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Plant Growth and Leaf N Content of *Solanum villosum* Genotypes in Response to Nitrogen Supply

Peter Wafula Masinde[1*] • **John Mwibanda Wesonga**[1] • **Christopher Ochieng Ojiewo**[2] • **Stephen Gaya Agong**[3] • **Masaharu Masuda**[4]

[1] Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000, code 00200 Nairobi, Kenya
[2] AVRDC, The World Vegetable Center, Regional Center for Africa, P. O. Box 10, Duluti, Arusha, Tanzania
[3] Maseno University, P.O. Private Bag, Maseno, Kenya
[4] Graduate School of Natural Science and Technology, Okayama University, 1-1-1 Tsushima Naka Okayama, 700-8530, Japan

Corresponding author: * **masinde_peter@yahoo.co.uk**

ABSTRACT

Solanum villosum is an important leafy vegetable in Kenya whose production faces low yields. Two potentially high leaf-yielding genotypes of *S. villosum*, T-5 and an octoploid have been developed. Field experiments were conducted at Jomo Kenyatta University of Agriculture and Technology to evaluate the vegetative and reproductive growth characteristics and leaf nitrogen of the genotypes under varying N levels. The experiments were carried out as split plots in a randomized complete block design with three replications. Nitrogen supply levels of 0, 2.7 and 5.4 g N/plant formed the main plots while the T-5, octoploid and the wild-type genotypes were allocated to the sub-plots. Periodic harvests were done at 5-10 days interval to quantify growth and leaf N. The octoploid plants had up to 30-50% more leaf area and up to 35-50% more leaf dry weight compared to wild-type plants. However, all the genotypes had similar shoot dry weight. The wild-type genotype had about 2-4 times higher flower/fruit dry weight as compared to the octoploid and T-5 genotypes. All the genotypes responded to N supply similarly by increasing leaf area and shoot dry weight 1.7-2.5 times. Leaf N on a dry weight basis was significantly higher in plants supplied with N, but these differences were not observed when the leaf N was expressed on leaf area basis. In conclusion, the *S. villosum* genotypes responded similarly to N limitation by drastic reduction in leaf area and dry matter production and maintaining the leaf N content on leaf area basis. The octoploid is a suitable candidate for increasing leaf yield of *S. villosum*.

Keywords: flower load, fruit load, leaf area, leaf dry weight, leaf yield, shoot dry weight

INTRODUCTION

Solanum villosum Mill. Subsp. *miniatum* (Bernh. ex Willd.) Edmonds is a key African nightshade grown and consumed as a leafy vegetable in Kenya and various parts of Africa (Edmonds and Chweya 1997; Chweya and Eyzaguirre 1999; Schippers 2000). African nightshades belong to traditional or indigenous vegetables whose consumption and value in Kenya is on the increase (ERA 2008). They can therefore be considered as new cash crops. Field and greenhouse studies have shown that the nightshades require high amounts of N application with recommendations of 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; Grindlay 1997). The main effect of N fertilization is an increase in leaf area index leading to increased light interception and dry matter production. In crops such as eggplant (*Solanum melongena* L.) (Rosati *et al*. 2001), lettuce (*Lactuca sativa* L. 'Vegas') (de Pinheiro Henriques and Marcelis 2000) and lucerne (*Medicago sativa* L.) (Lemaire *et al*. 2005), increasing the N supply has been shown to increase the leaf area index, light interception and dry matter production. Yin *et al*. (2003) presented quantitative relationships between leaf area index and canopy N content ranging from a logarithmic model in a fully developed canopy to a linear model in a young canopy for various crop species. In lucerne, a linear relationship has been shown between shoot N content and leaf area irrespective of the growing conditions (Lemaire *et al*. 2005). In their review papers, Lawlor (2002) and Ulukan (2008) have underscored the importance of N for vegetative growth in plants. Leaf growth is substantially affected by N and the response is more pronounced under increasing N supply when N is limiting (Lawlor 2002).

Leaf N concentration is an important physiological parameter that indicates the plant N status. This could be measured through N content on a dry weight basis or on a leaf area basis. SPAD measurements, which provide an estimate of chlorophyll content, could also be used as an indicator of N nutrition index (NNI), determined as the ratio between the actual crop N uptake and the critical N uptake indicating the minimum N content required for the maximum biomass production of a canopy (Lemaire *et al*. 2008; Mistele and Schmidhalter 2008). A good correlation has been shown between chlorophyll content and leaf N. However, this relationship is influenced by environmental conditions and genotypes (Lemaire *et al*. 2008). Demotes-Mainard *et al*. (2008) have shown that the SPAD values were positively correlated with leaf N on dry weight basis for ornamental woody species *Lagerstroemia indica*, and *Callicarpa bodinieri*, but not for *Viburnum tinus*. Muthuri *et al*. (2009) developed polynomial relationships between SPAD values and chlorophyll content as well as foliar N for maize (*Zea mays* L.). SPAD readings could be used for N management. The leaf N content correlates well with the leaf chlorophyll content, hence a low leaf N content as occurs during N deficiency leads to reduced photosynthesis resulting in lower biomass accumulation (Zhao *et al*. 2005).

One strategy that plants facing N limitation adopt is a reduction in leaf area and hence maintaining the leaf N concentration as has been shown in potato (*Solanum tuberosum* L.) (Vos and van der Putten 1998). In this case, plants exhibit a large reduction in biomass production under N deficiency. Cechin and de Fátima Fumis (2004) found that

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sunflower plants (*Helianthus annus* L. var. CATISSOL-01) grown at 282 ppm N had about 4-fold more dry matter than plants grown at 28.2 ppm N at the final harvest. Another strategy plants use is to maintain a high leaf area, but adapt the leaf N concentration per unit leaf area to N availability as demonstrated in maize (*Zea mays* L.) (Vos *et al.* 2005). Such plants exhibit relatively low reductions in leaf area and biomass production. Zhang *et al.* (2007) found that applying the equivalent of 5 g N/plant to maize in soil with about 0.096% total N increased mature dry matter weight by 9-26% compared to plants that received no N depending on variety and soil moisture. Additionally, Niu *et al.* (2007) found that the effect of N supply on biomass production in maize depended on the N use efficiency of the varieties. They showed that plants of an N-efficient variety of maize (Zi330) supplied with 4 mM N increased its biomass by 13% over plants supplied with 0.08 mM N, while for an N-inneficient variety (Chen 94-11), the increase was 57%. In grain amaranth (*Amaranthus* spp.), applying 50-100 kg N/ha did not increase the plant dry matter at flowering (Pospišil *et al.* 2006).

A clear understanding of the response of African nightshades to limited N supply in terms of leaf area expansion, dry matter production and leaf N content is vital in the efforts to develop appropriate N management strategies. *S. villosum* is a common species in Kenya. Generally it has erect plants with rhombic to ovate-lanceolate leaves and red or orange berries when ripe (Edmonds and Chweya 1997; Schippers 2000). Ojiewo *et al.* (2006a, 2006b) have recently developed two genotypes from the wild-type tetraploid (2n=4x=48) *S. villosum*. The octoploid (2n=8x=96) was developed by application of colchine to *S. villosum* wild-type tetraploid plants. 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. This has been shown in oranges (*Citrus sinensis* (L.) Osb.), where tetraploid plants had thicker leaves and lower gas exchange as compared to diploid plants (Romero-Aranda *et al.* 1997). Similarly in *Lolium* spp., Sugiyama (2005) reported larger leaf sizes in tetraploid plants compared to diploid plants and this was attributed mainly to a higher rate of leaf elongation. In *Catharanthus roseus*, Kulkarni *et al.* (1987) showed that a tetraploid strain 'Purple tetraploid bulk' (PTB) gave higher yield compared to a diploid strain 'Purple' variety (PDB) at closer plant spacings, especially in the absence of N application. The highest economic yield of the tetraploid PTB with the equivalent of 0.9 g N/plant was 26 % higher than the highest economic yield of PDB.

A novel male-sterile mutant with abnormal floral organs was isolated after the wild-type *S. villosum* was irradiated with carbon- ion beam 20 Gy $^{12}C^{5+}$ (Ojiewo *et al.* 2006b). The mutant, T-5, has flowers which are stamenless and infertile at day/night temperatures of 25/25°C and 30/20°C. The flowers are indeterminate at temperatures higher than 25°C. Floral structure and fertility restoration occurred at 20°C-25°C (day) and 15°C-20°C (night). 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, hence the potential to give higher leaf yields than the wild-type plants (Ojiewo *et al.* 2007). González-Real *et al.* (2008) showed that in sweet pepper (*Capsicum annuum* 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.

The apparent large size of the octoploid and increased leaf yield potential of the T-5 mutant raises question about their nutritional requirements. Will these genotypes have increased N use compared to the wild-type tetraploid? Are the genotypes higher leaf yielders under Kenyan field conditions? The objective of this study was to evaluate the effect of N supply on the vegetative and reproductive growth characteristics and leaf N of *S. villosum* genotypes T-5 and the octoploid, under field conditions in Kenya. In this study, it is hypothesized that the octoploid and T-5 genotypes have a higher N requirement and that they give higher leaf yields and leaf N compared to the wild-type tetraploid under field conditions in Kenya.

MATERIALS AND METHODS

Experimental sites

Field experiments were conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT) farm, Juja-Kenya, (lat. 1°10′48′S, long. 37°07′12′E, alt. 1525 masl) during the periods December 2007 to February 2008 (warm season), and April to August 2008 (cool season).

Experimental design and cultivation details

The plant materials consisted of the wild-type (wt), T-5 mutant, and the octoploid genotypes of *S. villosum* Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds. The experiments were carried out as split plots in randomized complete block design with three replications. Three N supply levels of 0, 2.7 and 5.4 g N/plant formed the main plots while the three genotypes were allocated to sub-plots. The sub-plots, each measuring 1 m × 2.5 m were prepared by raising the soil about 15 cm above the ground. The main plots were separated using a polythene paper placed vertically to a depth of 50 cm. Seedlings were sown on 12th December 2007 and 1st April 2008 for the two seasons, respectively in plastic trays filled with vermiculite. Emergence was observed after 4 days for all genotypes. After one week, the seedlings were transferred to plastic pots (10 cm diameter) filled with soil. Transplanting was done in the field on 12th January and 31st May 2008, for warm and cool seasons, respectively. Nitrogen treatments were applied in two splits, at two and four weeks after transplanting using calcium ammonium nitrate (CAN, 27%N). 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.

Quantifying vegetative and reproductive growth

At transplanting time, three seedlings of each genotype were cut and used to determine the initial plant size in terms of leaf area, plant height and shoot dry weight. Subsequently, two plants on each subplot were tagged and used for non-destructive measurements of plant height, leaf number, relative chlorophyll content (SPAD values) using SPAD meter (SPAD-502 Soil Plant Analysis Development, Minolta, Japan), number of flowers and fruits. This was done at 5-10 days-interval.

Harvests were done at 5-10 days interval to quantify plant size over time. At each harvest, one plant from each sub plot was cut at the base and divided into leaf blade, stems and, flowers and fruits. Plant leaf area was measured using a leaf area meter (model 3100, LI-COR Inc., Lincoln, NE, USA). The shoot parts were dried at 70°C for 48 hrs for dry weight measurement. Specific leaf area (SLA) was computed at every harvest as the leaf area divided by the leaf blade dry weight (Hunt 1978). Dried leaf blades were ground and used for N content analyses. Leaf area measurements were stopped when plants began flowering and fruiting profusely, which occurred at 43 and 64 days after transplanting in the warm and cool seasons, respectively. However, one more harvest for dry matter determination was done 7 days after these dates in both seasons. An additional harvest was done at 93 days after transplanting in the cool season mainly to capture the restoration of fertility in the T-5 plants that was observed.

Nitrogen determination

Total N was determined in leaf blades using the procedures outlined by Okalebo *et al.* (2002). Dried leaf samples were ground using a plant grinder. A sample of about 0.3 g of the ground leaf was digested in concentrated H_2SO_4 with a catalyst mixture of hydrogen peroxide and selenium and lithium sulphate at 300°C for

3 hrs, then removed and cooled. The digest was diluted to 50 volumetric flasks with distilled water. This was followed by steam distillation of the digested sample using excess NaOH. The distillate was collected in saturated H_3BO_3 and then titrated with 0.01N HCl. The percent N was calculated as:

$$\%N = \frac{(a-b)\times 0.2\times v\times 100}{1000\times w\times al}$$

where a = volume of titre HCl for sample, b = volume of titre HCl for blank, v = final volume of the digestion, w = weight of the sample taken and al=aliquot of the solution taken for analysis.

The leaf N content was calculated as the percent N multiplied by the leaf dry weight.

Data analyses

Statistical analyses were done using the GLM procedures of SAS (SAS 1999). An ANOVA was done with a split plot design for the field experiments for each date separately for leaf area, dry weights, specific leaf area and leaf N. Flower and fruit to shoot ratio data were first transformed to natural logarithms before analysis of variance. Data for developing relationships between leaf area and shoot dry eight, and leaf nitrogen and leaf area were subjected to the Durbin-Watson test to establish whether the data had correlated errors using the autoregression procedure of SAS. Linear regression was then done on the data without correlated errors using regression procedure of SAS (SAS 1999).

RESULTS

Effects of genotype and N supply on leaf area development in African nightshade

There was no significant interaction between genotype and N supply hence only main effects were considered. During the period of active vegetative growth, the octoploid had significantly higher leaf area than the wt and T-5 genotypes in both the warm and cool season (**Fig. 1A, 1B**). T-5 and the wt had similar leaf area throughout the growing period. The highest leaf area attained before intense flowering and fruiting in the warm season was 5182-5452 cm^2/plant for wt and T-5 compared to 7848 cm^2/plant for octoploid, while in the cool season, it was 6472-6612 cm^2/plant for wt and T-5 compared to 8367 cm^2/plant for octoploid. The genotypes responded similarly to N application by significantly increasing leaf area (**Fig. 1C, 1D**). The effect of N supply levels on leaf area was significant starting from 30 and 40 days after transplanting in the warm and cool seasons, respectively. In the warm season, plants supplied with 2.7 g and 5.4 g N/plant had similar leaf area except at 43 days after transplanting (DAT) when the latter had significantly higher leaf area. At this time, leaf area in plants supplied with 2-7-5.4 g N/plant was 6916-8506 cm^2/plant compared to 3299 cm^2/plant when no N was supplied. Intense flowering and fruiting was observed at 64 days after transplanting in the cool season. At this time, plants supplied with 2.7 g and 5.4 g N/plant had similar leaf area ranging between 8130-9097 cm^2/plant compared to 4222 cm^2/plant in plants that received no N.

Effects of genotype and N supply on shoot dry matter production and partitioning in African nightshade

Genotypes showed variation in leaf dry weight over time. The octoploid gave significantly higher leaf dry weight at 24 and 43 DAT in the warm season. It also maintained a higher leaf dry weight during the other harvest dates except at 50 DAT during the warm season **(Fig. 2A)**. In the cool season, the octoploid gave significantly higher leaf dry weight at 47 and 54 DAT. At 70 and 93 DAT, both the octoploid and T-5 gave significantly higher leaf dry weight compared to the wt (**Fig. 2B**). Applying N significantly increased the leaf dry weight. In both the warm and cool season, plants that received 2.7 g N/plant and 5.4 g N/plant gave similar leaf dry weight (**Fig. 2C, 2D**).

Shoot dry matter was not significantly different between genotypes in both the warm and cool seasons. At final harvests, shoot dry weight ranged between 77.6-92.5 g/plant in warm season and 108.9-162.6 g/plant in the cool season (**Fig. 3A, 3B**). N application increased shoot dry weight starting from 24 and 47 days after transplanting (DAT) during the warm and cool season, respectively. The effects

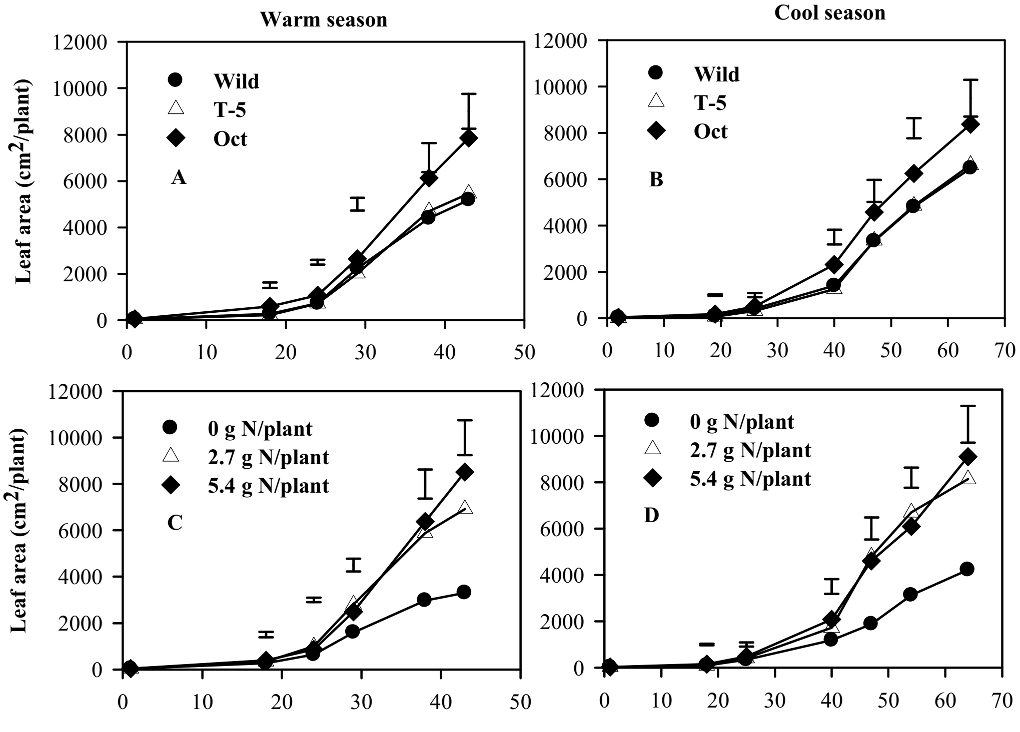

Fig. 1 Leaf area for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show $LSD_{0.05}$.

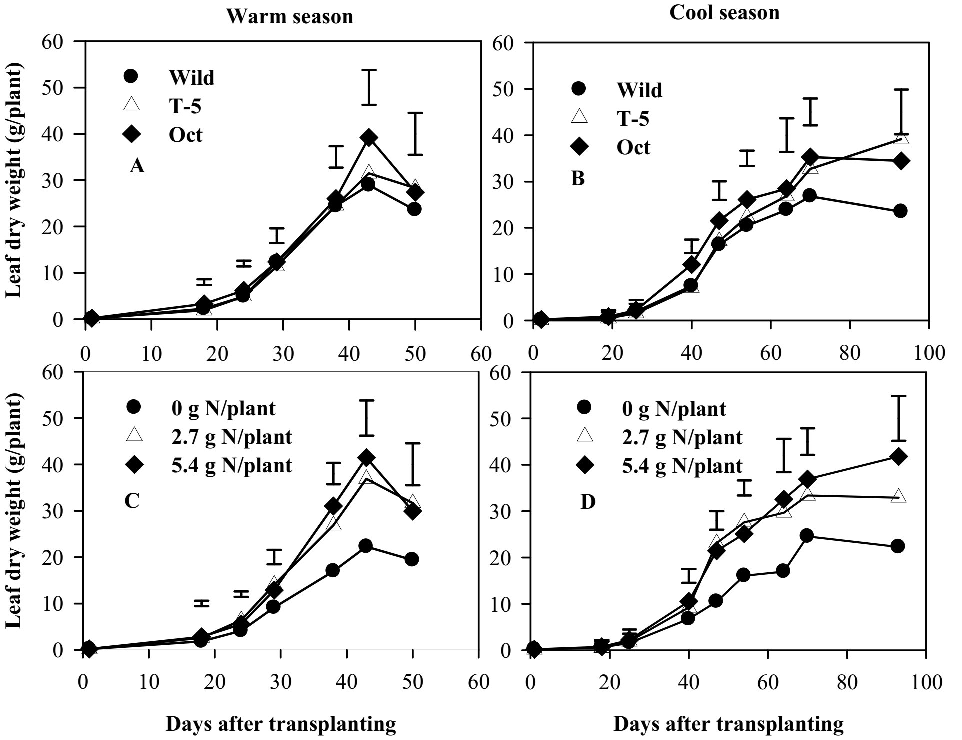

Fig. 2 Leaf dry weight for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show $LSD_{0.05}$.

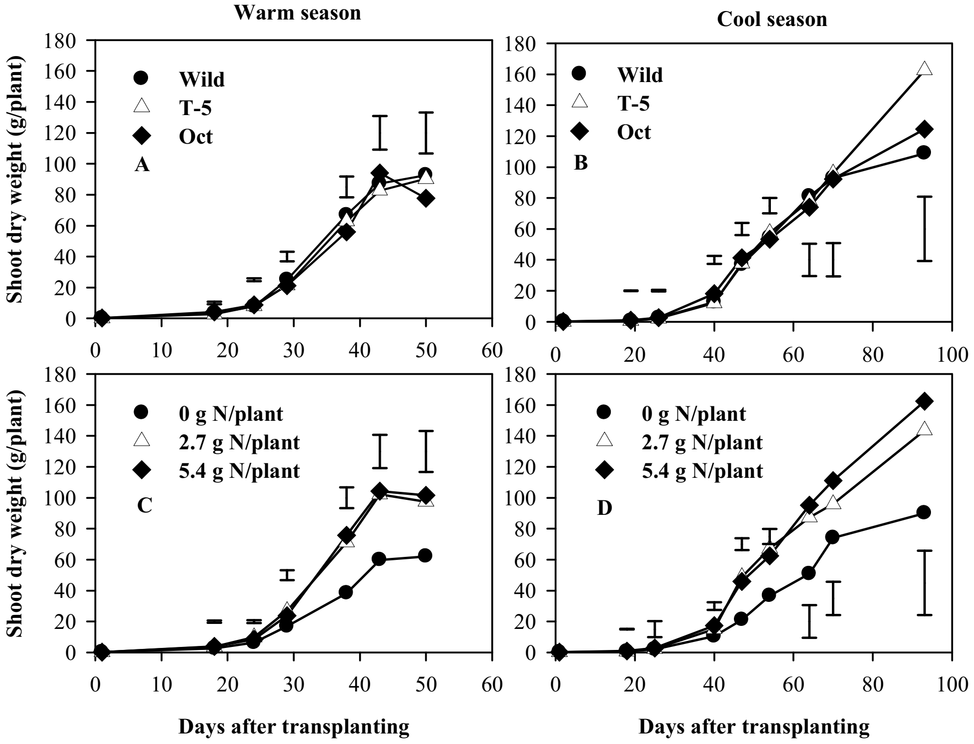

Fig. 3 Shoot dry weight for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season May-August 2008. Vertical bars show $LSD_{0.05}$.

were significant at 24, 38 and 43 DAT in the warm season, and at 47, 54 and 93 DAT in the cool season (**Fig. 3C, 3D**). The shoot dry weight ranged between 102.3-104.3 g/plant for plants supplied with 2.7-5.4 g N/plant compared to 59.9 g/plant in plants that were not supplied with N at 43 DAT in warm season, and this remained more or less constant at 50 DAT. In the cold season, the shoot dry weights between 87.4-95.3 g/plant and 50.8 g/plant for plants supplied with 2.7-5.4 g N/plant and 0 N g/plant, respectively at 64 DAT. Thereafter, the shoot dry weights increased to 143.7-162.4 g/plant and 90.1 g/plant, respectively. At all the harvests, the treatments 2.7 and 5.4 g N/plant gave similar shoot dry weights.

Specific leaf area (SLA) was significantly higher in octoploid plants at 24-38 DAT compared to the wt and T-5 plants in the warm season (**Fig. 4A**). This ranged between 174.0-234.8 cm^2/g for the octoploid and 110.8-203.6 cm^2/g for the wt and T-5 plants. In the cool season, the octoploid and wt plants had significantly higher SLA at 40-54 DAT compared to T-5 plants (**Fig. 4B**). This ranged between 174.9-294.4 cm^2/g for the octoploid and wt plants, and 174.4-243.4 cm^2/g for the T-5 plants. Plants that received N

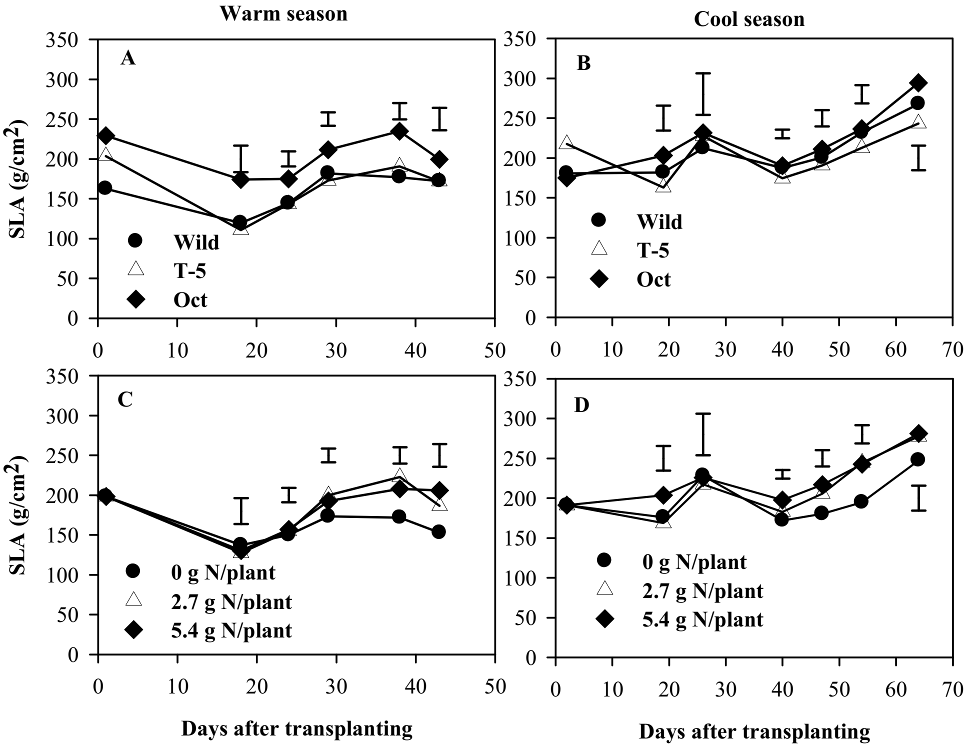

Fig. 4 Specific leaf area for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show LSD$_{0.05}$.

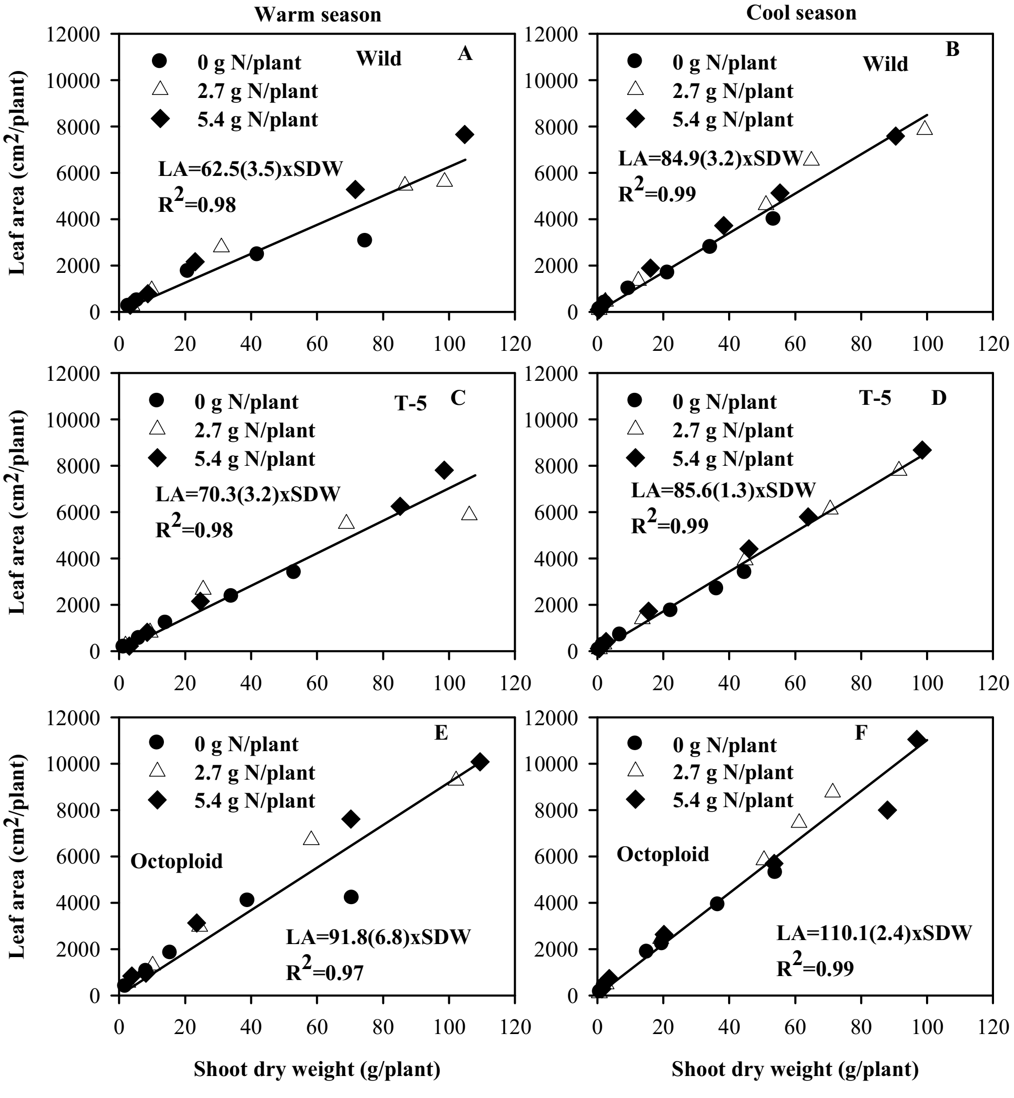

Fig. 5 The relationship between leaf area and shoot dry weight of African nightshade genotypes wild-type (**A**, **B**), T-5 (**C**, **D**) and octoploid (**E**, **F**) grown under different N levels at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Lines show the linear functions given and the SE of the slope is given in parenthesis.

gave a significantly higher SLA at 29-43 DAT in the warm season and 40-54 DAT in the cool season (**Fig. 4C, 4D**). There was no significant difference in SLA between plants that received 2.7 g N/plant and those that received 5.4 g N/plant. In both seasons, the SLA ranged between 152.9-247.4 cm^2/g for plants that received no N and 127.3-280.9 cm^2/g for plants that received 2.7 and 5.4 g N/plant.

The relationship between leaf area and shoot dry weight was not influenced by the N levels (**Fig. 5**). The slope of this relationship, which is the leaf area ratio (LAR) however was significantly higher in the octoploid plants ranging between 91.8-110.1cm^2/g as compared to a range of 62.5-85.6 cm^2/g for the wt and T-5 plants. Generally, the LAR was higher in the cool season compared to the warm season for all genotypes.

Effects of genotype and N supply on flower and fruit development in African nightshade

Wild-type plants had higher flower and fruit dry weight at all harvest dates in the warm season and this was significant at 24 and 38 DAT (**Fig. 6A**). Increase in flowering and fruiting occurred from 38 DAT onwards. In the cool season, wt plants also had significantly higher flower and fruit dry weight at 26, 47, 64 and 70 DAT (**Fig. 6B**). In this season, the intensity of flowering and fruiting shot up from 54 DAT. Fertilizer application had significant effect on the flower and fruit dry weight only at 38 DAT in the warm season, and 47 and 64 DAT in the cool season (**Fig. 6C, 6D**). In both cases, plants that received fertilizer had significantly higher flower and fruit dry weight.

During the warm season, the flower and fruit to shoot ratio was not significantly affected by N application. However, genotypes differed significantly in their flower and fruit to shoot ratio (**Fig. 7A**). Wild-type plants maintained significantly higher flower and fruit to shoot ratio, increasing from 0.05 at 18 DAT to 0.22 at 50 DAT. In octoploid plants, the flower and fruit to shoot ratio increased from 0.01 at 18 DAT to 0.12 at 50 DAT. T-5 plants maintained a flower and fruit to shoot ratio of about 0.05 throughout the experimental period (**Fig. 7A**). In the cool season, N application had no significant effect on the flower and fruit to shoot ratio except at 64 DAT when control plants had higher ratio (data not shown). Genotypes significantly differed in the flower and fruit to shoot ratio (**Fig. 7B**). The wt plants had higher ratios, increasing from 0.01 at 26 DAT to 0.19 at 70 DAT when leaf production declined. Thereafter the ratio rose to 0.33 at 93 DAT. In octoploid plants, the ratio increased from 0.01 to 0.11 in the period 26-70 DAT, and there after rose to 0.29 at 93 DAT. T-5 plants showed an

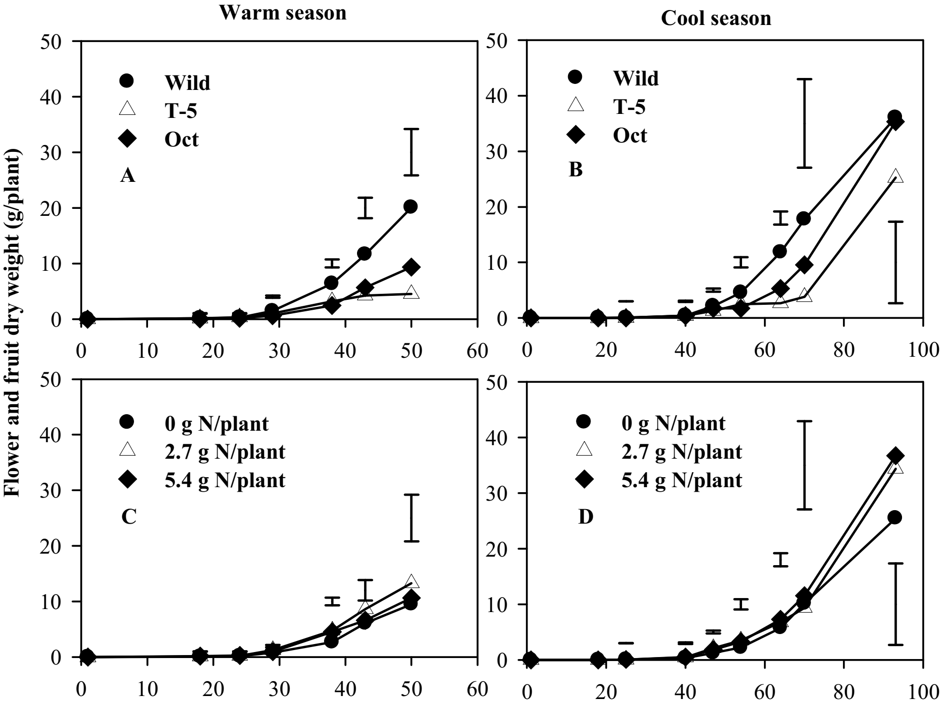

Fig. 6 Flower and fruit dry weight for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show $LSD_{0.05}$.

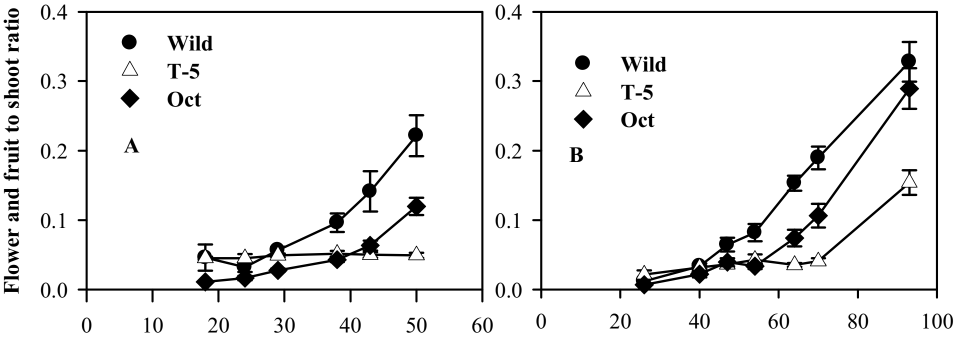

Fig. 7 Flower and fruit to shoot ratio for African nightshade as influenced by the genotypes grown at JKUAT farm during the warm season, December 2007- February 2008 (**A**) and cool season April-August 2008 (**B**). Vertical bars show SE.

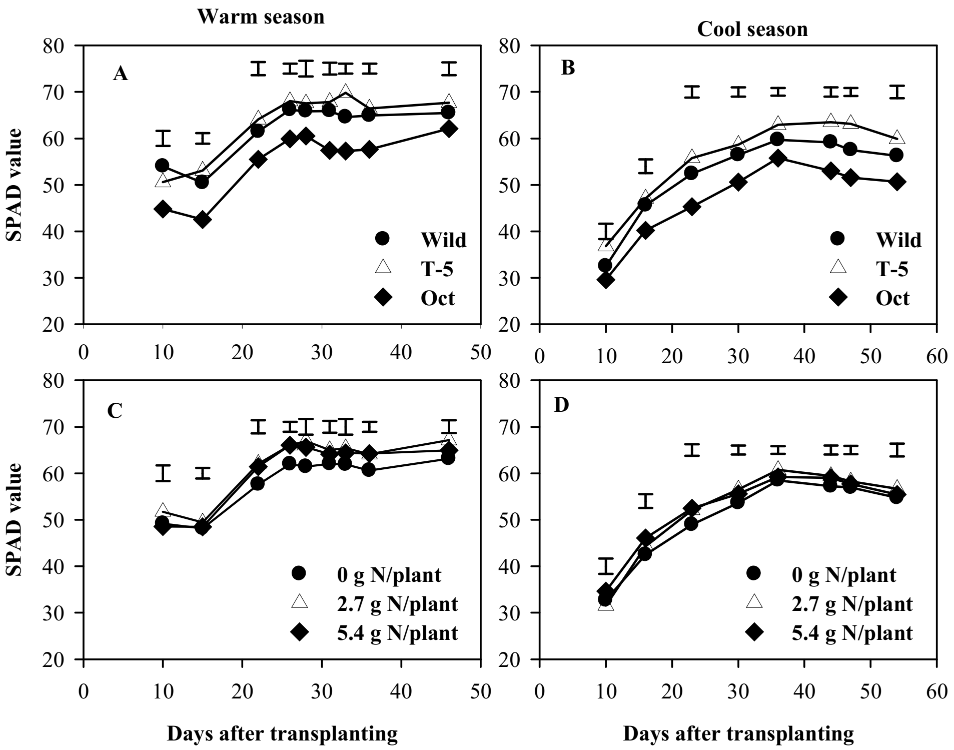

Fig. 8 SPAD value for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show $LSD_{0.05}$.

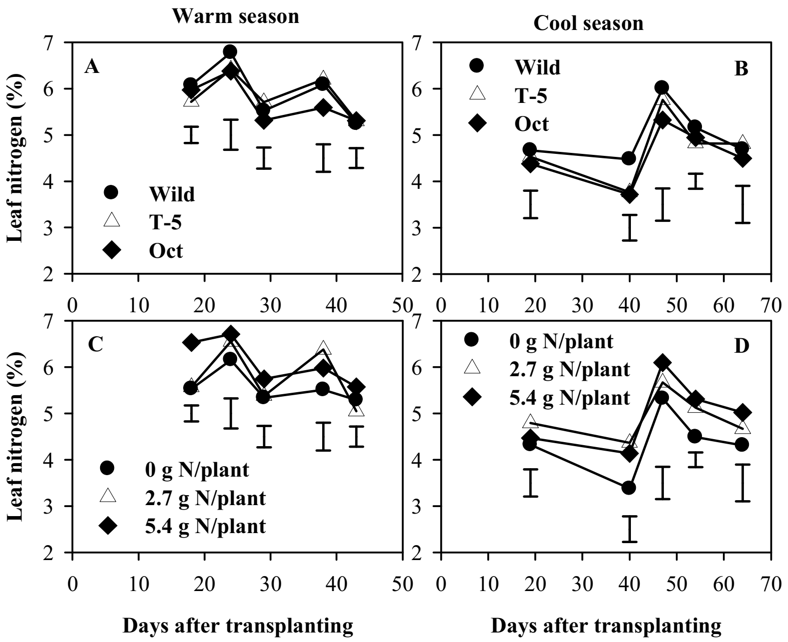

Fig. 9 The leaf N concentration (%) for African nightshade as influenced by the genotypes (**A**, **B**) and N levels (**C**, **D**), grown at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Vertical bars show $LSD_{0.05}$.

interesting pattern, where the ratio ranged between 0.02-0.04 in the period 26-70 DAT, but sharply rose to 0.15 at 93 DAT.

Effects of genotype and N supply on SPAD values and leaf N content in African nightshade

The wt and T-5 plants had similar and significantly higher relative chlorophyll content with SPAD values between 50-68 and 32-63 in warm and cool seasons, respectively, compared to 43-62, and 30-56 for the octoploid plants in the warm and cool seasons, respectively (**Fig. 8A, 8B**). Plants that received 2.7 and 5.4 g N/plant had significantly higher SPAD values compared to control plants (**Fig. 8C**). This ranged between 48-63, 49-67, and 48-65 for plants under 0, 2.7, and 5.4 g N/plant, respectively. In the cool season, N application significantly increased SPAD values at 23-36 DAT (**Fig. 8D**). The values ranged between 32-58, 31-60, and 34-59 for 0, 2.7, and 5.4 g N/plant treatments, respectively.

Leaf N content on dry weight basis was higher in wt and T-5 plants compared to octoploid plants but this was significant only at 18 DAT during the warm season (**Fig. 9A**). During the cool season, the wt and T-5 plants had higher leaf N content but this was not significantly different from octoploid plants (**Fig. 9B**). The leaf N content was also higher in plants supplied with 2.7-5.4 g N/plant compared to control plants but this was significant at 18 and 38

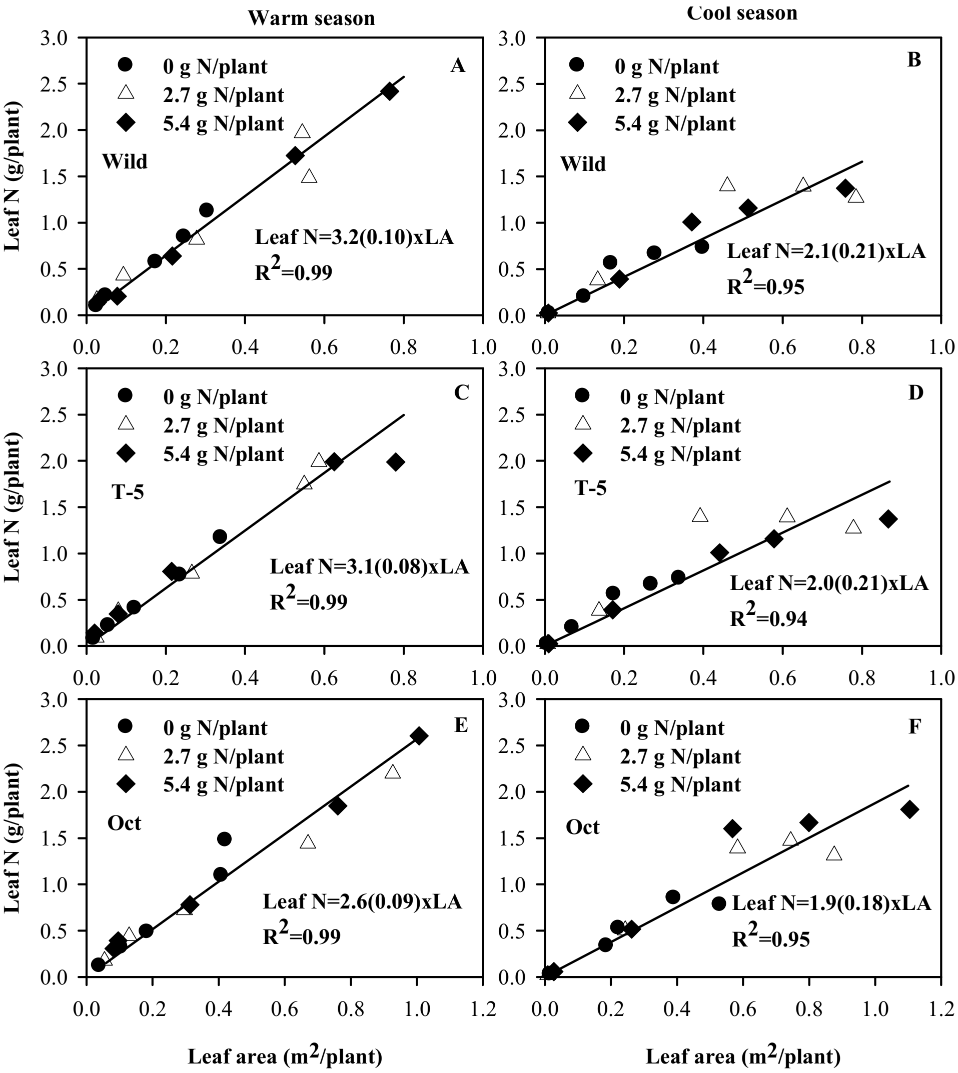

Fig. 10 Leaf N content (g/plant) as a function of leaf area for African nightshade genotypes wild-type (**A**, **B**), T-5 (**C**, **D**) and octoploid (**E**, **F**) grown under different N levels at JKUAT farm during the warm season, December 2007- February 2008 and cool season April-August 2008. Lines show the linear functions given and the SE of the slope is given in parenthesis.

DAT (**Fig. 9C**). Applying N increased the leaf N content but this was significant only at 54 DAT (**Fig. 9D**). On a leaf area basis, the leaf N content was not significantly different between the N levels during the warm season except at 43 DAT when control plants had significantly higher content than those supplied with N (data not shown). Similarly, on leaf area basis, wt and T-5 plants had significantly higher leaf N compared to octoploid plants at 29 and 38 DAT (data not shown). In the cool season, the wt and T-5 plants had significantly higher leaf N at 40 and 47 DAT, while application of N had no significant effect on leaf N content (data not shown). The leaf N content increased linearly with increase in leaf area (**Fig. 10**). The slope of the increase was higher for wt and T-5 plants but this was significantly different from the octoploid plants only in the warm season.

DISCUSSION

The octoploid and T-5 genotypes are possibly the first genotypes to be developed from wt *S. villosum*. Wild-type *S. villosum* tends to flower early and profusely, thus resulting in reduced leaf yields. One objective of developing these genotypes was to increase leaf yields by reducing or delaying their reproductive growth (Ojiewo *et al.* 2007). In this study, the leaf area in octoploid plants was 1.3-1.5 times higher than in wt plants, while T-5 had similar leaf area as the wt plants irrespective of the N supply (**Fig. 1A, 1B**). It was generally observed that the octoploid plants had larger leaves, implying better quality considering that size is also is a quality aspect. Application of N is essential for leaf area development in *S. villosum*. The response to N supply was the same in the three genotypes (**Fig. 1C, 1D**). Supplying 2.7-5.4 g N/plant increased the leaf area 2.1-2.6 times in warm season, and about 2 times in cool season compared to plants that received no N. Plants that received 2.7 and 5.4 g N/plant had generally similar leaf area.

Vos and van der Putten (1998) have outlined two extreme strategies that plants species use when faced with N deficit. On one hand, some species maintain leaf size rather than maintaining maximum leaf N. In this strategy, the plant maximizes leaf area expansion and light interception at the cost of reduced rate of photosynthesis per unit leaf area. On the other hand, other species maximize productivity per unit area through reduced leaf area expansion to maintain maximum leaf N. In this case, there is reduced light interception. The latter strategy fits the response given by the three *S. villosum* genotypes in terms of drastic leaf area reduction under low N supply. In potato, Vos and van der Putten (1998) found that at extreme N application rates of 250 mg and 2500 mg N/pot per application, leaf area differed by a factor of 3. They concluded that leaf size in potato is very responsive to N supply due to the effect on leaf expansion rate. They also supplied 4 and 12 g N/plant to potato (*Solanum tuberosum* L. cv. 'Vebeca') and found that there was more than a two-fold difference in plant green leaf area between the two N levels (Vos and van der Putten 2001, estimated from **Fig. 5A, 5B**). This conforms to the second strategy outlined above. Akanbi and Togun (2002) applied 3 t/ha (12 g/plant) maize-stover compost with 30 kg N/ha (0.12 g N/plant) to amaranth (*Amaranthus cruentus* L.), and found that this increased leaf area to 469.1 cm^2 compared to 31.8 cm^2 in control plants. Similarly, large reductions of 68% in leaf area were reported in sorghum (*Sorghum bicolor* (L.) Moench) plants grown in half-strength Hoagland's nutrient solution without N supply from 25 days after sowing as compared to plants with 100% N supply in the solution (Zhao *et al.* 2005). In lettuce (*Lactuca sativa* L. cv. 'Corsica'), applying inorganic N levels of 0.0-3.38 N g/ plant gave significantly different fresh yield depending on the season (Pavlou *et al.* 2007). In that study, it was found that applying the higher levels of N 2.08-3.38 g N/plant gave fresh yield that was 1.3-2.3 times the yield of plants that received no N. In addition, the leaf length and width of plants that received N was 1.1- 2.3 times that of plants that received no N. This is contrary to the case of maize where Vos *et al.* (2005) reported that the final area of the largest leaf and total plant leaf area differed by 16 and 29% from the lowest to highest N supply (0.5-6.0 g N/plant).

The leaf dry weight followed the same patterns as the leaf area between the genotypes in both warm and cool seasons (**Fig. 2A, 2B**). The octoploid tended to have higher leaf dry weight compared to the other genotypes. This was attributed to bigger leaf sizes in the octoploid plants. However, the total shoot dry weight showed little variation between the genotypes. The effect of N supply on dry matter production corresponded with leaf area growth. Plants supplied with 2.7 and 5.4 g N/plant had similar but significantly higher dry weights than plants that did not receive N starting from 24 and 47 days after transplanting in the warm and cool seasons, respectively. During these periods, both leaf and shoot dry weights in plants supplied with 2.7 and 5.4 g N/plant were 1.3-2.3 times that in plants that received no N. The drastic reduction in leaf area may have largely contributed to the reduced dry matter production in plants not supplied with N (**Figs. 2C, 2D, 3**). This maybe attributed to a reduction in cumulative light interception. Rosati *et al.* (2001) supplied the equivalent of 2, 8 and 14.2 g N/plant to eggplant and found a canopy leaf area index of 1.79-2.38, 2.60-3.00, and 3.09-3.48, and a canopy light interception of 0.77, 0.79, and 0.82 of incoming photosynthetically active radiation (PAR) for the three N levels, respectively. Similarly, Tei *et al.* (2002) found that supplying the equivalent of 3.1-6.3 g N/plant in tomato (*Lycopersicon esculentum* Mill.) gave optimum leaf area index, which increased absorbed PAR to 2222-2923 molm^{-2} as compared to 1860-2272 molm^{-2} in control plants.

The dry matter increases with N supply in this study ranging between 1.3-2.3 times are relatively high considering that the soils had moderate total N levels of 0.12-0.18%. This increase is comparable to that reported by Vos and van der Putten (2001, estimated from **Figs. 2C, 5A, 5B**) who found about a 2-fold increase in leaf dry weight and about 1.4 times higher whole plant dry weight of potato when N supply was increased from 4 to 12 g N/plant. Similar increases in dry weight due to N supply have been reported in three woody ornamental species *Lagerstroemia indica* cv. 'Red Imperator' (deciduous, Lythraceae), *Callicarpa bodinieri* cv. 'Profusion' (deciduous, Verbenaceae) and *Viburnum tinus* cv. 'Macrocarpa' (evergreen, Caprifoliaceae) (Demotes-Mainard *et al.* 2008). These species were irrigated with a nutrient solution containing 4 or 88 mg/L N and it was found that the difference in dry weight of plants was 1.6-2.3 times between the N treatments. Khan *et al.* (1995) found that black nightshade (*Solanum nigrum* L.) plants supplied with 1.80 g N/plant in a pot experiment gave 14.4% higher plant dry weight than those not supplied with N. A reduction of 41% in total dry matter was reported in sorghum plants grown in Half-strength Hoagland's nutrient solution without N supply from 25 days after sowing as compared to plants with 100% N supply in the solution (Zhao *et al.* 2005).

A greater effect of N has been reported by van Averbeke *et al.* (2007) who found that at the optimum N level of 2.66 g N/plant for *Solanum retroflexum* Dunn., a nightshade indigenous to South Africa, the dry weight of above ground was 32.2 g/plant compared to only 2.5 g/plant at 0 N g/plant. The same authors found that the dry weight of *Brassica rapa* L. subsp. *Chinensis* peaked at N supply of 3.9 N g /plant. Similarly, in sunflower (*Helianthus annuus* L. var. CATISSOL-01), plants supplied with high N produced nearly four-fold the dry matter produced by plants supplied with low N (Cechin and de Fátima Fumis 2004). Further, N supply has been shown to increase dry matter production in soybean (*Glycine max* (L.) Merr.) (Taylor *et al.* 2005). de Pinheiro Henriques and Marcelis (2000) found a strong decrease in dry matter production of lettuce (*Lactuca sativa* L.) with decreasing rate of N supply, which was accompanied by a marked decline in leaf area index. N supply of 25-100 kg N/ha significantly increased dry matter production in soybean (*Glycine max* (L.) Merr.) to levels of 705-860 kg/ha as compared to 630 kg/ha in control plants (Taylor *et al.* 2005). Aujla *et al.* (2007) tested the growth and yield of eggplant (*Solanum melongena* L.) supplied with 90-180 kg N/ha under varying irrigation levels. They found that the fruit yield increased significantly with increasing N level up to 150 kg N/ha which corresponded to about 4 g N/plant.

Thus the *S. villosum* genotypes tested in this study showed a relatively high decline in dry matter production at lower N supply levels, which could be attributed to the strategy of drastic reduction in leaf area in response to N limitation. This is in contrast to the strategy of maintaining leaf area and reducing leaf N content, which results in lower dry matter reductions. Zhang *et al.* (2007) found that applying the equivalent of 5 g N/plant to maize in soil with about 0.096% total N increased mature dry matter weight by 9-26% compared to plants that received no N depending on variety and soil moisture. Niu *et al.* (2007) found that Zi330, a N efficient line of maize had 13% increase in whole plant dry weight compared to 54% increase in Chen 94-11, an N inefficient line, when both were grown at 225 kg N/ha and 0 kg N/ha. In wheat, leaf growth rate (g/plant/day) increased by 25-54% when plants were grown in 19.9 mM N compared to 4.5 mM N depending on the day/night temperatures (Lawlor 2002).

SLA is an important parameter that can be used to describe plant morphological adaptation to the growth environment. In this study, the octoploid plants tended to have a higher SLA compared to the wt and T-5 plants (**Fig. 4A, 4B**). This suggests a more vigorous growth in octoploid plants compared to the other genotypes, which is a desirable trait for high leaf yields. SLA has been shown to be positively correlated with relative growth rate (RGR) of plants in various species and growing conditions. Hunt and Cornelissen (1997) assessed the RGR, leaf area ratio (LAR), leaf weight fraction (LWF), specific leaf area (SLA) and root to shoot ratio of 21 herbaceous monocotyledons, 22 herbaceous and 16 woody dicotyledonous species. The results showed that RGR in all the species was strongly dependent upon LAR. Further, Hunt and Cornelissen (1997) found that among SLA and LWF, both sub-components of LAR, SLA was more strongly and positively related with RGR. If this correlation holds for African nightshades, then RGR can be estimated using measurements of SLA. However, Shipley (2006) assessed published data on 241 species of woody dicots, 39 species of conifers, 153 species of herbaceous dicots and 181 species of herbaceous monocots and found only a very weak correlation between SLA and RGR, leading to the conclusion that SLA is not a very good indicator of RGR. Shipley (2006) however indicated that the importance of SLA in determining RGR may be higher at low (less than 15 mol/m^2/day) rather than high (more than 25 mol/m^2/day) irradiance. In this study, plants supplied with 2.7-5.4 g N/plant gave significantly higher SLA compared to control plants (**Fig. 4C, 4D**). Similar significant effects of N supply on SLA or the reciprocal specific leaf weight (mass) have been reported. de Pinheiro Henriques and Marcelis (2000) have shown a linear decrease in SLA with decreasing plant N content, which in turn depended on N supply in lettuce. They attributed the decrease in SLA to increase in leaf dry matter percentage. Additionally, they found a higher SLA at low irradiance, which they attributed to low dry matter percentage and small leaf thickness. In grasses, Knops and Reinhart (2000) found that SLA increased significantly with N fertilization. Vos and van der Putten (2001) reported significant decreases in specific leaf weight, the reciprocal of specific leaf area, for potato.

In this study, no measurements for leaf thickness were done. However, the low SLA observed in the wt and T-5 genotypes, and plants that were not supplied with N suggests thicker leaves as opposed to thinner leaves which gave a high SLA in the octoploid plants and plants that received 2.7-5.4 g N/plant. Niinemets (1999) and Abrams *et al.* (1994) have shown that leaf thickness was more strongly and positively correlated to leaf mass per unit area, the reciprocal of SLA in various shrubs and trees. Using original and published data covering widely differing geographical areas, species and growth forms, Vile *et al.* (2005) have shown a strong negative correlation between leaf thickness and SLA. A reduction in SLA under N deficiency has also been associated with accumulation of starch in leaves (Grindlay 1997) as has been observed in tomato (Le Bot *et al.* 1998). It is thought that 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). Thin large leaves are desirable for traditional leafy vegetables since leaf size is a quality attribute. Thus genotypes that maintain a relatively high SLA under low N conditions are high yielding. Gulias *et al.* (2003) measured the net photosynthesis on mass basis (A_m), leaf N on mass basis (N_m) and SLA of 78 species that included crops, endemic and non-endemic species growing in a Mediterranean climate. They found that in all species including the crops, A_m and N_m were positively correlated with SLA. Additionally, Reich *et al.* (1999) have shown that in more than 100 species, maximum photosynthesis, leaf N_m and SLA were positively correlated.

Leaf area ratio (LAR) provides a measure of the leafiness of a plant (Hunt 1978). It therefore follows that with a higher LAR, the octoploid plants were leafier than the wt and T-5 plants (**Fig. 5**). Application of N did not change LAR suggesting that plants adjusted their photosynthesizing to respiring materials to maintain a constant ratio under different N conditions. The higher LAR for octoploid plants may be attributed to its higher leaf area and SLA.

In both seasons, N supply did not show consistent effect on the partitioning of dry matter between the reproductive and vegetative growth (**Fig. 6**). In the warm season, the two genotypes, the octoploid and T-5, had significantly lower reproductive growth during the period of active vegetative growth. T-5 maintained about 5% of its dry matter in the flowers and fruits, while the octoploid allocated up to 12% of its dry matter to flowers and fruits (**Fig. 7**). This is contrast to the wt plants, which allocated up to 22% of its dry matter to flowers and fruits. In this regard, the breeding objective of reducing the reproductive fractions in *S. villosum* was achieved. Most of the reproductive fraction in T-5 was the flowers and only few fruits formed due to sterility. In the cool season, the genotypes maintained the patterns, with maximum of 4, 11 and 19% of dry matter allocated to flower and fruit in T-5, octoploid and wt, respectively during the period of active vegetative growth. Plants were left late into the reproductive phase, and the allocations increased to 15, 29 and 33% in T-5, octoploid and wt plants, respectively. It was noted that T-5 plants restored fertility in this late phase and had fertile fruits. During the warm season, the site experienced an average day/night temperature of 26.9°C/13.6°C and 27.4°C/13.4°C in January and February, respectively with lowest temperatures of 10.0-10.3°C. The relative humidity at 12.00 noon ranged between 44-47% with a total radiation of 1434.61 MJm^{-2}. In the cool season, the average day/night temperature was 23.7°C/13.3°C and 23.1°C/13.3°C for June and July, respectively. The lowest temperatures ranged between 9.5 and 7.8°C in June and July, respectively and a relative humidity at 12.00 noon of 42-54%. The total radiation received for June and July was 762.05 MJm^{-2} (weather data collection stopped on 26th July 2008 for renovations). The fertility restoration in cool season was attributed to the low night temperatures of 9.5-15.6°C, and 7.8-15.1°C the plants had been exposed to in the months of June and July, respectively. The actual restoration to fertility occurred in August 2008, but weather data for this month was not available due to the station being renovated. This result concurs with description of T-5 as a season dependant male-sterile mutant with temperatures of 20-25°C/15-20°C (day/night) restoring fertility (Ojiewo *et al.* 2007). Cultivation of T-5 for leaf production should therefore be done in the warm season where flower and fruit production is limited, while seed production can be done during the cool season when fertility is restored.

In this study, the octoploid plants had consistently lower SPAD values and also had lower leaf N concentration on a dry weight basis (**Figs. 8A, 8B, 9A, 9B**). These plants had a higher leaf area, but the shoot dry weight was not different from the wt and T-5 plants. On one hand, it suggests that given similar soil N status, the wt and T-5 plants partitioned a higher amount of N to the leaf. However, even with the higher leaf N, these two genotypes produced similar dry matter as the octoploid plants, which had lower leaf N. Plants supplied with 2.7-5.4 g N/plant maintained higher SPAD values and leaf N concentration on a dry weight basis as compared to those that received no N (**Figs. 8C, 8D, 9C, 9D**). SPAD values can be used in N nutrition management. In rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), Singh *et al.* (2002) showed that N management using SPAD readings saved 12.5 to 25% of the existing fertilizer N recommendation. They found that SPAD value of 37.5 was critical for the region of their trials. In our study, no critical SPAD value for African nightshade was established but at the optimum N level of 2.7 g N/plant, the SPAD value was seasonal dependant ranging between 49-67 and 31-60 in warm and cool seasons, respectively. These values are within the range Muthuri *et al.* (2009) reported for maize.

There is a positive correlation between leaf N concentration and both the leaf chlorophyll concentration and the rate of photosynthesis (Sinclair and Horie 1989; Zhao *et al.* 2005; Muthuri *et al.* 2009). In our study, African nightshade plants that received 2.7-5.4 g N/plant had a higher leaf N on weight basis compared to control plants. Such a response has been reported in soybean where plants supplied with N had higher plant N concentration (Taylor *et al.* 2005). Similar results have been reported in 22 herbaceous species (Meziane and Shipley 2001), wheat (*Triticum aestivum* L.) (Sinclair *et al.* 2000), and sorghum (Zhao *et al.* 2005).

However, when leaf N content was expressed on a leaf area basis the wt and T-5 plants had a higher content than the octoploid plants, but this was significant only in the warm season. In addition, the differences between N levels were not significant (**Fig. 10**). This suggests that the *S. villosum* genotypes when faced with N limitation try to maintain high leaf N content probably through reduction in leaf growth. The wt and T-5 plants had 3.1-3.2 g N/m^2 and 2.0-2.1 g N/m^2 in warm and cool seasons, respectively compared to 2.6 and 1.9 g N/m^2 for octoploid plants in the warm and cool seasons, respectively. The results suggests a maximum leaf N concentration on per area basis and raises questions as to whether it is dependant on light intensity. Demotes-Mainard *et al.* (2008) found that in three ornamental woody species, N content on leaf area basis was less sensitive to N fertilization than N content on dry weight basis and this they attributed to the fact that N fertilization decreased leaf mass per unit area (the reciprocal of SLA). This partly compensated for the increase in N content on dry weight basis. It appears that the octoploid plants accumulates less N in leaves per unit leaf area as compared to the wt and T-5 plants and more work needs to be done to establish if this may also lead to variation in nitrate accumulation among the genotypes. Ripley *et al.* (2008) supplied 0, 3.5 and 7 g N/m^2/year to grass subspecies *Alloteropsis semialata* (R.Br.) Hitchc. subsp. *semialata* and subspecies *Alloteropsis semialata* (R.Br.) Hitchc. subsp. *eckloniana* (Nees) Gibbs Russell. They found that leaf N content was significantly affected by N supply when expressed as a percentage of dry weight, but not on a leaf area basis. The leaf N content on area basis obtained in this study for T-5 and wt plants are relatively higher than those reported for durum wheat (*Triticum durum*) having 1.36-2.03 g N/m^2 between cultivars and N supply levels of 40-120 kg N/ha at plant density of 350 seeds/m^2 (Fois *et al.* 2009). Vos and van der Putten (2001) reported values ranging between 1.0-3.4 g N/m^2 for potato (estimated from **Fig. 3C**). In various arctic plant species, van Wijk *et al.* (2005) reported a leaf N content range of 1.27-3.32 g N/m^2.

It is concluded that the African nightshade plants responded to N deficiency by large reductions in leaf area and consequently dry matter production. This enabled the N deficient plants to maintain a leaf N concentration on area basis comparable to plants well supplied with N. The octoploid plants were leafier as compared to T-5 and wt plants and therefore showed higher potential as a leafy vegetable. The T-5 and wt plant had lower amounts of the reproductive fractions. In addition, T-5 and wt plants tended to accumulate more N in the leaves per unit leaf area as compared to the octoploids. Irrespective of the genotypes for *S. villosum*, applying 2.7 g N/plant in soils with moderate N content is sufficient to give higher leaf yields.

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