RESPONSE OF LEAF YIELD AND NUTRITIVE VALUE OF AFRICAN NIGHTSHADE (*Solanum villosum*) GENOTYPES TO NITROGEN APPLICATION

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ABSTRACT

The wild-type *Solanumu villosum* is an important leafy vegetable in Kenya, which however is low-yielding mainly due to the early flowering and prolific fruiting. Two new genotypes of *S.* v*illosum*, T-5 and octoploid with different flowering and fruiting characteristics from the wild-type have been developed. Field experiments were conducted to evaluate leaf yield, plant height and iron content of the genotypes under varying nitrogen levels. The experiments were laid out as split plots in a randomized complete block design with three replications. Nitrogen rates 0, 2.7 and 5.4 g/plant formed main plots while the wild-type, T-5 and octoploid genotypes formed sub-plots. Plants were cut at 5-10 days interval to quantify plant height, leaf area, fresh weight and iron content. The leaf fresh weight in octoploid plants was 1.3-1.6 times higher than in T-5 and wild-type plants. Applying nitrogen at 2.7-5.4 g N/plant increased leaf fresh weights by 1.5-2.3 times over the control plants. The octoploid plants tended to have fewer but larger leaves than the wild-type and T-5 plants. All the genotypes formed 6-7 leaves per unit increase in plant height irrespective of the nitrogen rate. The three genotypes had similar iron content, ranging from 5.5-16.5 mg/100 g dry weight across the nitrogen rates. Thus the octoploid is an improved variety over the wild-type parent. T-5 productivity potential lies in its capacity to give leaf yields for longer periods than the wild-type. The genotypes octoploid and T-5 are recommended for African nightshade production using 2.7 g N/plant for leaf yield and subject to further field trials and plant chemical analyses.

Key words: Iron content, Leaf fresh weight, Leaf number, Leaf size, Plant height, *Solanum* spp.

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INTRODUCTION

Solanum villosum Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds is among the widely consumed African nightshade species in Kenya and many parts of sub-Saharan Africa (Abukutsa-Onyango, 2007; Smith and Eyzaguirre, 2007). The readily available genotype of *S. villosum* in Kenya is largely wild-type that bears small leaves and has high incidence of flowering and fruiting. Breeding for large leaves, reduced flowering and fruiting is important for increased leaf vegetable production. Two new genotypes, the octoploid and T-5, have been developed from the wild-type *S.* v*illosum* (Ojiewo et al., 2006 a, b). The octoploid plants have larger stomata, fewer but larger leaves, and are late flowering as compared to the wild-type tetraploid (Ojiewo et al., 2006a; Masinde et al., 2007). Increase in ploidy level results in increased vigour and more growth of plants as reported in *Lolium* spp. (Sugiyama, 2005). However, in small burnet (*Sanguisorba minor* Scop.), tetraploids and octaploids had similar growth vigor, habit and yields (Peel et al., 2009).

T-5 is a novel, weather dependant male-sterile mutant, which is stamenless and infertile at day/night temperatures of 25°C/25°C and 30°C/20°C (Ojiewo et al., 2006b). Under warm conditions as occurs during the growing season in Kenya, the plants are expected to be male-sterile, bearing no fruits. This trait should enable the T-5 plants to invest more of its photosynthates into leaf production and hence the potential to give higher leaf yields than the wild-type plants (Ojiewo et al., 2007). Gonza´ lez-Real et al. (2008) has shown that in sweet pepper (*Capsicum annum* L. cv. Cornado), distribution of dry matter and N in the plant is preferentially diverted to the fruits and that this coincides with decreasing specific leaf weight as fruit load increases.

Studies have shown that nightshades require high amounts of nitrogen fertilizer (up to 5 g N/plant) for higher leaf yield and nutritive quality (Khan et al., 1995; Murage, 1990; Opiyo, 2004; van Averbeke et al., 2007). Nitrogen is important for plant growth partly due to its influence on leaf area index and consequently light interception (Jones, 1992) as has been shown in crops such as eggplant (*S. melongena* L.) (Rosati et al., 2001) and lucerne (*Medicago sativa* L.) (Lemaire et al., 2005). Lawlor (2002) and Ulukan (2008) have underscored the importance of nitrogen for vegetative growth in plants. Leaf growth is substantially affected by nitrogen and the response is more pronounced under increasing nitrogen application when nitrogen is limiting (Lawlor, 2002).

It is important to characterize the new *S. villosum* genotypes for leaf yields and nutritive quality under field conditions. This objective of this study was to evaluate leaf yield characteristics and nutrient content of the octoploid and T-5, compared to their parental wild-type *S. villosum*, under varying nitrogen fertilization rates. The hypotheses tested were that the new genotypes give higher leaf vegetable yields and have similar nutrient contents as the parental wild-type *S. villosum*.

MATERIALS AND METHODS

Experimental Design and Cultivation Details

Field experiments were conducted at Jomo Kenyatta University of Agriculture and Technology farm, Juja-Kenya during the warm season (December 2007 to March 2008) and cool season (April to August 2008). The farm lies at latitude 1°10′48′S, longitude 37°07′12′E and altitude 1525 m. The soils on the farm are well drained, moderately deep to deep dark brown, friable and gravely clay over petroplinthite and are classified as eutric cambisols (FAO/UNSECO, 1974; Muchena et al., 1978). However, amendments have been done including adding new soils to horizon A.

The plant materials consisted of wild-type, T-5 mutant, and the octoploid genotypes of *S. villosum* Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds. The experiments were laid out as split plots in randomized complete block design with three replications. Three nitrogen application rates of 0, 2.7 and 5.4 g/plant formed main plots, while the three genotypes were allocated to sub-plots. The sub-plots consisted of 15 cm raised beds of 1 m × 2.5 m. The main plots were separated using a polythene paper placed vertically to a depth of 50 cm. Seeds were sown in plastic trays filled with vermiculite on 12th December 2007 and 1st April 2008 for the two seasons, respectively. Emergence was observed after 4 days for all genotypes. After one week, the seedlings were transferred to 10 cm diameter plastic pots filled with soil. Transplanting was done in the field at a spacing of 50 cm between rows and 30 cm between plants on 12th January and 31st May 2008, for warm and cool seasons, respectively. Nitrogen rates were applied using calcium ammonium nitrate (CAN 27% N) in two equal splits, at two and four weeks after transplanting. Irrigation was done daily by hand to keep the soil moisture above 80% field capacity. This was ensured through periodic soil sampling and gravimetric soil moisture determination.

Determination of Leaf Yield and Plant Growth

At transplanting time, three seedlings of each genotype were cut and used to determine the fresh leaf weight (leaf yield). Harvests were done at 5-10 days interval. At each harvest, one plant from each sub-plot was cut at the base.

Leaves were removed and weighed immediately to avoid moisture loss. This was done early in the morning and all harvesting was finished within 1 hour to minimize variation in weight due to changes in environmental conditions.

In each subplot, two plants were tagged and used for plant height and leaf number measurements at 5-10 days-interval. Leaf size was determined by harvesting the 6^{th} leaf from the top and the largest leaf on a plant and measuring the leaf area using a leaf area meter (model 3100, LI-COR Inc., Lincoln, NE, USA) at 45 days after transplanting in the warm season. Photos of fruits were taken.

Determination of Iron Content

Iron content in the leaf blades was determined according to the procedure of Okalebo et al. (2002). A finely ground leaf sample of 0.3g was digested in a mixture of sulphuric acid, salicylic acid, hydrogen peroxide and selenium powder at $300^{o}C$ for at about 3 h. The digest was then aspirated into a flame from an air-acetylene mixture and detected with an atomic absorption spectrophotometer at a wavelength of 248.3nm.

The concentration of the iron in the dried sample expressed in mg/kg was calculated using the following formula:

$$Fe=\frac{(a-b)\times v\times f\times 1000}{1000\times w}$$

Where a = concentration of Fe in the solution, b = concentration of Fe in the mean values of the blanks, v = final volume of the digestion process, w = weight of the sample.

Data Analyses

Statistical analyses were done using the GLM procedures of SAS (SAS 1999). An ANOVA was done for each date separately for leaf area, dry weights, and specific leaf area and leaf nitrogen. Regressions were done using regression procedures of SAS.

RESULTS

Influence of Genotype and Nitrogen Application on Leaf Fresh Weight

There was no significant interaction between genotype and nitrogen application hence the factors are considered separately. The octoploid had significantly higher leaf fresh weight than the wild-type and T-5 genotypes in periods 24-43 days after transplanting (DAT) and 40-70 DAT in the warm and cool seasons, respectively (Figure 1A and B). T-5 and the wild-type had similar leaf fresh weights throughout the growing periods in both seasons. In the warm season, octoploid plants attained the highest fresh leaf weight of 312 g/plant compared to 197-211 g/plant for the wild-type and T-5 plants. These highest points were followed by reductions in leaf fresh weight at 54 DAT with the biggest decline in the octoploid plants and smallest in T-5 plants (Figure 1A). During the cool season, the highest fresh leaf weights attained were 309, 241 and 213 g/plant for the octoploid, T-5 and wild-type plants, respectively. Leaf fresh weight declined at 70 DAT in the octoploid and wild-type while it continued to increase in the T-5 plants (Figure 1B).

Nitrogen significantly increased leaf fresh weight irrespective of genotype in both warm and cool seasons (Figure 1C and D). Plants supplied with 2.7 and 5.4 g N/plant had similar but significantly higher leaf fresh weights compared to the control plants at 24-43 and 40-70 DAT in the warm and cool seasons, respectively. The highest leaf fresh weight for the plants supplied with 2.7-5.4 g N/plant was 279-315 and 279-310 g/plant compared to 138 and 186 g/plant for control plants in the warm and cool seasons, respectively.

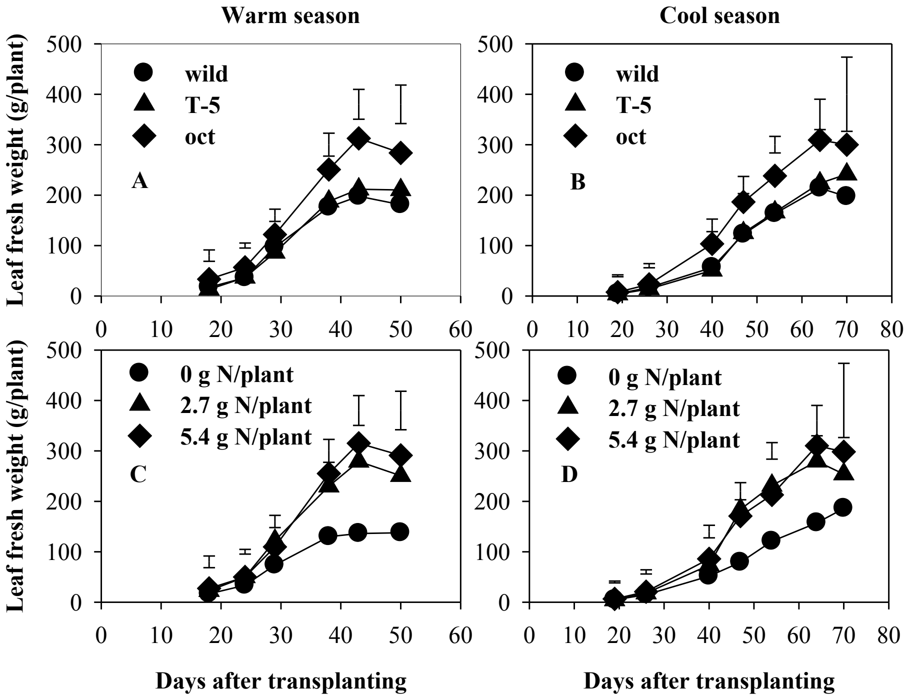

Figure 1. Leaf fresh weight for African nightshade as influenced by the genotype (A, B) and nitrogen rate (C, D) during the warm season, December 2007-February 2008 and cool season April-August 2008. Vertical bars show LSD.

Influence of Genotype and Nitrogen Application on Number of Leaves, Leaf and Fruit Sizes

In the warm season, the octoploid plants had significantly fewer leaves per plant at 10, 15, 22 and 26 DAT (Figure 2A). They also maintained fewer leaves per plant in the cool season though not significantly different from the wild-type and T-5 plants (Figure 2B). Nitrogen had no significant effect on the number of leaves in the warm season. In the cool season the effect was significant only at 47 DAT with plants supplied with 2.7 g N/plant giving the highest number of leaves per plant (Figure 2C and D). However, in both seasons, plants supplied with 2.7-5.4 g N/plant had higher number of leaves compared to control plants.

The sizes of the 6th leaf from the top of the plant and the largest leaf were significantly greater in the octoploid plants as compared to the wild-type and T-5 plants (Table 1; Photo 1A). Plants that received 5.4 g N/plant also had greater sizes of the 6th leaf and the largest leaf. The octoploid plants had relatively fewer but larger regular shaped fruits compared to the wild-type plants. T-5 plants had limited but irregular shaped fruits (Photo 1B).

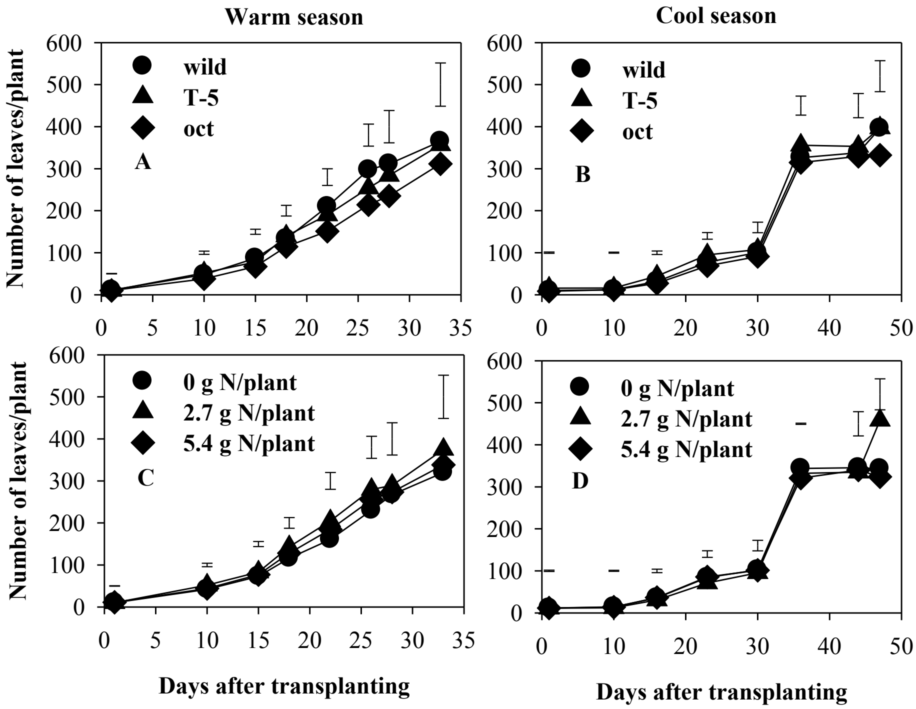

Figure 2. Leaf number for African nightshade as influenced by genotype (A, B) and nitrogen rate (C, D) during the warm season, December 2007-February 2008 and cool season April-August 2008. Vertical bars show LSD.

Table 1. Leaf area of the 6th leaf from the top of main stem, and largest leaf at 45 DAT for African nightshade as influenced by genotype and nitrogen rate during the warm season of December 2007-February 2008

	Leaf area (cm^2)	
Genotype	6th leaf	Largest leaf
Wild	23.96a	39.75a
T-5	24.11a	34.42a
Octoploid	41.52b	81.16b
$LSD_{0.05}$	5.49	4.84
Nitrogen (g N/plant)		
0	18.77a	36.18a
5.4	40.38b	67.38b
$LSD_{0.05}$	4.47	3.96

Means followed by the same letter down the column are not significantly different at P=0.05.

Photo. 1. Largest leaf (A) and fruit sizes and shapes (B) for African nightshade genotypes during the warm season, December 2007-February 2008 and cool season, April-August 2008

Influence of Genotype and Nitrogen Application on Plant Height

Plant height was not significantly different among the genotypes although the octoploid plants were generally shorter than the wild-type and T-5 plants in the warm season (Figure 3A). The same trend was observed in the cool season but in this case, the octoploid plants were significantly shorter than the wild-type plants at 30-47 DAT, while the T-5 plant were intermediate (Figure 3B). Increase in plant height due to nitrogen application was only significant at 46 DAT (Figure 3C). No such significant differences in plant height due to nitrogen rate were observed in the cool season (Figure 3D).

There was a positive linear relationship between number of leaves and plant height (R^2 = 0.94-0.98). The slope of the function, a measure of the increase in number of leaves per unit increase in plant height, was not significant and ranged from 5.8-7.1, 6.2-7.2 and 6.1-6.6 for the octoploid, T-5 and wild-type plants, respectively (Figure 4).

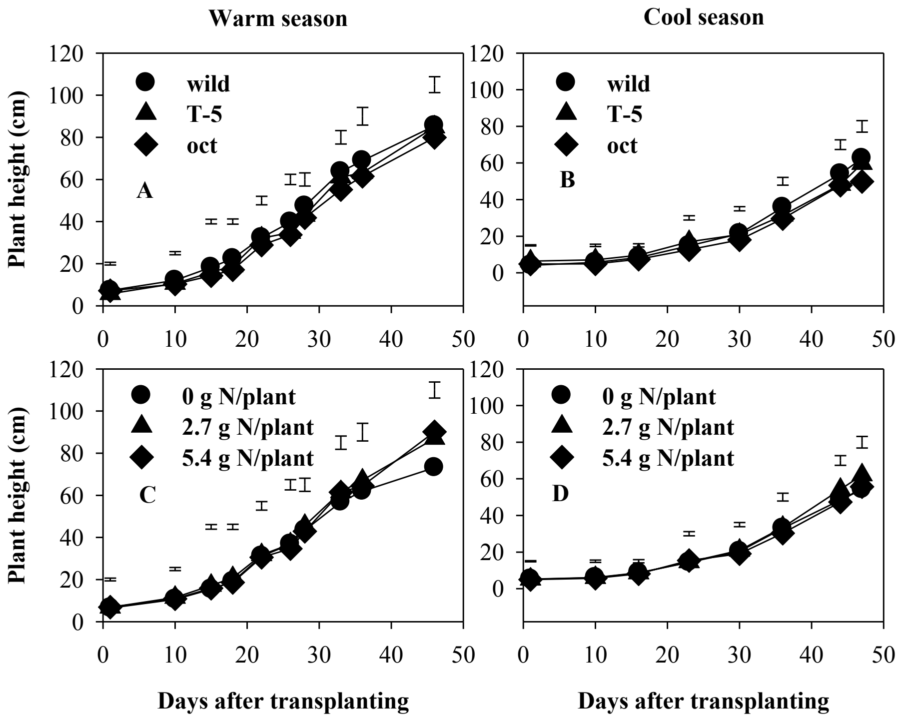

Figure 3. Plant height for African nightshade as influenced by genotype (A, B) and nitrogen rate (C, D) during the warm season, December 2007-February 2008 and cool season, April-August 2008. Vertical bars show LSD.

Influence of Genotype and Nitrogen on Iron Content in Leaf Blades

The iron content in leaf blades was not significantly different among the genotypes in both the warm and cool seasons (Table 2). Generally, the iron content ranged from 6.4-14.0, 6.4-12.9 and 5.5-16.5 mg/100 g dry weight for the octoploid, T-5 and wild-type plants, respectively. Considering nitrogen application, control plants had significantly higher iron content at 24 and 29 DAT in the warm season, while the opposite was true at 40 DAT in the cool season (Table 2).

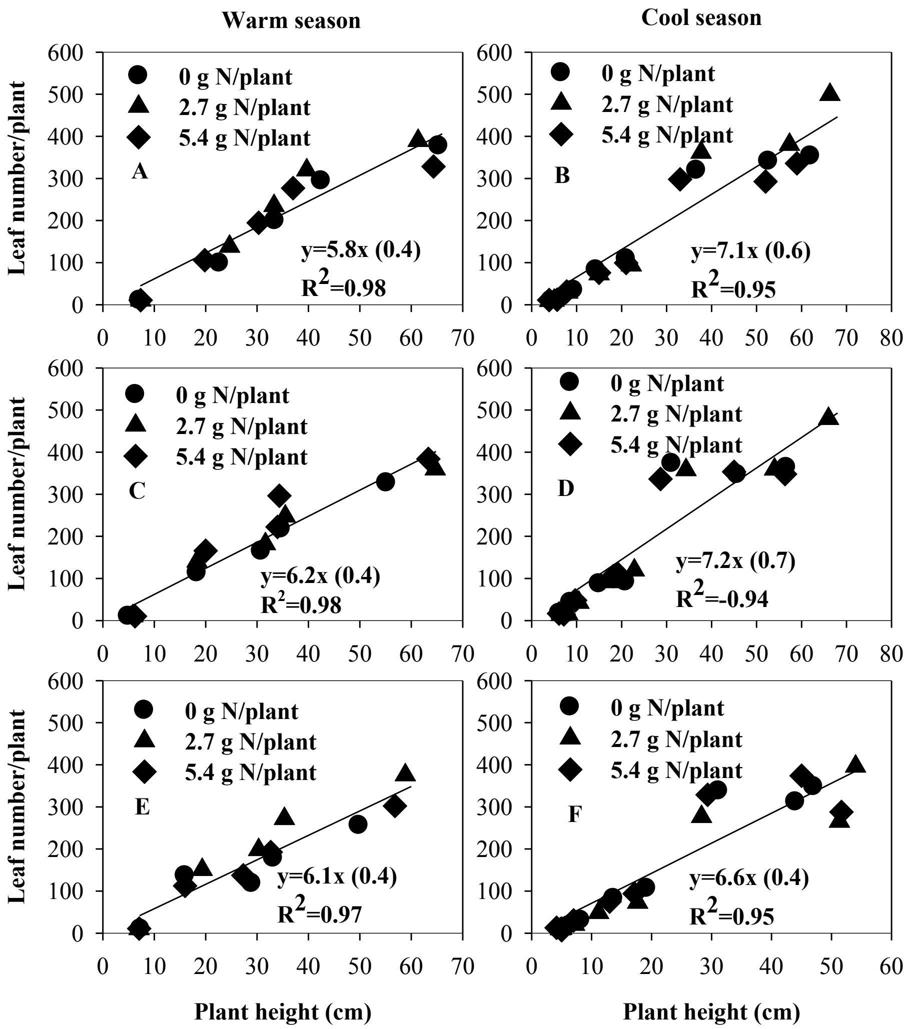

Figure 4. Relationship between leaf number and plant height for the octoploid (A, B), T-5 (C, D) and wild (D, E) African nightshade genotypes grown under varying nitrogen rates during the warm season, December 2007-February 2008 and cool season April-August 2008. Linear regression equations are given in the figures. Values in parenthesis show the SE of the slope.

Table 2. Iron content in leaf blades of African nightshade as influenced by the genotype and nitrogen rates during the warm season, December 2007-February 2008 and cool season, April-August 2008

	Iron (mg/100 g dry weight)									
Treatment	DAT[1] (warm season)					DAT[1] (cool season)				
Variety	18	24	29	38	43	19	40	47	54	64
Wild	10.7	5.6	11.5	11.0	9.2	5.5	5.8	9.0	5.7	16.5
T-5	11.1	12.4	12.1	9.1	10.7	5.6	6.5	7.1	6.4	12.9
Oct[2]	10.6	13.0	10.8	10.3	11.5	5.7	6.4	7.4	6.4	14.0
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N (g/plant)										
0	10.4	18.4a	13.6a	9.9	7.8	5.2	5.0a	8.0	5.9	14.1
2.7	11.0	7.5b	10.9b	9.6	9.8	6.0	6.6ab	8.6	6.9	14.8
5.4	10.7	8.3b	10.1b	11.0	14.3	5.6	7.1b	6.8	5.7	14.4
$LSD_{0.05}$	ns	5.7	2.7	ns	ns	ns	0.9	ns	ns	ns

Means followed by the same letter down the column are not significantly different at P=0.05

DISCUSSION

The octoploid and T-5 genotypes were developed with the objective of increasing leaf yields (Ojiewo et al., 2007). Leaf yield in African nightshades can be considered in terms of fresh leaf weight as well as leaf number. Lack of significant interactions indicated that the genotypes responded similarly to nitrogen application in terms of fresh leaf weight, leaf number and iron content. Generally, the leaf yield in octoploid plants was 1.3-1.6 times higher than in the wild-type and T-5 plants (Figure 1A, B). Applying nitrogen at 2.7-5.4 g N/plant increased leaf fresh weights by 1.5-2.3 times the control plants. Applying more than 2.7 g N/plant did not significantly increase leaf yield. van Averbeke et al. (2007) reported that total aboveground fresh weight increased with increasing N application rates in *S. retroflexum* until the rate of 2.66 g N/ha. Fresh leaf yields of up to 2.7 fold were reported in spiderplant (*Cleome gynandra* L.) plants supplied with 20.8 kg N/ha compared to control plants (Mauyo et al., 2008). Nitrogen is important for plant growth as it influences leaf area index and consequently light interception (Jones, 1992; Grindlay, 1997). This explains why nitrogen fertilization leads to increased leaf yields.

Fewer and larger leaves in the octoploid (Figure 2A, B; Table 1, Photo 1) suggest a trade-off between leaf size and number. At a given leaf biomass, plants may have fewer, large or many, small leaves, depending on environmental conditions. Kleiman and Aarssen (2007) found a negative isometric relationship between leaf size and number in trees. Large size of leaves for the octoploid plants may mean better quality for some consumers, which may endear the genotype to the market. T-5 plants were similar to the wild-type parent in terms of plant height, number of leaves, size of leaves and leaf fresh weight. However, T-5 plants had irregular shaped fruits and limited fruiting, which could explain why its leaf fresh weights did not decline at the same time with those of the octoploid and wild-type plants.

Genotypes had different plant heights (Figure 3.) but in all of them, there was a strong relationship between leaf number and plant height. The slope of the function, which represented the increase in number of leaves per unit increase in plant height, was not significantly different among the genotypes and nitrogen rates (Figure 4). All the genotypes formed 6-7 leaves per unit increase in plant height. Developing simple empirical relationships between plant height and number of leaves could help predict leaf yield in African nightshade. Plant height can be easily measured as compared to counting the number of leaves. A similar significant linear relationship between number of leaves and plant height has been reported in *Impatiens pallida*, in which plants formed about 2 leaves per unit increase in plant height under uncrowded conditions (Berntson and Weiner, 1991).

The three genotypes had similar iron content in leaf blades, ranging from 5.5-16.5 mg/100 g DW (Table 2) across the nitrogen rates suggesting equal genetic capacity to accumulate iron. Moderately high levels of iron ranging from 66-80 mg/100 g DW have been reported in various genotypes of *S. nigrum* (Sheela et al., 2004; Akubugwo et al., 2007). On the other hand, lower levels of iron of 2.5 mg/100 g DW have been reported in *S. nigrum* leaves in Tanzania (Lyimo et al., 2003).

CONCLUSION AND RECOMMENDATIONS

The octoploid is an improved variety over the wild-type parent considering its higher leaf fresh weight and larger leaf sizes in addition to similar characteristics to the parent in terms of plant height, number of leaves, number of leaves increment per unit plant height increase, and leaf blade iron content. The genotype T-5 is better than the wild-type parent in its potential to give leaf yields for a longer period. These two are recommended for production subject to further trials and plant chemical analyses. They responded to nitrogen application in the same way as the wild-type parent and they will need no more than 2.7 g N/plant to give better leaf yields.

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