion had lower photosynthetic capacity since the SLN was lower and both leaf net carbon content and stomatal conductance declined gradually until the base of the vine. With ageing, from about 20 to 60 days, Bhagsari (1988) found that individual leaves' net photosynthesis for yam decreased by about 52%. Rodriguez-Montero (1997) observed that respiration rate increased again between 30 and 50 days. Reduction in photosynthetic rate as leaves age has been attributed to reduction in concentrations of enzymes involved in the various photosynthetic reactions, chloroplast membrane composition (Zima & Sestak, 1979) and stomata closure (Shenxi & Xianshi, 2003).

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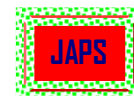
As the photosynthesis rate of the entire crop canopy depends on the photosynthesis of individual leaves, photosynthetic efficiencies of individual leaves over their lifespan may vary with growth stage and nutritional status. Perhaps, more important, the yam crop is characterized by a critical yield-formation period in which changes in leaf photosynthetic capacity could compromise the yield. Thus, once established, plants must also continue photosynthesizing during the tuber filling period to achieve high yield (Below, 2001). Where fertilizer was applied, plants could have lost water more easily as their stomatal conductance was higher and, thus, fixed more carbon for photosynthesis activity. This may increase cell division and hence the higher expansion rate and larger leaf area compared to the non-fertilized plants. Many studies have shown that in dicotyledonous plants, the impact of N supply on leaf growth is mostly due to increased cell growth rate and leaf water potential (Radin & Ackerson, 1981; Radin *et al.*, 1982). The photosynthetic capacity of individual leaves increased from the vegetative growth phase to the tuber bulking phase.

Conversely, insufficient N supply to crops results directly in a decrease in final leaf area of individual leaves (Muchow, 1988; Sinclair, 1990) due to its key role in cell division. If cell division is stopped, the leaf area decreases (Hai-Yan *et al.*, 2006). The important consequence of the inhibited leaf area growth resulting from inadequate leaf N is that the amount of solar radiation intercepted by a leaf canopy decreases, thereby decreasing the ability of crop canopy to assimilate carbon dioxide.

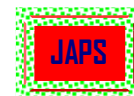
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