

# Plant Growth, Water Relations and Gas Exchange of Octoploid and Tetraploid *Solanum villosum* Mill. ssp. *miniatum* (Bernh. Ex Wild.) Edmonds under Water Deficit Conditions

P. W. Masinde<sup>1\*</sup> • C. O. Ojiewo<sup>2</sup> • S. G. Agong<sup>1</sup> • M. Masuda<sup>2</sup>

<sup>1</sup> Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, code 00200 Nairobi, Kenya

<sup>2</sup> Faculty of Agriculture, Okayama University, 1-1-1 Tsushima Naka, Okayama, 700-8530, Japan

Corresponding author: \* masinde\_peter@yahoo.co.uk

## ABSTRACT

*Solanum villosum* Mill. ssp. *miniatum* (Bernh. Ex Wild.) Edmonds is a leafy vegetable in Kenya, which has had limited genetic improvement. An octoploid line with larger stomata and leaves, and late flowering characteristics has been recently developed from the wild tetraploid. A study was conducted to evaluate the adaptive responses of this octoploid to water deficit in comparison to its wild tetraploid parent. Three pot experiments were carried out between January and August 2006 with the aim of quantifying plant responses to water deficit in terms of expansive processes, leaf area, plant dry matter production and partitioning, relative water content, stomatal conductance, transpiration and photosynthesis. There were no significant differences in the response to water deficit between the octoploid and tetraploid plants. Relative leaf expansion, relative leaf appearance and relative stem elongation rates began to decline at a fraction of available soil water (FASW) thresholds of 0.46-0.48, 0.34 and 0.47-0.51, respectively. Water deficit caused a 3–10-fold reduction in total plant leaf area and a 2–4-fold reduction in plant dry weight. The leaf relative water content of drought-stressed plants relative to the watered ones began to decline at a FASW of 0.27-0.32 while stomatal closure and consequently reduction in transpiration occurred when the FASW declined to below 0.41-0.57. Thus, reduction in expansive growth processes as well as in stomatal conductance occurred before reduction in relative water content suggesting presence of root signals. Stomatal closure caused relatively less reduction in photosynthesis rate as compared to transpiration rate, thereby increasing the water use efficiency. In conclusion, both the octoploid and tetraploid responded to water deficit mainly by reduction in leaf area and stomatal closure.

**Keywords:** fraction of available soil water, leaf area, leaf expansion, relative water content, stem elongation, stomatal conductance

## INTRODUCTION

*Solanum villosum* Mill. ssp. *miniatum* (Bernh. ex Wild.) is an important African nightshade grown and consumed in Kenya and various African countries (Schippers 2000). Mostly growing wild, semi-wild or as weedy forms and volunteer plants in crop fields, African nightshades flower early and produce large amounts of fruits and seeds, probably as a survival adaptation. Domesticated production has remained largely on a kitchen garden scale, with low leaf yields (Edmonds and Chweya 1997; Chweya and Eyzaguirre 1999). Genetic improvement of the species has been recognized as an important research requirement for increased production (Edmonds and Chweya 1997). Ojiewo *et al.* (2006) have recently developed an octoploid ( $2n=8x=96$ ) *S. villosum* from wild type tetraploid ( $2n=4x=48$ ) plants using colchicine. The octoploid and tetraploid plants can be used in back-cross breeding schemes that will result in heteroploids such as pentaploids ( $2n=3x=60$ ). These are expected to be sterile hence could concentrate much of their growth in vegetative parts potentially increasing leaf yields. Increase in ploidy level can alter plant leaf characteristics, which can influence plants' responses to water deficit. Romero-Aranda *et al.* (1997) have shown that in oranges (*Citrus sinensis* (L.) Osb.), tetraploid plants had thicker leaves and lower gas exchange as compared to diploid plants. Tetraploid *Lolium* spp., plants have been shown to have larger leaf sizes than diploid plants and this was attributed mainly to a higher elongation rate (Sugiyama 2005). In *Betula papyrifera* seedlings, pentaploids and hexaploids were found to be more tolerant to water deficit

as compared to diploids (Li *et al.* 1996). In these seedlings, increasing ploidy level was associated with fewer stomata per unit leaf area, thicker lower and upper epidermis and increased pubescence, all which led to reduced transpiration. In addition, photosynthesis in the diploids was found to be more sensitive to water deficit. Erdei *et al.* (2002) found that within hexaploid cultivars of *Triticum aestivum*, 'Kobomugi' showed no decrease in fresh and dry mass, and had higher carbohydrate levels when subjected to osmotic stress as compared to 'Othalom' and 'Regina'. They also found that *T. dicoccum*, a tetraploid and *T. aestivum* cv. 'Kobomugi', a hexaploid showed increased root length under osmotic stress as compared to *T. monococcum*, a diploid, *T. durum* and *T. spelta*, both tetraploids.

The developed octoploid *Solanum villosum* has exhibited morphological differences from the tetraploid, tending to have larger stomata, fewer but larger leaves, and late flowering. The apparent large size of the octoploid raises concerns that it could be more sensitive to water deficit than the tetraploid parent. Plant size and morphological features have an important role to play in the plant response to soil moisture conditions. Blum and Sullivan (1997) have shown that in wheat (*Triticum aestivum* L. cv. 'Bersee'), small sized plants (plant height and shoot biomass) were affected relatively less in terms of stomatal closure and reduction in shoot biomass when exposed to top-root drying as compared to large sized plants. Blum *et al.* (1997) reported a higher osmotic adjustment in large plants of wheat as compared to small plants although both had similar leaf relative water content. They concluded that large plants were more sensitive to water deficit as compared to small plants. A tall

hybrid of sunflower (*Helianthus annuus* L.) has been shown to be less drought tolerant than dwarf varieties with the latter having a higher osmotic adjustment (Angadi and Entz 2002). However, this tall hybrid had a higher productivity under conditions of adequate water supply.

Leaf size plays an important role in drought adaptation. In rice (*Oryza sativa* L.), 'IR20', a small-leaved cultivar accumulated more abscisic acid (ABA) than in '63-83', a large-leaved cultivar (Henson 1983). ABA plays an important role in stomatal closure when plants are growing in a drying soil (Wilkinson *et al.* 1998; Holbrook *et al.* 2002). Eastern nightshade (*Solanum ptycanthum*), which has more stomata, larger but fewer leaves transpired more water than hairy nightshade (*Solanum sarrachoides*) under conditions of adequate water and light (Tan and Weaver 1997). Transpiration has been shown to be linearly related to leaf area in soybean (*Glycine max* (L.) Merr. 'Corsoy' (Eavis and Taylor 1979). Generally, a large leaf area exhausts soil water more rapidly in the event of terminal drought (Ludlow and Muchow 1990; Jones 1992).

Production of African nightshade in Kenya is mainly rainfed. Rainfall is erratic and hence exposes the crops to episodes of drought. This should be an important consideration during genetic improvement of these crops. Chaves and Oliveira (2004) noted the importance of improving cultural practices and crop genotypes for drought-prone areas as a way to achieve efficient water use in agriculture. Development of the octoploid *S. villosum* is a step towards genetic improvement of African nightshades. The wild type tetraploid *S. villosum* has been shown to have limited osmotic adjustment in the range of 0.16-0.19 MPa and responded to water deficit largely by reducing leaf area (Masinde *et al.* 2006). It is necessary to understand the mechanisms of response to water deficit by the octoploid *S. villosum* in order to provide information for its use as a new variety or a breeding parent. The objective of this study therefore was to evaluate the adaptive responses of the octoploid *S. villosum* to water deficit in terms of leaf area, plant dry matter production and partitioning, relative water content, stomatal conductance and transpiration. The hypothesis tested is that the apparent large size of the octoploid plants could increase their sensitivity to water deficit as compared to the tetraploid plants.

## MATERIALS AND METHODS

### Experimental site and design

Three pot experiments were conducted during January-February, April-June and June-August 2006 in the greenhouse at the Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT), (lat. 1°10'48'S, long. 37°07'12'E, 1525 m above sea level). Plants were grown under natural conditions. The January-February season was relatively warmer with maximum and minimum temperatures of 27.5°C and 12.4°C, respectively, total radiation of 559 MJ/m<sup>2</sup> and relative humidity (RH) of 40% at noon. During the April-June and June-August periods, the weather conditions were moderate with maximum and minimum temperatures of 24.1°C-24.8°C and 12.4°C-14.5°C, respectively, total radiation of 416-564 MJ/m<sup>2</sup> and RH of 52-55% at noon.

The pot experiments were set up in a completely randomized design with two genotypes, two water levels and three replications. A total of 96 pots were used, 48 for each genotype divided equally between the water levels. The genotypes were a wild type tetraploid (2n=4x=48) *S. villosum* obtained from western Kenya and an octoploid (2n=8x=96) produced from the wild type tetraploid (Ojiewo *et al.* 2006). Water levels consisted of drought and watered treatments. During the establishment of the plants, the soil moisture was kept high by daily watering of all pots. Water treatments began at two weeks after transplanting in the January-June experiments and three weeks after sowing in the June-August experiment, just before the onset of flowering. At the onset of the drought treatment, soil moisture in all pots was raised to 90%-95% pot water holding capacity (WHC). Thereafter, droughted pots received no more water while watered pots were irrigated daily to

maintain the soil moisture at 90%-95% WHC.

### Sowing details

Plastic pots with a capacity of 20 L were used in this study. During the January-February and June-August experiments, the pots were filled with soil obtained from a nursery area of the Department of Horticulture, JKUAT. The soil had bulk density of 1.00 g/cm<sup>3</sup> with gravimetric soil water content of 29.30% at 100% WHC. In the April-June experiment, a forest soil with bulk density of 0.49 g/cm<sup>3</sup> and gravimetric soil water content of 102.43% at 100% pot capacity was used. During the January-February and April-June experiments, seedlings were raised in small pots (10 cm diameter) for four-five weeks before being transplanted into the pots at a density of one seedling per pot. In the June-August experiment, about 5-10 seeds of the genotypes were sown directly into the pots. On emergence, the seedlings were then thinned to one seedling per pot two weeks after planting. Two weeks after transplanting (and three weeks after sowing), 1.0 g N/plant was applied using calcium ammonium nitrate (CAN, 26% N). The soil surface was then covered to a depth of about 5 cm with quartz gravel to minimize soil moisture evaporation.

### Soil moisture determination

The soil moisture was estimated by pot weight measurements and gravimetric methods. Water loss through transpiration was determined by weighing the pots daily. The difference in weights between two consecutive measurements was considered as the water lost through transpiration. Water lost was returned through irrigation to the watered pots. The weighing balance used had a low precision (10-100 kg ± 200 g). To determine soil moisture more precisely, soil samples were taken from the pots at a depth of 30 cm using a soil auger at the time of harvesting. The soils were then dried at 105°C for 48 hours and the gravimetric soil water content (G<sub>W</sub>) determined and expressed on a dry weight basis:

$$G_W = \frac{F_{WT} - D_{WT}}{D_{WT}} \quad (1)$$

where G<sub>W</sub> refers to the gravimetric soil water content, F<sub>WT</sub> refers to the fresh weight of soil and D<sub>WT</sub> refers to the dry weight of soil.

The plant available soil water was expressed as the fraction of available soil water (FASW) for each pot in the drought-stressed plants. FASW at day i for each pot was calculated as:

$$FASW = \frac{G_w \text{ at day } i - G_{Wend}}{G_{W100} - G_{Wend}} \quad (2)$$

where G<sub>Wend</sub> refers to the gravimetric soil water content at the end of the experiment when plants wilted and G<sub>W100</sub> refers to the gravimetric soil water content at 100% WHC.

### Expansive growth measurements

At the onset of the drought treatment, four plants per water level for each genotype were marked. These plants were used for daily measurements of leaf length (L) and width (W) at the widest part for leaf numbers 15, 16 and 17 (numbering from the bottom along the main stem). The product, LxW was used as an estimate of the leaf area. A linear relationship between LxW and actual leaf area (LA) measured by leaf area meter (model 3100, LI-COR Inc., Lincoln, NE, USA) was established (R<sup>2</sup>=0.98, n=98). The total number of leaves was counted daily on these plants as were measurements of plant height.

### Harvesting and relative water content measurement

Six harvests were done periodically; at the onset of water treatments, when average soil moisture content had dropped to about 80, 60, 50 and 40% WHC and lastly when plants wilted and did not recover for three days. During harvesting, plants were cut at

the base and separated into petioles, stems and blades. Leaf area was measured using a Licor 3100 leaf area meter. Roots were carefully picked by hand and washed. All plant parts were then dried at 100°C for 48 hours and weighed. Specific leaf area (SLA) was calculated as leaf area divided by the leaf dry weight. The SLA of drought-stressed plants was divided by that of watered plants to give the SLA ratio, which was then expressed as a function of FASW.

Leaf relative water content (RWC) was determined on plants that were being harvested. The fourth or fifth leaf from the youngest leaf on the main stem, which was fully formed was picked from the plants at 11.00-13.00 h, and quickly weighed to obtain the fresh weight. They were then placed in distilled deionised water in a Petri dish and left at 20°C in dim illumination for 24 hours. The turgid weight was obtained after blotting; thereafter the leaves were dried at 100°C to a constant weight to obtain the dry weight. RWC was computed as:

$$\text{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \quad (3)$$

The RWC of drought-stressed plants was divided by that of watered plants to give the RWC ratio, which was then expressed as a function of FASW.

### Gas exchange measurements

Stomatal conductance, transpiration and photosynthesis rate were measured using the portable photosynthesis system (CIRAS-1; PP System, Hitchin, Herts, UK). The measurements were done at 11.00-13.00 h on the fourth or fifth leaf from the youngest leaf on the main stem, which was fully formed and well exposed to sunlight. Measurements were done at 1-6 days interval. Values of plants in the drought treatment were divided by corresponding values in watered plants to give relative stomatal conductance, transpiration and photosynthesis rate, which were then expressed as functions of FASW.

### Data analyses

Statistical analyses were performed using the GLM procedure of SAS (SAS 1999). Analyses of variance were executed for each date separately for leaf area, plant biomass, specific leaf area, relative water content, stomatal conductance and transpiration. The relationships between relative parameters, i.e. SLA ratio, RWC ratio, relative stomatal conductance, relative transpiration rate and relative photosynthesis rate, and fraction of available soil water (FASW) were developed using a linear plateau regression using the non linear procedure of SAS:

$$\text{Relative parameter} = 1 \text{ if } \text{FASW} > \text{FASW}_t \quad (4)$$

$$\text{Relative parameter} = 1 + A \times (\text{FASW} - \text{FASW}_t) \text{ if } \text{FASW} < \text{FASW}_t \quad (5)$$

where A is the slope of the linear decline, and  $\text{FASW}_t$  is the FASW threshold at which the relative parameter began to decline.

$R^2$  values were calculated as:

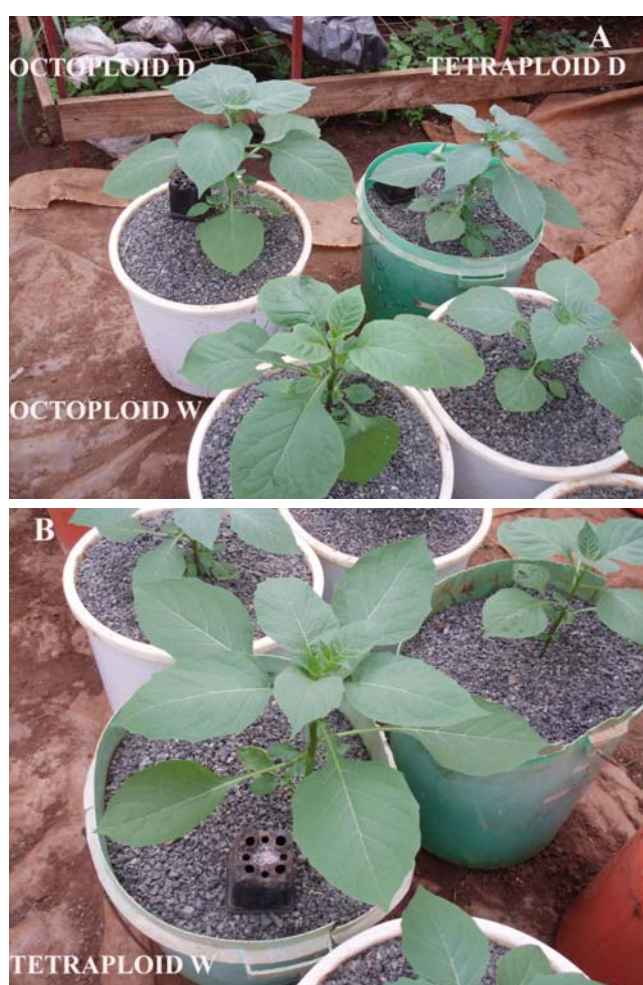
$$R^2 = 1 - \frac{\text{SSE}}{\text{CSE}} \quad (6)$$

where SSE is the sum of squares of the residual and CSE is the total corrected sum of squares.

## RESULTS

### Plant characteristics and soil moisture extraction

The octoploid had significantly fewer leaves and less fruit weight per plant than the tetraploid but both genotypes had a similar leaf area in the January-February and June-August experiments (Table 1, Photo 1). The gravimetric soil water content under watered conditions was kept at 30%, 84% and 25% on average during the January-February, April-June and June-August experiments, respectively (Fig. 1A-C). These corresponded to pot WHC ranges of 82-88%. Under drought conditions, there was a similar decline in gravimetric soil water content in both genotypes. Plants wilted when the content was about 9%, 40% and 11% in the three experiments, respectively. The soil moisture extraction characteristics in terms of fraction of available soil water



**Photo 1** Octoploid plants under drought stress (D) and watered conditions (W) and tetraploid plants under drought stress (A), and tetraploid plants under watered conditions (B), two weeks after the onset of drought treatment in the greenhouse in June-August at Juja, Kenya.

**Table 1** The number of leaves, fruit dry weight and leaf area at the end of the experimental period for octoploid and wild African nightshades (means across soil water levels) grown in the greenhouse in January-February, April-June and June-August 2006 at Juja, Kenya.

Genotypes	Experimental period							
	January-February		April-June			June-August		
	Fruit weight (g/plant)	Leaf area (cm <sup>2</sup> /plant)	Number of leaves/plant*	Fruit weight (g/plant)	Leaf area (cm <sup>2</sup> /plant)	Number of leaves/plant*	Fruit weight (g/plant)	Leaf area (cm <sup>2</sup> /plant)
Octoploid	1.05 a	4175.41 a	270 a	1.93 a	2472.91 a	125 a	0.34 a	3841.89 a
Wild	4.50 b	3172.83 a	385 b	3.02 b	1354.59 b	187 b	0.74 b	3407.68 a
LSD <sub>0.5</sub>	1.37	1232.30	66.2	0.66	524.24	19.3	0.20	572.87

\* Number of leaves was counted for the April-June and June-August periods only.

declined over time were similar between both genotypes under drought-stressed conditions (Fig. 1D-F).

### Leaf growth and stem elongation

The genotypes showed no significant differences in their response under the two water levels in terms of leaf and stem growth. The relative leaf expansion rate began to decline at the fraction of available soil water (FASW) range of 0.46-0.48 during the April-August experiments (Fig. 2A, 2B). Relative leaf appearance rate as a plateau function of the fraction of available soil water (FASW) was significant only in the June-August experiment, with the decline starting at a FASW of 0.34 (Fig. 2C, 2D). Relative stem elongation rate began to decline at a FASW of 0.47-0.51 (Fig. 2E, 2F).

Considering the whole plant leaf area and specific leaf area (SLA), there were no significant interactions between the water levels and the genotypes, hence only the main effects are shown. Plants in the drought-stress treatment had significantly lower leaf area 9, 25 and 20 days after the onset of drought in the January-February, April-June and June-August experiments, respectively (Fig. 3A-C). Leaf dry weight showed similar trends as the leaf area (Fig. 3D-F). Drought significantly reduced SLA, especially in mature plants (Fig. 4A-C). The SLA of the plants in the drought-stressed treatment relative to the plants in the watered treatment began to decline when the fraction of soil available water fell below 0.32-0.49 for both genotypes (Fig. 4D-F).

### Dry matter production and partitioning

There were no significant interactions between the water levels and the genotypes for dry matter production and partitioning. Plants in the drought-stressed treatment had significantly lower root (data not shown) and total plant dry weights than those in the watered treatment in the mature stages (Fig. 5A-C). At the onset of the drought treatment, the root to shoot ratio between watered and drought-stressed plants was similar. Towards the end of the experiments, plants in the drought treatment had a significantly higher root to shoot ratio than those in the watered treatment (Fig. 5D-F).

### Relative water content, stomatal conductance, transpiration rate and photosynthesis

Interaction between the genotypes and water levels for relative water content, stomatal conductance, transpiration rate and photosynthesis were not significant. The relative water content (RWC) in the watered plants was maintained between 75% and 90% in the three experiments (Fig. 6A-C). In the drought-stressed treatment, RWC in the early stages of the experiments was generally above 80% but declined to levels of 53%-66% (Fig. 6A-C). RWC of drought-stressed plants relative to the watered ones began to decline at a fraction of soil available water of 0.27-0.32 for both the octoploid and tetraploid genotypes (Fig. 6D-F).

In all the experiments, stomatal conductance and transpiration rate were similar between watered and drought-stressed plants at the onset of the treatments. Under severe water deficit, towards the end of the experiments, drought-stressed plants had a significantly lower stomatal conductance and transpiration rate compared to watered plants (Fig. 7A-D). Photosynthetic rate was lower in drought-stressed than watered plants towards the end of the experiments but the differences were not significant (Fig. 7E, 7F). The relative rates as a function of FASW were significant for relative stomatal conductance in the June-August experiment, and relative transpiration rate in the June-August experiment. The decline for both rates occurred at a FASW range of 0.41-0.57 (Fig. 8A-D). The relative photosynthetic rate did not show any significant functions with FASW (Fig. 8E, 8F). No functions were fitted for the April-June

experiments due to lack of plateau phases for both the relative stomatal conductance and transpiration rates.

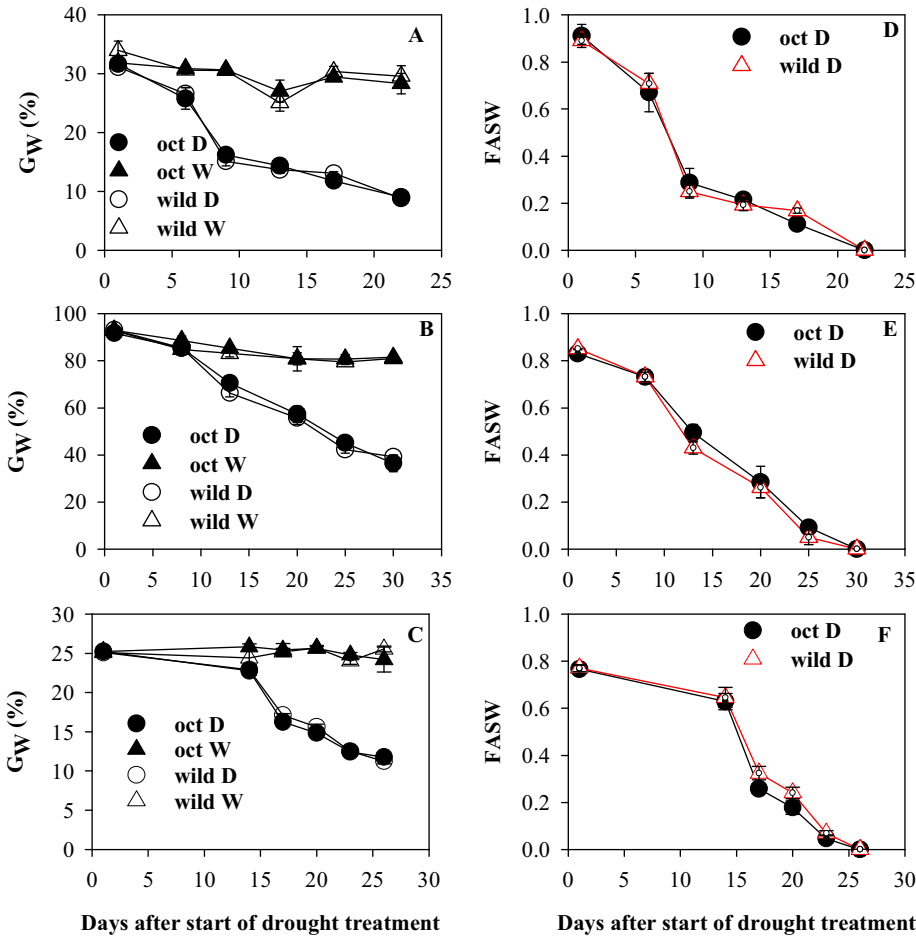
### DISCUSSION

The two genotypes differed in their leaf sizes, numbers and flowering characteristics. The octoploid had fewer but larger leaves than the tetraploid. It also tended to flower later and had less fruit load as compared to the tetraploid (Table 1). However, within the experimental period of this study, the differences in growth characteristics of the two genotypes did not translate into differences in soil water extraction (Fig. 1). Since the octoploid plants had larger leaves, it was hypothesized that water deficit could have more severe effects on its growth and yield. However, with fewer leaves per plant, its leaf area was similar to that of the tetraploid plants (Photo 1). Soil moisture extraction is known to be correlated with plant leaf area (Eavis and Taylor 1979; Jones 1992; Gómez del Campo 1999). Plants with a smaller leaf area have a slower rate of water use as compared to plants with a bigger leaf area (Sinclair and Muchow 2001).

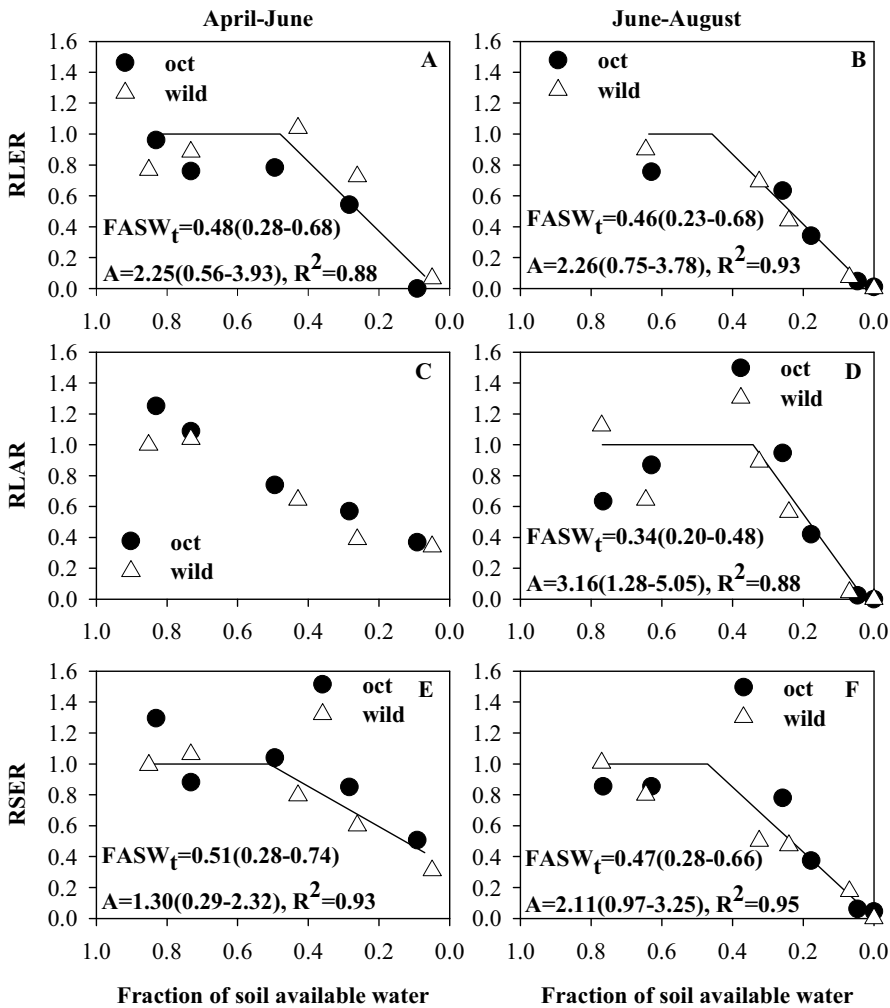
Leaf growth is important for yield of African nightshades considering that the edible part is the leaf. The impact of water deficit on leaf growth is therefore crucial for the productivity of these vegetables in drought-prone areas. Leaf area development is a combination of leaf emergence rate, the rate and duration of leaf expansion and the duration of individual leaves (Marcelis *et al.* 1998). In this study, the leaf area of *S. villosum* was considered to be dependant on the total number of leaves per plant and plant height, which was an indirect determinant of branching. Both the octoploid and the tetraploid showed similar sensitivities to water deficit in terms of leaf expansion, stem elongation and leaf appearance (Fig 2). The relative leaf expansion and stem elongation rates (RLER, RSER, respectively) remained close to 1.0 until the fraction of available soil water (FASW) fell below the range of 0.46-0.51 when it declined linearly. For the relative leaf appearance rate (RLAR), a linear decline occurred at a FASW of 0.34. This suggests that the plant expansive processes, defined by leaf expansion and stem elongation, were more sensitive to water deficit than leaf emergence. The FASW threshold for leaf expansion and stem elongation for *S. villosum* is similar to values of 0.40-0.45 reported in pea (*Pisum sativum* L.), tomato (*Lycopersicon esculentum* Mill. cv. 'Aisla Craig') and sorghum (*Sorghum bicolor* (L.) Moench), (Rosenthal *et al.* 1987; Lecoeur and Sinclair 1996; Sobeih *et al.* 2004), while for sunflower (*Helianthus annuus* L.), the threshold was in the range of 0.5-0.8 (Sadras *et al.* 1993).

In vegetable amaranth (*Amaranthus* spp.), leaf expansion has been shown to be more sensitive to soil water deficit, declining at soil moisture thresholds of 0.67-0.77 (Liu and Stützel 2002). On the other hand, Muchow and Sinclair (1991) reported a lower threshold of 0.3 for relative leaf area development in maize (*Zea mays* L.). While leaf appearance in this study appeared relatively less sensitive to water deficit, in grapes (*Vitis vinifera* L.), leaf appearance declined at soil moisture thresholds of 0.32-0.57, with higher sensitivity on the branches as compared to the main stem (Lebon *et al.* 2006).

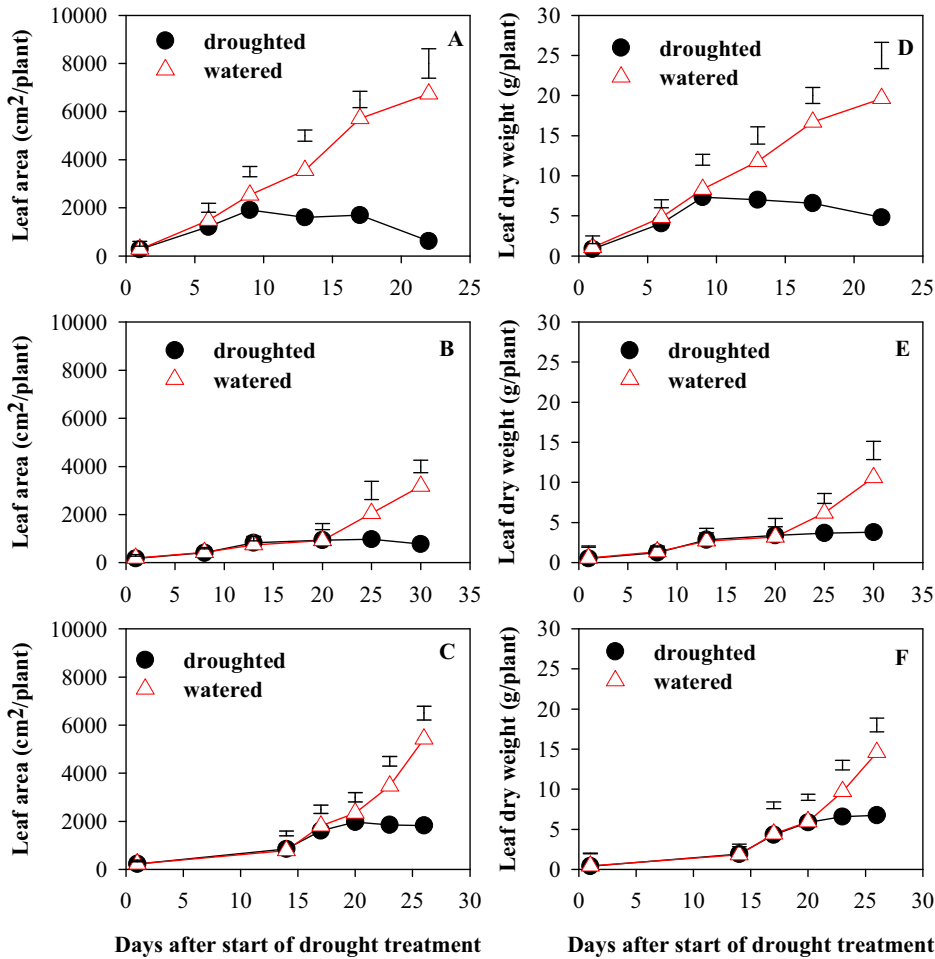
Water deficit caused reductions in total plant leaf area ranging 10-, 4- and 3-fold in the January-February, April-June and June-August experiments, respectively (Fig. 3A-C). The higher reduction in January-February was mainly due to leaf shedding which was experienced at the last harvest. Leaf dry weight showed a 2-4 fold reduction in leaf dry weight under water deficit (Fig. 3D-F). This translated into a reduction in specific leaf area (SLA) as the water deficit intensified with FASW thresholds of 0.32-0.49 (Fig. 4). At the plant level, this reduction in leaf area was a combination of reduced leaf expansion, leaf emergence and stem elongation (branch formation). This result suggests that both the octoploid and tetraploid make drastic reductions in leaf area under water deficit conditions to cut down



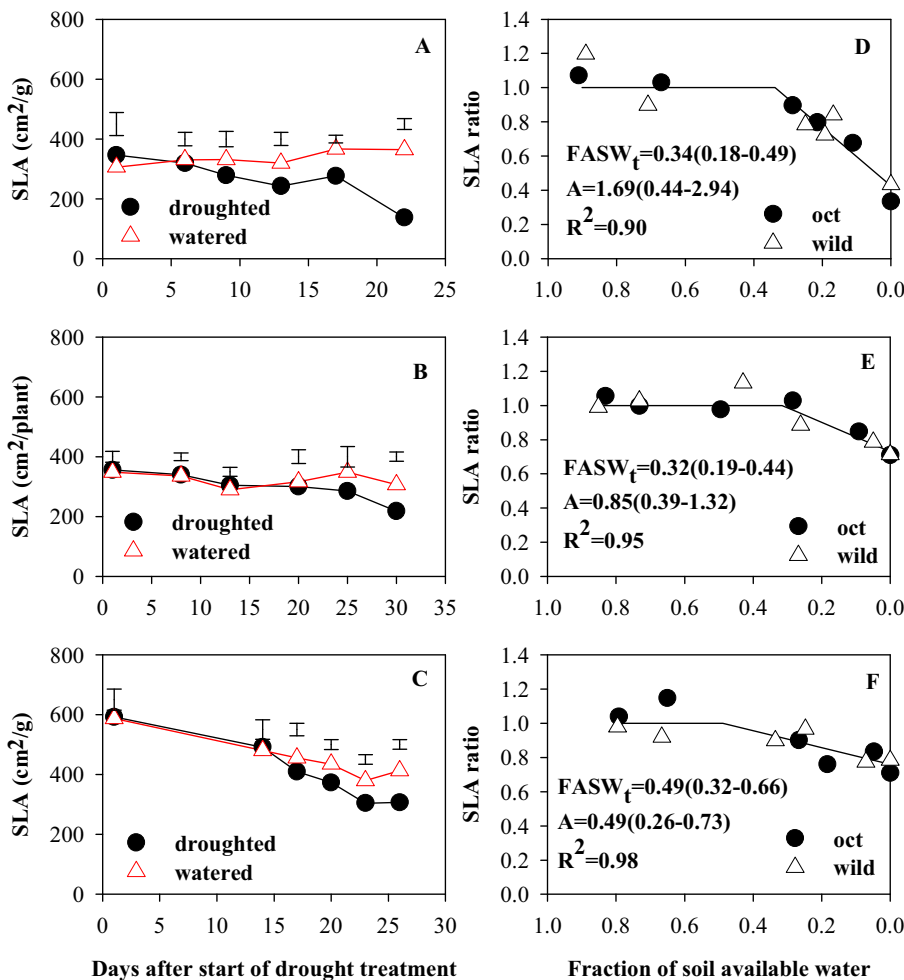
**Fig. 1** The gravimetric soil water content ( $G_w$ %) and fraction of soil available water (FASW) for the octoploid (oct) and tetraploid (wild) genotypes of African nightshade grown under drought-stressed (D) and watered (W) conditions in January-February (A, D), April-June (B, E) and June-August (C, F) 2006 at Juja, Kenya. Vertical bars show SE ( $n=3$ ).



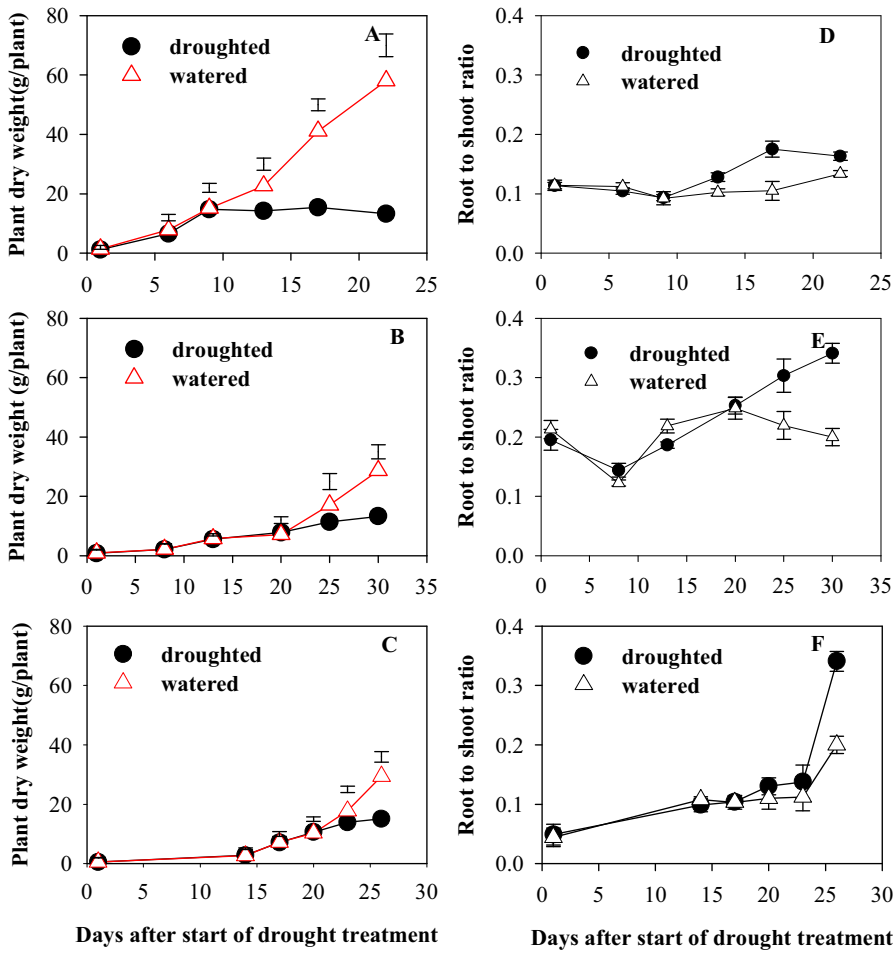
**Fig. 2** The relative leaf expansion rate (RLER), relative leaf appearance rate (RLAR) and relative stem elongation rate (RSER) as functions of fraction of soil available water for the octoploid (oct) and tetraploid (wild) genotypes of African nightshade grown in the greenhouse in April-June (A, C, E) and June-August (B, D, F) 2006 at Juja, Kenya. Lines show plateau regression functions. The fraction of available soil water threshold ( $FASW_t$ ) and slope of the decline (A) with their 95% confidence intervals (in parentheses) are shown in the figure.



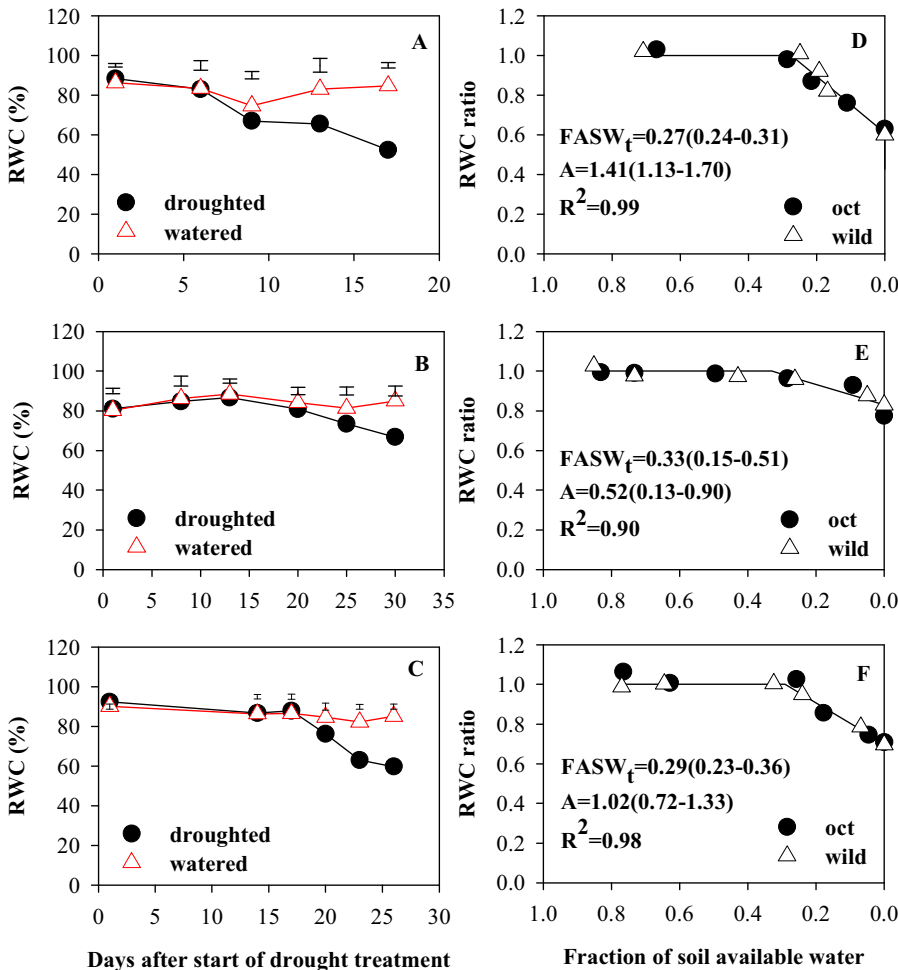
**Fig. 3** The leaf area and leaf dry weight of African nightshade grown in the greenhouse under drought-stressed and watered conditions in January-February (A, D), April-June (B, E) and June-August (C, F) 2006 at Juja, Kenya. Vertical bars show LSD<sub>0.05</sub>.



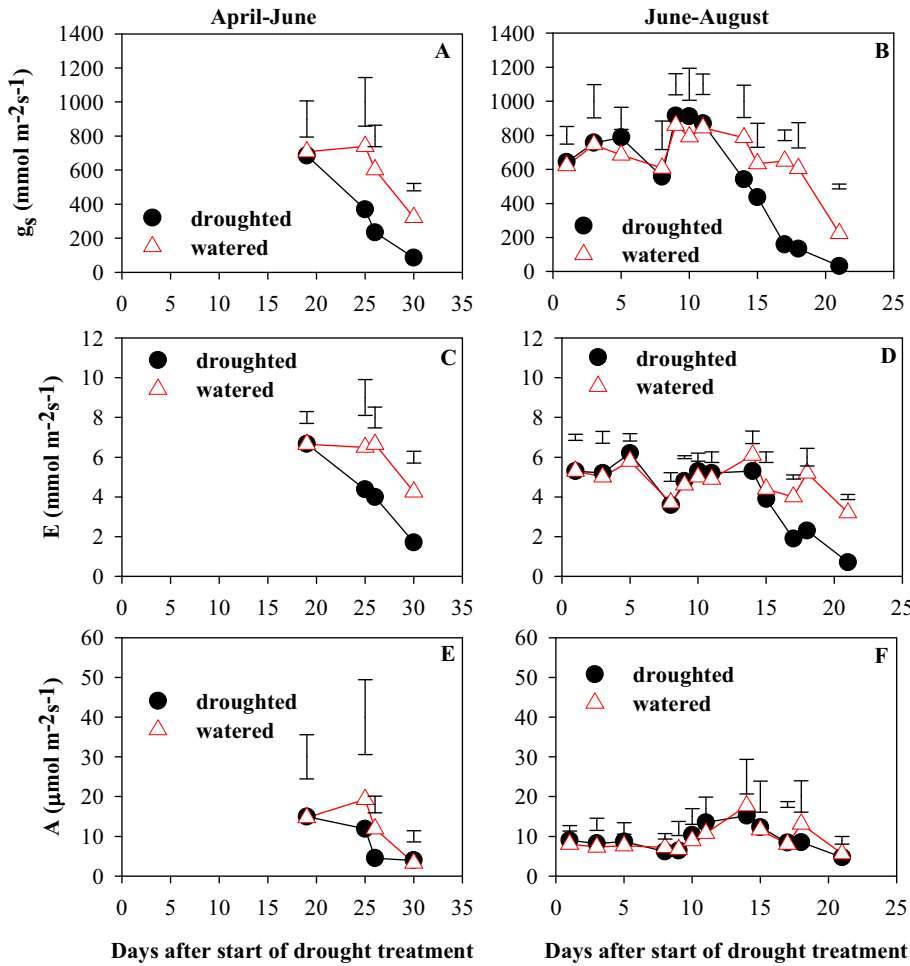
**Fig. 4** The specific leaf area (SLA) and specific leaf area ratio (SLA ratio) between drought-stressed and watered plants for the octoploid (oct) and tetraploid (wild) genotypes of African nightshade grown in the greenhouse in January-February (A, D), April-June (B, E) and June-August (C, F) 2006 at Juja, Kenya. Vertical bars show LSD<sub>0.05</sub>. Lines show plateau regression functions. The fraction of available soil water threshold (FASW<sub>t</sub>) and slope of the decline (A) with their 95% confidence intervals (in parentheses) are shown in the figure.



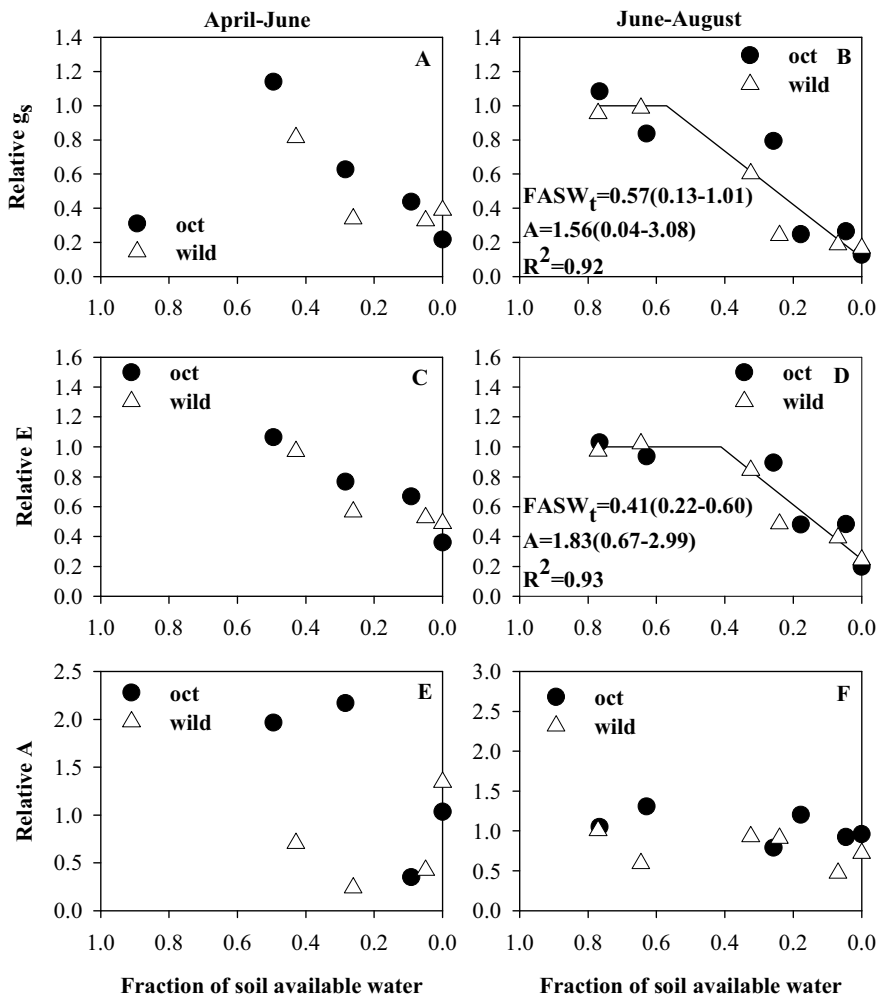
**Fig. 5** The plant dry weight and root to shoot ratio of African nightshade grown in the greenhouse under watered and drought-stressed conditions in January-February (A, D), April-June (B, E) and June-August (C, F) 2006 at Juja, Kenya. Vertical bars show  $LSD_{0.05}$  for plant dry weight and SE (n=6) for root to shoot ratio.



**Fig. 6** The relative water content (RWC) and RWC ratio between drought-stressed and watered plants for the octoploid (oct) and tetraploid (wild) genotypes of African nightshade grown in the greenhouse in January-February (A, D), April-June (B, E) and June-August (C, F) 2006 at Juja, Kenya. Vertical bars show  $LSD_{0.05}$ . Lines show plateau regression functions. The fraction of available soil water threshold (FASW<sub>t</sub>) and slope of the decline (A) with their 95% confidence intervals (in parentheses) are shown in the figure.



**Fig. 7** Stomatal conductance,  $g_s$  (A, B), transpiration rate,  $E$  (C, D) and photosynthetic rate,  $A$  (E, F) of African nightshade grown in the greenhouse under drought-stressed and watered conditions in April-August 2006 at Juja, Kenya. Vertical bars show LSD<sub>0.05</sub>.



**Fig. 8** The relative stomatal conductance, transpiration rate and photosynthesis rate as functions of fraction of soil available water for the octoploid (oct) and tetraploid (wild) genotypes of African nightshade grown in the greenhouse in April-August at Juja, Kenya. Lines show plateau regression functions. The fraction of soil available water threshold ( $FASW_t$ ) and slope of the decline ( $A$ ) with their 95% confidence intervals (in parentheses) are shown in the figure.



on transpiration. Plants facing water deficit reduce their leaf area, which helps to conserve water but at the same time reduces productivity through reduced light interception (Ludlow and Muchow 1990). The reduction in SLA maybe due to the relatively larger reduction in leaf area than in leaf dry weight, as well as smaller and thicker leaves. Plants occurring in dry environments such as *Aegilops* spp. are thought to benefit from making thicker leaves hence a lower SLA (Bultynck *et al.* 2006). A smaller SLA suggests a smaller leaf area and thicker leaves, hence reduced transpiration and better adaptation to drought stress. Romero-Aranda *et al.* (1997) found a higher leaf tissue density, the reciprocal of SLA, in tetraploid as compared to diploid 'Valencia' sweet oranges (*Citrus sinensis* (L.) Osb.). This implied a decrease in SLA with increase in ploidy level. They, however, did not observe a similar response in 'Femminello' lemon (*C. limon* (L.) Burm. f.). In this study, ploidy level had no effect on the SLA of African nightshade under watered and drought stress conditions.

Plant dry weight was reduced by water deficit 2-4 fold, which could be attributed to the large reductions of leaf area (Fig. 5A-C). Leaf area is important in light interception and hence dry matter production (Jones 1992). In addition, plants under drought-stressed conditions tended to allocate more dry matter to the roots than those in watered conditions as shown by the higher root to shoot ratios in both the tetraploid and octoploid (Fig. 5D-F). Marcelis (1993) reported that in various greenhouse crops dry matter distribution towards the roots increases with decreasing supply of water. The increase in the root to shoot ratio for both the octoploid and tetraploid African nightshades could also be due to a higher sensitivity of the shoot to water deficit, experiencing about 50% reduction as compared to 40% for the root. Blum and Sullivan (1997) also reported a higher sensitivity of the shoot to water deficit as compared to the root in wheat (*Triticum aestivum* cv. 'Bersee'). They found that under stress, shoot dry weights declined while root dry weights increased. In lupin (*Lupinus albus* L.), drought led to a significant increase in the root length density and to a non-significant increase in the root dry weight (Rodrigues *et al.* 1995).

Relative water content (RWC) provides a measure of the leaf water status. The well watered plants maintained a RWC above 75% which is reported to be the range for plants that are well watered (Sinclair and Ludlow 1985). Stress development within the leaves coincided with the fraction of available soil moisture (FASW) of 0.27-0.33 (Fig. 6). Leaf expansion and stem elongation began to decline at higher FASW range than RWC. It appears that the reaction of the expansive processes could have been triggered by causes other than changes in RWC.

Stomatal conductance and transpiration rates declined significantly as water deficit intensified (Fig. 7). Lack of plateau phases for both the relative stomatal conductance and transpiration rates as functions of FASW for the April-June experiment was due to long measurement intervals. This resulted in insufficient data points especially in the early stages of the experiment. For the June-August experiment, the declines in the relative stomatal conductance and transpiration rates occurred at a FASW range of 0.41-0.57, which was above the range for RWC (Fig. 8). Reduction in leaf and stem growth, stomatal conductance and transpiration rate in both octoploid and tetraploid African nightshades occurring before changes in the RWC suggests the presence of root signals in the drying soil.

Plants growing in a drying soil can produce chemical root signals, which are transported to the shoot to regulate stomatal behavior and expansive growth independent of hydraulic signals (Davies *et al.* 2002). Plant growth and gas exchange declining before changes in RWC or leaf water potential under drought stress has been reported in various crops. In cucumber (*Cucumis sativus* L.), Melkonian and Wolfe (1995) reported a decrease in stomatal conductance before predawn leaf water potential decline when the plants were exposed to water deficit. However, they ruled out

effects of root signals using further experiments. Onset of stomatal closure in lupin (*Lupinus cosentinii*) occurred before any significant changes in leaf water potential and relative water content, suggesting presence of root signals (Henson *et al.* 1989). Liu *et al.* (2005) have also found significant reduction in stomatal conductance of potato (*Solanum tuberosum* L.) before declines in RWC and leaf water potential under soil drying conditions. Under slow soil drying, leaf growth, specific leaf area and stomatal conductance of rape (*Brassica napus* L.) decreased before significant fall in leaf water potential while under fast soil drying stomatal closure occurred nearly simultaneously with decreasing leaf water potential (Jensen *et al.* 1996). These results were attributed to root signals under slow soil drying and hydraulic signals under fast soil drying. Root signals may consist of the hormone abscisic acid (ABA), (Davies *et al.* 2002; Medrano *et al.* 2002), precursors of ABA, cytokinins and xylem pH (Holbrook *et al.* 2002).

The range of FASW at which stomatal conductance and transpiration in both the octoploid and tetraploid *S. villosum* declined is similar to that reported in tomato (*Lycopersicon esculentum* Mill. cv. 'Aisla Craig'), where the decline in stomatal conductance of partial root drying plants occurred when the soil moisture of the drying soil had decreased by 45% (Sobeih *et al.* 2004). This is also similar to the threshold of 0.45 reported for stomatal conductance in grapes (Lebon *et al.* 2006). In maize, stomatal closure occurred at a fraction of transpirable soil water range of 0.36-0.60 for various hybrids (Ray and Sinclair 1997).

The photosynthetic rate of *S. villosum* showed only a slight but non-significant decline under drought. Consequently, the correlation between FASW and photosynthetic rate was low. In effect, the water use efficiency computed as the ratio of photosynthetic rate to the transpiration rate therefore was significantly higher in drought-stressed plants considering the significant decline in stomatal conductance. Cases of photosynthetic rate being less sensitive than stomatal conductance during soil drying have also been reported in potato (Liu *et al.* 2005), lupin (Henson *et al.* 1989) and rape (Jensen *et al.* 1996). Monti *et al.* (2005) showed a stronger correlation between stomatal conductance and soil drying as compared to photosynthetic rate and soil drying in chicory (*Cichorium intybus* L.). *S. villosum* could benefit from the concept of partial root drying (PRD) in water management of crops. PRD is based on the theory that part of the root system in drying soil sends root signals to the shoot thereby closing the stomata to reduce transpiration. This partial closure of the stomata may reduce transpiration substantially with little effect on photosynthesis. Kang and Zhang (2004) have found that maize (*Zea mays* L.) plants grown under PRD had similar photosynthesis rate as plants grown under conventional irrigation. In addition, the maize plants under conventional irrigation had higher transpiration rate compared to those under PRD.

In conclusion, both the octoploid and tetraploid had similar adaptive responses to water deficit despite showing differences in individual leaf size and flowering characteristics. This was mainly due to the fact that both had similar overall plant size in terms of leaf area and dry weight. Both of them responded to water deficit mainly by large reductions in leaf area attributed to reduction in expansive processes i.e. individual leaf expansion and stem elongation, and leaf appearance. Stomatal conductance also declined with a corresponding reduction in transpiration. This however, did not cause significant reduction in photosynthesis, thereby increasing the water use efficiency. The reduction in expansive processes as well as stomatal conductance occurred before reduction in relative water content suggesting presence of root signals. Physiological reactions in terms of growth processes and gas exchange of the plants occurred when the available soil moisture fell below 0.40-0.60 while for leaf water status this occurred at about 0.30.

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