THE INFLUENCE OF NITROGEN APPLICATION ON THE GROWTH AND MINERAL CONTENT OF TWO AFRICAN NIGHTSHADE SPECIES (*SOLANUM SPP.*) CULTIVATED IN KENYA

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Abstract
Plant growth, leaf nitrogen and nitrate-N, and chemical content of two African nightshades, *Solanum villosum* and *S. sarrachoides* under different nitrogen levels was investigated in field experiments in 2001 and 2004. Plants were supplied with 0, 1.3, 2.6 and 5.2 g N/plant. Both African nightshade species responded similarly to nitrogen supply. Leaf area and dry matter production of plants supplied with nitrogen was 4-8 times that of plants not supplied with nitrogen. Plants supplied with nitrogen had significantly higher specific leaf area but had a lower leaf to stem ratio ($p \le 0.05$). Leaf nitrogen concentration was significantly higher ($p \le 0.05$) in plants supplied with nitrogen. However, when expressed on a leaf area basis, the differences in the leaf nitrogen content between nitrogen treatments were minimal. Leaf blade nitrate-N tended to be high in young plants especially those that were supplied with nitrogen. The species showed significant differences ($p \le 0.05$) in the mineral content. It was concluded that African nightshade responded to limited nitrogen by drastic reduction in leaf area to maintain the leaf nitrogen content.

Key words: Leaf nitrogen, leaf to stem ratio, mineral content, nitrates, *Solanum sarrachoides*, *Solanum villosum*

1.0 Introduction

African nightshades are grown and consumed as leafy vegetable crops in Kenya and various parts of Africa (Chweya and Eyzaguirre, 1999; Smith and Eyzaguirre, 2007). They are promising alternative cash crops considering their increasing availability in retail and supermarkets and have been recently ranked second to amaranth in priority among indigenous vegetables in Kenya (Agong and Masinde, 2006; Ndegwa et al., 2011). Limited agronomic technologies have been identified as a constraint facing African nightshade production (Ndegwa et al., 2011). The application of nitrogen to increase yield in leafy vegetables is a well-recognized practice. It is known that nitrogen deficiency exerts its effects on plant growth through reduced leaf area index and hence low light interception and low dry matter production (Jones, 1992). Also, the leaf nitrogen content correlates well with the leaf chlorophyll content, hence N deficiency leads to reduced photosynthesis resulting in lower biomass accumulation (Zhao et al., 2005). Under limited nitrogen supply, plants may respond by reducing leaf area and hence maintain the leaf nitrogen concentration as has been shown in potato (Solanum tuberosum L.) (Vos and van der Putten, 1998). Another strategy is to maintain a high leaf area, but adapt the leaf nitrogen concentration to nitrogen availability as demonstrated in maize (*Zea mays L.*) (Vos et al., 2005). A clear understanding of the response of African nightshades to limited nitrogen supply is vital in the efforts to integrate them into the mainstream agriculture. Growers may apply excessive N to realise high leaf yield and presumably high economic returns but this can lead to environmental contamination as well as nitrate accumulation in the vegetables, thereby posing health hazards to consumers (Gashaw and Mugwira, 1981; Wright and Davison, 1964).

Some agronomic studies to develop optimal cultivation practices for improved yield and nutritive quality of African nightshades have been conducted (Murage, 1990; Khan et al., 1995; Opiyo, 2004). These studies have shown that production of leafy Solanum spp. required high amounts of nitrogen application with recommendations of upto 5 g N/plant under field conditions. This study focuses on Solanum villosum Mill. subsp. miniatum (Bernh. ex Willd.) Edmonds and S. sarrachoides Sendtn. (Edmonds and Chweya, 1997; Schippers, 2000). The objective of this study was to evaluate the mechanisms of adaptation to nitrogen stress by these species and asses the influence of nitrogen application on the nutrient content of the two African nightshade species.

2.0 Materials and methods

2.1 Experimental site and plant materials

Field experiments were conducted in July - October 2001 and December 2003 - March 2004 at Jomo Kenyatta University of Agriculture and Technology (JKUAT) farm, Juja, Kenya (1525 m above sea level). The plant materials consisted of two African nightshade species; *Solanum sarrachoides* Sendtn. obtained from

Genebank of Kenya (GBK 028726) and also from IPK Genebank, Gatersleben, Germany (Sol 262/97) and S. villosum Mill. subsp. miniatum (Bernh. ex Willd.) Edmonds purchased from Kenya Seed Company, Nairobi, Kenya.

2.2 Experimental design and crop culture

The experiments were carried out as split plots in a completely randomised block design with three replications. The experimental factors consisted of 0, 2.6, 5.2 g N/plant in 2001 and 0, 1.3, 2.6 and 5.2 g N/plant in 2003, and two African nightshade species. The main plots comprised of the nitrogen levels, while the sub-plots consisted of the two species. The sub-plots, each measuring 2 m $\times$ 2.5 m were prepared by raising the soil about 15 cm above the ground. Seedlings were first raised in pots (10 cm diameter) in July 2001 and December 2003. The field was ploughed and harrowed and sub-plots raised about 15 cm were made to fine tilth. Soil samples were taken for nitrogen analysis using the Kejdahl method (Okalebo et al., 1992). Transplanting to the field was done in August 2001 and January 2004 at a spacing of 40 x 40 cm in 2001 and 30 x 30 cm in 2004. The crops were irrigated using sprinklers 2-3 times in a week depending on weather conditions. Nitrogen treatments consisting of Calcium Ammonium Nitrate (CAN, 26%N) were applied one week after transplanting using. The fertilizer was weighed and applied uniformly by hand along drills ensuring that each plant row had drills on either side. Phosphorus was applied at planting along the drills at levels of 92 kg P2O5/ha using triple superphosphate (TSP, 46% P2O5).

2.3 Harvesting

At transplanting time, some seedlings were excised to determine the initial plant size in terms of leaf area, plant height and shoot dry weight. Subsequent harvests were carried out 5-16 days interval to quantify plant size over time. Harvesting was ended when plants were flowering profusely. At each harvest, one plant in a central row from each plot was cut at the base and partitioned into leaf blade, petioles and stems. Plant leaf area was measured using a leaf area meter model AAM-8, Hayashi Denko Co. LTD., Tokyo for 2001 crop, and model 3100, LI-COR, Lincoln, Nebr. for 2004 crop. The shoot parts were dried at 100°C for 48 hours in the oven for dry weight measurement. Dried leaf blades were ground and used for nutrient analysis. Nitrogen content was analysed using the Kjeldahl method (Okalebo et al., 2002). Nitrate content was analysed using the colorimetric method while determination of Ca and Fe contents was done by dry ashing followed by use of atomic absorption spectrophotometer for Ca and Fe (Okalebo et al., 2002).

2.4 Data analyses

Statistical analysis was done using the GLM procedure of SAS (SAS, 1999). An ANOVA was executed with a split plot design for the field experiments for each date separately for the parameters; leaf area, dry weights, nitrogen and nitrate contents and mineral contents. Significance level was set at $P \leq 0.05$.

3.0 Results

3.1 Effect of nitrogen and species on growth of African nightshade

The soils nitrogen content at 0.10% N, which was low. The interactions between nitrogen applied and species were not significant. Nitrogen application significantly increased leaf area from the age of 16 and 15 days after transplanting in 2001 and 2004, respectively (Figure 1). In 2001, plants supplied with 2.6 and 5.2 g N/plant had similar leaf area in all the harvests except at 53 days after transplanting when the 5.2 g N/plant treatments plants had significantly higher leaf area. Similarly, in 2004 plants supplied with 5.2 g N/plant had significantly higher leaf areas followed by plants in the 1.3 and 2.6 g N/plant treatments, while plants in the 0 g N/plant had the lowest leaf area. Leaf area was generally higher in sarrachoides as compared to villosum but this was significant only at 53 days after transplanting in 2001 and beyond 15 days after transplanting in 2004. At the time of terminating the experiments, the leaf area of sarrachoides ranged 6029.83-10448.41 cm^2 while that of villosum ranged 3050.06-6930.74 cm^2.

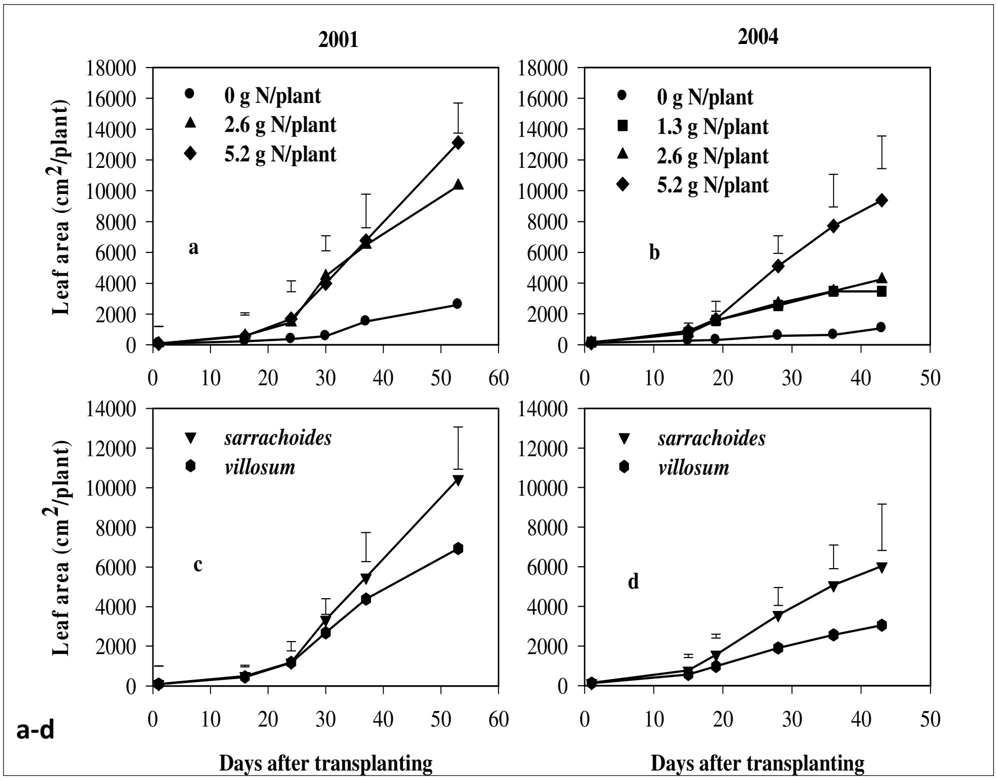

Figure 1(a-d): The leaf area as influenced by nitrogen rates (a, b) and species (c, d) for African nightshade grown at JKUAT farm in August-October 2001 and January-March 2004. Vertical bars show LSD0.05.

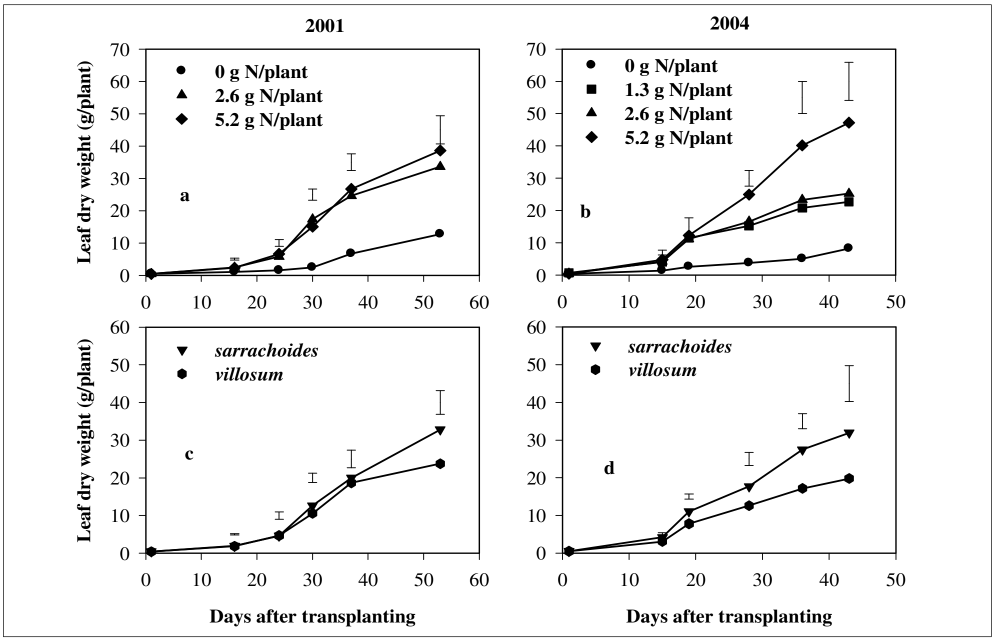

Figure 2(a-d): The leaf dry weight as influenced by nitrogen rates (a, b) and species (c, d) for African nightshade grown at JKUAT farm in August-October 2001 and January-March 2004 Vertical bars show LSD0.05

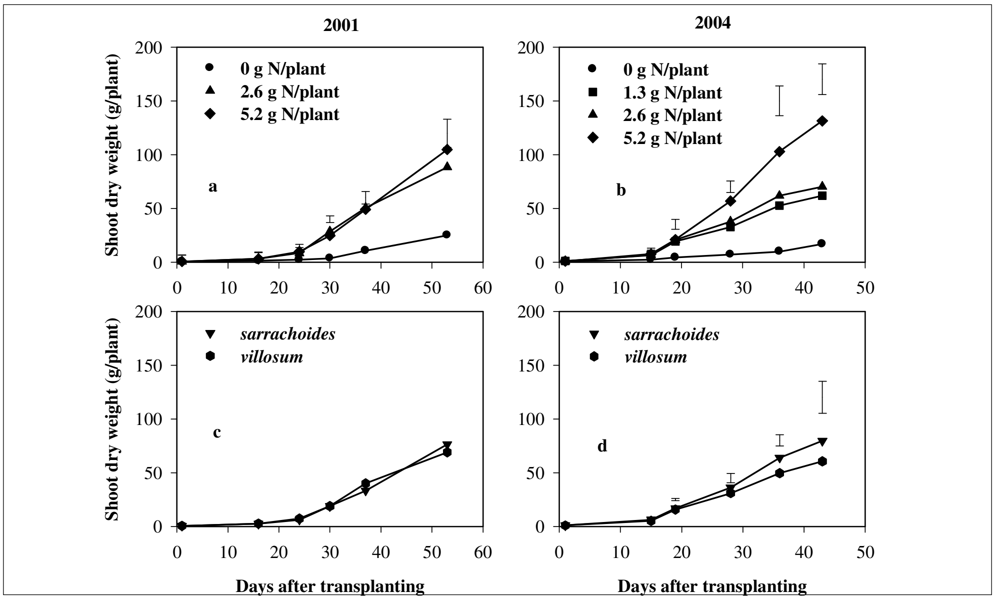

Figure 3(a-d): The shoot dry weight as influenced by nitrogen rates (a, b) and species (c, d) for African nightshade grown at JKUAT farm in August-October 2001 and January- March 2004
Vertical bars show LSD0.05

Leaf dry weights followed similar patterns as leaf areas. Nitrogen application gave significantly higher leaf dry weights from 16 and 15 days after transplanting in 2001 and 2004, respectively (Figure 2). In 2001, there was no difference in the leaf dry weight between plants supplied with 2.6 g and 5.2 g N/plant while in 2004, plants in the 5.2 g N/plant treatment gave consistently higher leaf dry weight. The influence of the species on leaf dry weight corresponded closely to that of leaf area with *S. sarrachoides* having significantly higher weights than *S. villosum* in older plants.

The influence of nitrogen application on shoot dry weight was similar to leaf area and leaf dry weight (Figure 3). On the other hand, the shoot dry weight was not significantly different between the two species except at 36 days after transplanting in 2004 when *S. sarrachoides* had higher shoot dry weight than *S. villosum*.

3.2 Effect of nitrogen and species on the specific leaf area (SLA) and dry matter partitioning of African nightshade

Plants supplied with nitrogen maintained a higher specific leaf area (SLA) in both species than plants, which did not receive any nitrogen, and this difference was significant in mature plants (Figure 4). In both years, treatment 5.2 g N/plant gave the highest SLA but this was significantly different from the other nitrogen treatments only at 53, and 28 to 43 days after transplanting in 2001 and 2004, respectively. *Solanum sarrachoides* had significantly higher SLA ranging 179.57-328.94 cm2/g compared to 156.56-264.53 cm2/g for *S. villosum* in the mature plants.

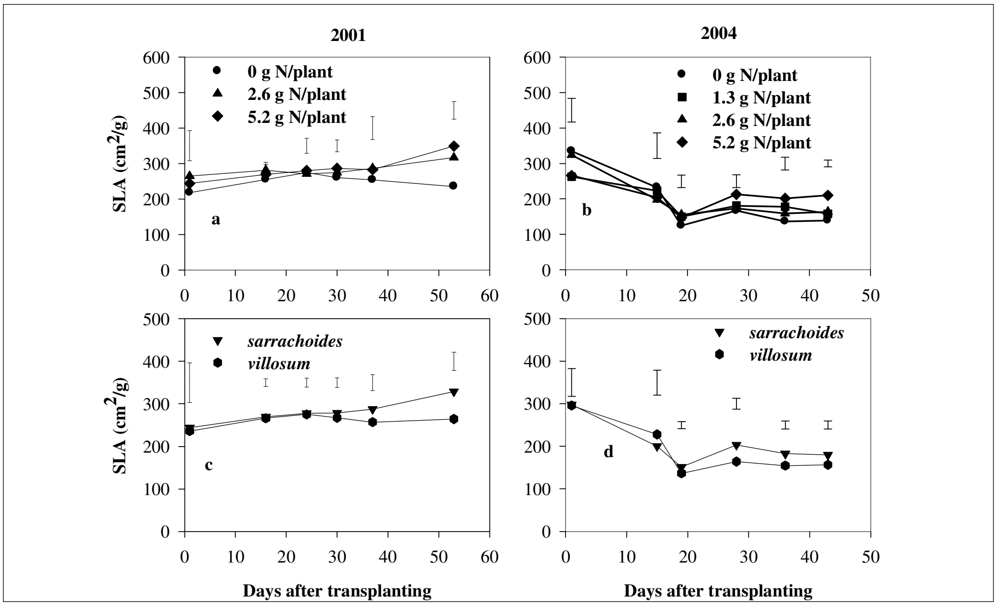

Figure. 4(a-d): The specific leaf area as influenced by nitrogen rates (a, b) and species (c, d) for African nightshade grown at JKUAT farm in August-October 2001 and January-March 2004. Vertical bars show LSD0.05

Leaf to stem ratios declined over time in both years. Plants supplied with nitrogen had significantly lower leaf to stem ratios from 22 and 28 days after transplanting in 2001 and 2004, respectively (Figure 5). The differences in the leaf to stem ratio between the nitrogen treatments 1.3 - 5.2 g N/plant were minimal. Solanum sarrachoides maintained a significantly higher leaf to stem ratio than S. villosum for all or most of the harvesting period in both years.

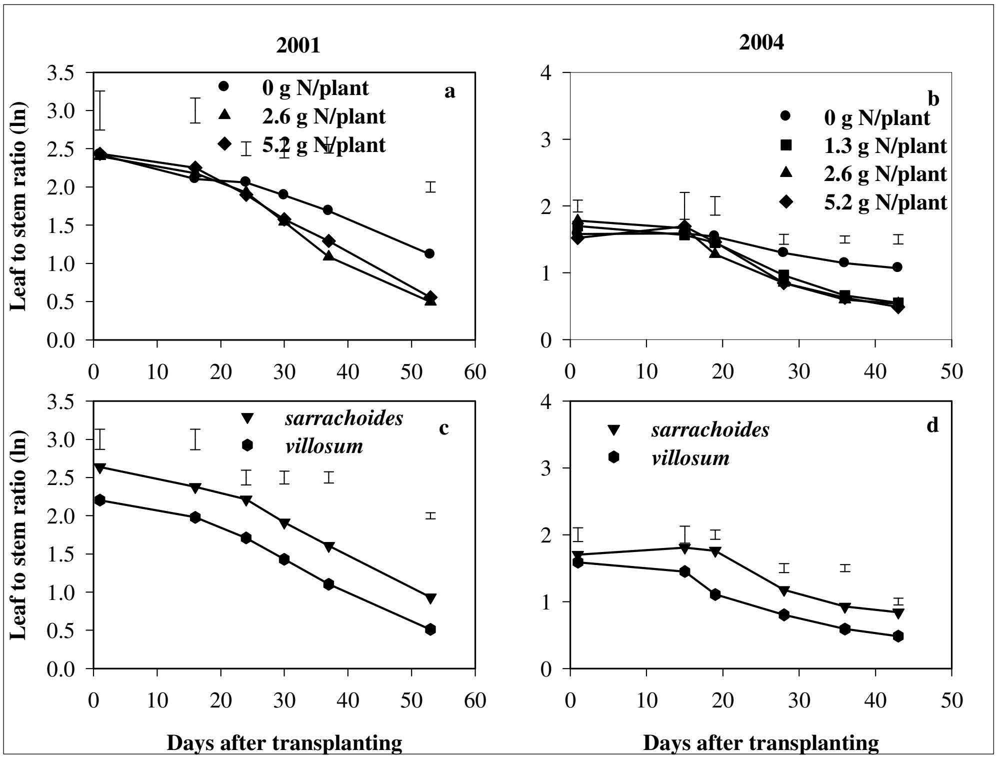

Figure. 5(a-d). Leaf (harvestable part, blade+petiole) to stem ratio in natural logarithims as influenced by nitrogen rates (a, b) and species (c, d) for African nightshade grown at JKUAT farm in August-October 2001 and January-March 2004. Vertical bars show LSD0.05.

The leaf blade to stem ratio in 2001 declined with increasing plant height (Figure 6). The power function fitted well to the data for S. sarrachoides only in 2001, and for *S. villosum* in both years. The two species had similar coefficients in 2001, while *S. villosum* had a significantly higher power coefficient in 2004 as compared to in 2001 (Table 1). Nitrogen treatments had no significant effect on the leaf blade to stem ratio as a function of plant height.

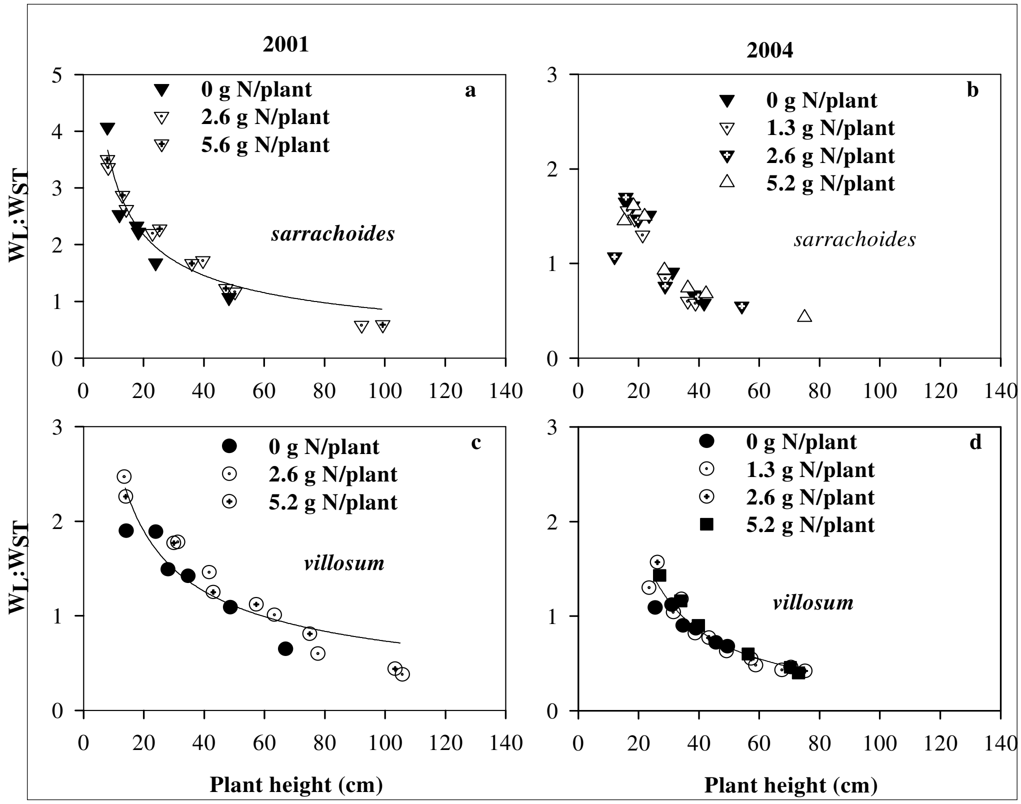

Figure. 6(a-d) The blade to stem (stem+petiole) ratio as functions of plant height for African nightshade species S. sarrachoides (a, b) and S. villosum (c, d) under different nitrogen levels grown at JKUAT farm in August-October 2001 and January-March 2004. Lines show power function $y=ax^{-b}$ where the coefficients are significant. The coefficients a and b are shown in Table 2.

Table 1: Coefficients and their 95 % confidence intervals for the blade to stem ratio as functions of plant height

Species	Year	a (95%CI)	b (95% CI)	R2
S. sarrachoides	2001	12.3 (7.1, 17.5)	-0.58 (-0.73, -0.42)	0.95
	2004	14.9 (-11.7, 41.5)	-0.80 (-1.39, -0.22)	n.s*
S. villosum	2001	11.1 (0.6, 21.5)	-0.59 (-0.88, -0.30)	0.99
	2004	45.9 (18.3, 73.6)	-1.08 (-1.25, -0.91)	0.99

3.3 Effect of nitrogen and species on the mineral contents of African nightshade leaf blades

The leaf blade nitrogen concentration was significantly higher in plants supplied with 5.2 g N/plant as compared to plants that received no nitrogen (Figure 7). Plants that received 2.6 g N/plant had intermediate leaf blade nitrogen concentration. The nitrogen concentration ranged 3.26-5.37%, 3.84-6.09% and 5.33-6.29% in control, 2.6, and 5.2 g N/plant treatments. Similarly, plants supplied with 5.2 g N/plant had higher leaf blade nitrate concentration, significant at 16 and 37 days after transplanting (Figure 7). The nitrate concentrations declined in more less the same ways as leaf blade nitrogen concentration, with the decline being highest in the 2.6 and 0 g N/plants treatments. For both leaf blade nitrogen and nitrate concentrations, there were no differences between the species.

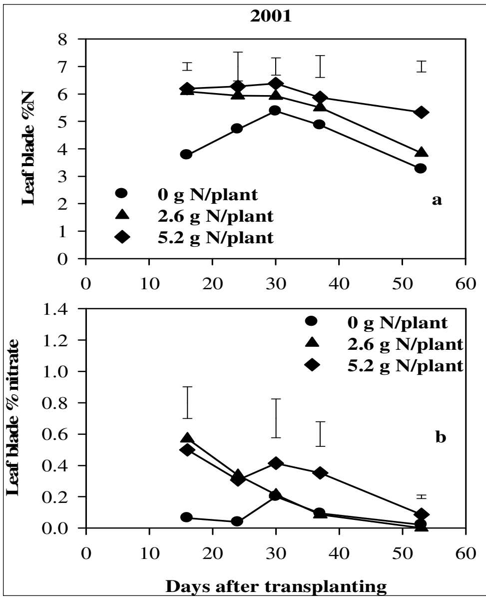

Figure 7: The leaf blade nitrogen (a) and nitrate (b) contents of African nightshade grown at JKUAT farm in July-October 2001 as influenced by nitrogen rates. Vertical bars show LSD0.05.

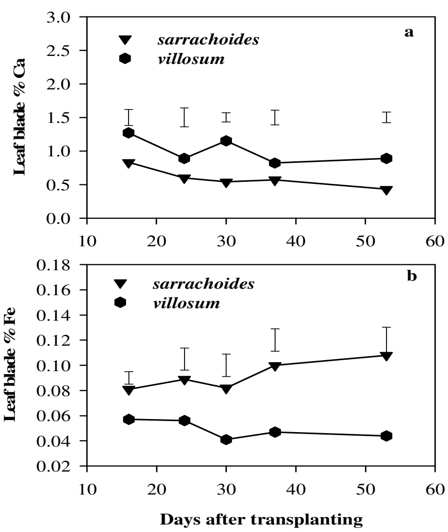

Figure 8: The leaf blade calcium (a) and iron (b) contents of African nightshade grown at the JKUAT farm between July-October 2001 as influenced by species.

Application of nitrogen had limited influence on the % Ca and % Fe content in leaf blades. Significant differences were observed only at 53 days after transplanting where plants supplied with 5.2 g N/plant had higher % Ca while those supplied with 0 and 2.6 g N/plant had higher % Fe (data not shown). On the other hand, *S. villosum* had significantly higher % Ca in leaf blades as compared to *S. sarrachoides* while *S. sarrachoides* had significantly higher % Fe as compared *to S. villosum* (Figure 8).

4.0 Discussion

The objective of this study was to evaluate the growth characteristics leaf nitrogen concentration and mineral nutrient content of *Solanum villosum and S. sarrachoides* under different nitrogen levels. In both years, the two species responded similarly to nitrogen supply as shown by lack of significant interactions between the species and nitrogen levels. Leaf area in mature plants was 5-8 times higher in plants supplied with 5.2 g N/plant than in plants that were not supplied with nitrogen. Potato, which has a similar strategy, was shown to vary the leaf area 4-fold between non-limiting and limiting nitrogen supplies (Vos and Van der Putten, 1998). On the other hand, maize, which does not have this response, increased leaf area only by 30% in plants supplied with non-limiting nitrogen rates as compared to those in the limiting rates (Vos *et al*., 2005).

Dry matter production response corresponded with that of the leaf area. Both leaf and shoot dry weights were 4-8 times higher in the plants supplied with non-limiting nitrogen then plants in the nitrogen limiting treatment. This can be attributed to the reuction in leaf area, which results in reduction in the cumulative light interception (Jones, 1992). It has been shown that in tomato (*Lycopersicon esculentum Mill.*), increasing nitrogen supply led to an increase in the accumulated absorbed photosynthetically active radiation through increase in leaf area index (Tei *et al*., 2002). Nitrogen supply has been shown to increase dry matter production in crops such as soybean (Glycine max (L.) Merr) (Taylor *et al*., 2005), sunflower (*Helianthus annuus* L. var. CATISSOL-01) (Cechin and de F`atima Fumis, 2004) and safflower (*Carthmus trinctorious L.*) (Dordas and Sioulas, 2009). The leaf area and leaf dry weight being higher in *S. sarrachoides* suggests that it would be a better leaf yielder as compared to *S. villosum*. Differences in the shoot dry weight between the two species were minimal or absent due to a bigger stem in *S. villosum.*

The specific leaf area (SLA) was higher in plants that were supplied with higher levels of nitrogen. This could be explained by the higher leaf area in these plants, which may imply a higher leaf expansion as well as leaf appearance. The low SLA in the African nightshades under nitrogen stress suggests that plants were restricting leaf area probably to maintain a high leaf nitrogen concentration. Meziane and Shipley (2001) working on 22 herbaceous species have shown that increased nutrient supply increased specific leaf area at low irradiance. Similarly, higher nutrient additions resulted in higher SLA in Arabidopsis thaliana (Bonser *et al*., 2010). A low SLA suggests thicker leaves as opposed to thinner leaves which have a high SLA. Whereas thicker leaves have a greater concentration of the photosynthetic apparatus per unit leaf area, broad thinner leaves can intercept more light (White and Consuelo Montes, 2005). The higher SLA in *S. sarrachoides* as compared to *S. villosum* especially in mature plants may suggest a higher light interception and photosynthetic activity in the former species. However, photosynthesis was not measured in this study. In addition, there was no clear evidence of differences in shoot dry matter production between the species.

The partitioning of dry matter in the two species was considered through the leaf to stem ratio and the leaf blade to stem ratio. The former is a measure of the harvest index, in which case the leaf consisted of the leaf blade and petiole, which comprise the harvestable part. The latter is an indication of partitioning of dry matter between the photosynthetic tissues (leaf blades) and the support structures (stems and petioles). It seems that application of nitrogen while increasing overall leaf growth, increases stem growth relatively more, hence the low leaf to stem ratios. The differences emerged at 22-28 days after transplanting and widened with time. In mature plants, the dry matter allocated to the leaves was about 60% of that allocated to stems in plants that were supplied with nitrogen. In comparison, mature plants that received no nitrogen supply had similar dry matter allocation between the leaves and stems. In this regard, nitrogen supply could be seen as having reduced the harvest index.

In the second consideration of dry matter partitioning, the leaf blade to stem ratio was fitted as a function of plant height. It is recognized that as the plant leaf area increases, a greater proportion of new dry matter needs to be invested in structural material (mainly stems) to maintain the plant's integrity and strength (Stützel *et al*., 1988). Thus, the leaf blade to stem ratio declines with increase in plant size in a power function. In this study, attempts were made to evaluate the effect of nitrogen application and species on the function. Both factors had no significant effect on the function. This suggests that the partitioning of new above-ground dry matter was directly dependent on plant size rather than nitrogen level or species.

Leaf nitrogen concentration is an important physiological parameter that indicates the plants nitrogen status. In this study, plants supplied with the highest nitrogen level that is 5.2 g N/plant maintained higher leaf nitrogen as compared to other treatments. Such a response has been reported in soybean (Taylor *et al*., 2005), and 22 herbaceous species and wheat (Sinclair *et al*., 2000; Meziane and Shipley, 2001). However, the leaf nitrogen concentration in plants supplied with no nitrogen ranged 61%-84% of the concentration in plants supplied with 5.2 g

N/plant. The differences therefore were much smaller than those in leaf area and dry matter production. Moreover, when the leaf nitrogen concentration was expressed per unit leaf area, the range of leaf nitrogen content in plants not supplied with nitrogen was 64%-95% of that in plants supplied with 5.2 g N/plant. This suggest that the African nightshade species under nitrogen stress tried to maintain a high leaf nitrogen concentration. This was probably through reduction in leaf growth. It has been shown that the maximum canopy photosynthesis in cauliflower, maize and rice was correlated to leaf nitrogen concentration (Vos *et al*., 2005).

Supplying nitrogen to African nightshade can increase the nitrate content in blades significantly. In this study, the equivalent NO-3 content in fresh blades using the observed moisture content of 88% ranged 2652-3029 mg/kg to 5-462 mg/kg in plants supplied with nitrogen, and 1063 mg/kg to 112 mg/kg in plants not supplied with nitrogen, in young to mature plants, respectively. It therefore appears that in the early vegetative stages, African nightshade can be classified together with crop species that accumulate moderate nitrates up to 2500 mg/kg such as endives (*Cichorium endivia* L.), leeks (*Allium ampeloprasum var porrum* (L.) J. Gay, parlsey (*Petroselinum crispum*) and rhubarb (*Rheum rhabarbarum*) (Hill 1991, Santamaria and Elia 1997). However, in cases where high nitrogen has been supplied, the young plants have high amounts of nitrates (above 2500 mg/kg), similar to high accumulating species like celery (*Apium graveolens* L.), lettuce (Lactuca sativa L.) and spinach (*Spnacia oleracea* L.) (Van Der Boon *et al*., 1990; Hill, 1991; Andersen and Nielsen, 1992; Behr and Wiebe, 1992; Martignon *et al*., 1994; Jaworska, 2005; Prasad and Chetty, 2008). Mature plants have relatively low nitrates in the leaf blades irrespective of whether nitrogen was supplied or not. The European Commission Regulation (EC) No 1881/2006 has established maximum levels (MLs) for nitrate in lettuces and spinach at 2500-3500 mg mg/kg for summer harvest and 3000-4500 mg/kg for winter harvest (Cited in Correia *et al*., 2010). Thus there is always the risk of accumulating nitrates more than the acceptable levels especially in young African nightshades, which have been supplied with high nitrogen levels. The differences in the mineral content between the two species means they can play complementary roles in meeting the consumers nutrient requirement. *Solanum sarrachoides* will be valued for its high iron content while *S. villosum* provides high calcium content.

5.0 Conclusion

The two species of African nightshade responded to limited nitrogen supply by drastic reduction in leaf area. This could be seen as a strategy to maintain a high leaf nitrogen content and therefore keep photosynthesis high. On the contrary, this led to a similar drastic reduction in dry matter production which could be attributed to reduced light interception. To avoid these reductions and keep the leaf yield high, it is recommended that that growers may need to supply up to 5.2 g

N/plant, although the risk of nitrate accumulation has also to be considered. Nitrate fertilizer levels that may lead to toxic nitrates levels need further investigation.

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