Growth and yield responses of two ginger species to different levels of nitrogen

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Abstract

Nitrogen (N) is a critical determinant of plant growth and productivity, but there is limited information on the agronomic parameters of medicinal plants. Three levels of N fertiliser (0, 1.375 and 1.625 g plant−1) were used to investigate the effects of fertilisation on the growth and yield parameters of two ginger species (Siphonochilus aethiopicus and Zingiber officinale). The experiment was conducted in the glasshouse with six treatments (two ginger species and three levels of N fertiliser) arranged in a randomised complete block design and replicated four times. The results indicated that N application significantly affected plant height, leaf number, chlorophyll content and rhizome yield of the species for two cropping seasons. Scanning electron microscopy analyses of stomata opening revealed that a higher N level increased the number of open stomata in both ginger species. The results showed that with application of 1.625 g N plant−1 both ginger growth and yield were higher compared with the lowest N fertiliser application levels. However, the growth parameters of Z. officinale were variably greater than those of S. aethiopicus. Therefore, the present study demonstrates that application of 1.625 g N plant−1 to commercial ginger and African ginger can be used to improve growth and yield.

Keywords: ginger, growth, nitrogen fertiliser rate, nitrogen use efficiency, yield

Introduction

Owing to their ability to produce bioactive compounds used in a wide variety of medicines and food products, medicinal plant derivatives are considered important sources of active ingredients to be used in drug development and synthesis. The demand for medicinal plants has increased enormously due to use of biologically active compounds in food, the pharmaceutical and health-care industries (Singh et al. 2014). The growing importance and utilisation of medicinal plants can be valued from the economic outlook with global trade in herbs estimated at over US$14 billion per annum and projected to increase to US$5 trillion in 2050 (Bhowmik et al. 2009).

African ginger *(Siphonochilus aethiopicus)* and commercial ginger (*Zingiber officinale*) are members of the Zingiberaceae family used in various food ingredients and medicines. Both plant species produce underground rhizomes varying in shape (Figure 1). The species have been reported to possess antidiabetic properties, antioxidant properties and volatile oils (Ghasemzadeh et al. 2010). These species have immense potential for economic development and poverty reduction through income generation for smallholder farmers. The production and cultivation of medicinal plants is not fully utilised to its potential because of lack of improved agronomic practices. Among agronomic practices employed in medicinal plant production, nitrogen (N) is a key measure in improving growth and yield. Nitrogen regulates plant metabolic processes and is critical to synthesise amino acids, which are the building elements of protein, nucleotides, chlorophyll, and numerous other metabolites and cellular components (Nunes-Nesi et al. 2010). The application of N fertilisers at the correct recommended rate and timing has potential to improve growth and yield (Chaturvedi 2006). In contrast, high N application has been found to decrease secondary metabolites in medicinal plants (Ibrahim et al. 2011). Optimum N fertilisation has a favourable effect on root growth and distribution in the soil (Wang et al. 2014). The application of N and other elements (phosphorus [P] and potassium [K]) significantly increases vegetative growth of moringa plants (Isaiah 2013). In addition, N fertilisation significantly increases plant height, fresh weight and dry weight of *Ficus deltoidea* (Sheikh and Ishak 2016).

Although studies of cultivation techniques in medicinal plants have been undertaken previously, the agronomic and physiological responses to different N levels are often still unknown. Due to increasing demand for ginger species for medicinal purposes, it is therefore necessary to investigate ways to improve its growth and quality. Therefore, the aim of this study was to investigate the effect of different N levels on the growth and yield of two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) under controlled conditions.

Materials and methods

Study site and treatments

A glasshouse experiment was conducted at the Experimental Farm of the University of Pretoria, South Africa. Two ginger species, African ginger (*Siphonochilus aethiopicus*) and commercial ginger (*Zingiber officinale*) (Figure 1) were grown for two cropping seasons (2015/16

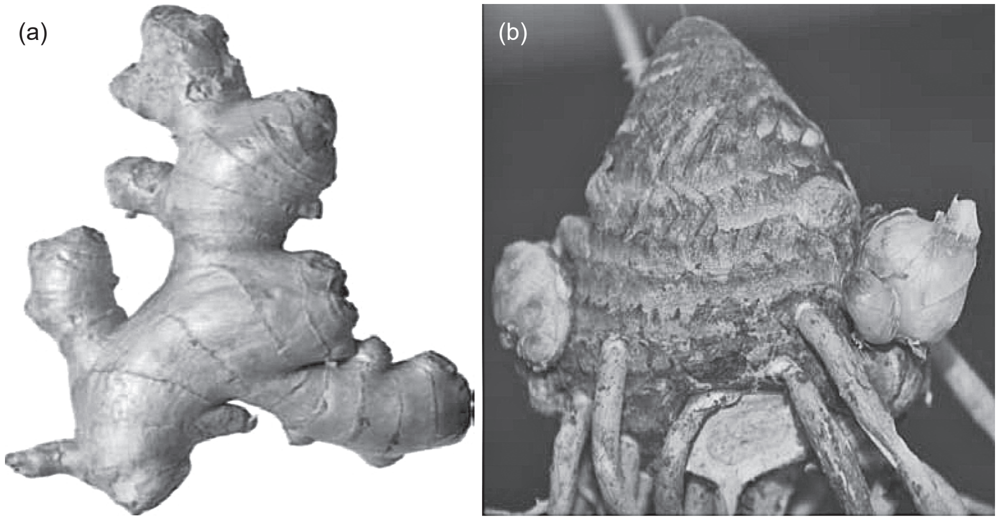

Figure 1: Rhizomes of the two ginger species studied. (a) Commercial ginger (*Zingiber officinale*) and (b) African ginger (*Siphonochilus aethiopicus*)

and 2016/17). Six treatments were arranged in a randomised complete block design replicated four times. Artificial growth media (perlite and coir medium) were used and supplemented with N fertiliser at different rates (Table 1). Ginger rhizomes (*S. aethiopicus* and *Z. officinale)* weighing 20–30 g were planted in polypropylene bags filled with 30 L perlite. Three months after planting, once the rhizomes were enlarged, 20 L coir medium was added on top of the perlite to increase the total medium volume to 50 L (Figure 2). Phosphorus and potassium were applied as a basal application at a rate of 1.312 g/plant. Three months after planting, N treatments were applied at the rate of 0, 1.375 or 1.625 g plant^{-1}. Nitrogen was applied in the form of limestone ammonium nitrate (LAN; 28%), P as 14% superphosphate and K as 50% potassium chloride. Plants were irrigated three times a week using drip irrigation for 30 min at each application.

Table 1: Glasshouse experiment design for ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) in response to nitrogen levels. CG = commercial ginger, AG = African ginger

Ginger species	Treatment (g N plant^{-1})	Treatment equivalent (kg N ha^{-1})
CG	0	0
	1.375	220
	1.625	260
AG	0	0
	1.375	220
	1.625	260

Temperature monitored in the glasshouse during the experiment

Temperature (*T*) data were obtained from an automatic portable data HOBO logger (MicroDaQ.com Ltd, Contoocook, NH, USA). The data logger was installed in the glasshouse at the experimental site. The monthly means over the two cropping seasons starting in February and ending in August are presented in Figure 3.

Growth parameters

Growth parameters, i.e. plant height, number of stems and leaves per plant were measured and sampled on a monthly basis for each treatment. Plant height (cm) was measured from the base of the plant to the apex using a measuring tape. Number of stems and leaves were monitored by manual counting. Data on plant growth were measured at monthly intervals for both seasons.

Figure 2: Plants of African ginger (a) and commercial ginger (b) growing under glasshouse conditions

Plant chlorophyll content

Plant chlorophyll content was measured once a month. Three leaves were marked for data representation and chlorophyll measurements were taken at different stages of plant development. Chlorophyll was measured using a SPAD meter (Minolta, Osaka, Japan) to estimate plant N status.

Open stomata parameter

Fresh leaves were collected from each polypropylene bag to evaluate the stomatal variation between the treatments. A leaf area of 10 mm × 10 mm was cut from each sample and fixed immediately in 30% (w/v) aqueous glutaraldehyde solution in 0.05 M phosphate buffer (pH 7.0). The samples were then post-fixed in osmium tetraoxide (1%, w/v) for 2 h and dehydrated in an ethanol concentration series ranging from 30%, 50%, 70%, 90% and 100% (w/v) for 15 min and repeated three times (Eiasu et al. 2012). The samples were dried in a critical point drying apparatus (Bio-Rad e300, Watford, UK). After drying, the samples were mounted on copper stubs and coated with gold in a vacuum coating unit (Polaron E5200C, Watford, UK). To observe the total and open stomata numbers, the coated samples were analysed under a JSM 840 scanning electron microscope (JEOL, Tokyo, Japan) at 350× magnification.

Yield

All plants in each pot replicate were labelled and sampled for yield. Yield was measured at harvest 10 months after planting. Rhizomes were removed from the polypropylene bags and the fresh rhizomes were weighed. The rhizomes were dried in an oven at 50 °C for 3 d, and the dry weights were recorded.

Nitrogen-use efficiency

Nitrogen-use efficiency (NUE) is the N accumulation capacity from the initial plant parts to the harvested plant parts (Roberts 2008). Both fresh and dry rhizomes were used to calculate NUE using the formula:

$$AE = (Y - Y_o)/F$$

where AE is agronomic efficiency, Y is crop yield with applied N (g plant^{-1}), Y_o is the control treatment without N application, and F is the amount of N fertiliser applied (g plant^{-1}).

Data analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.4 (SAS Institute, Cary, NC, USA). Significantly different means were separated using Duncan's multiple range test ($p \leq 0.05$).

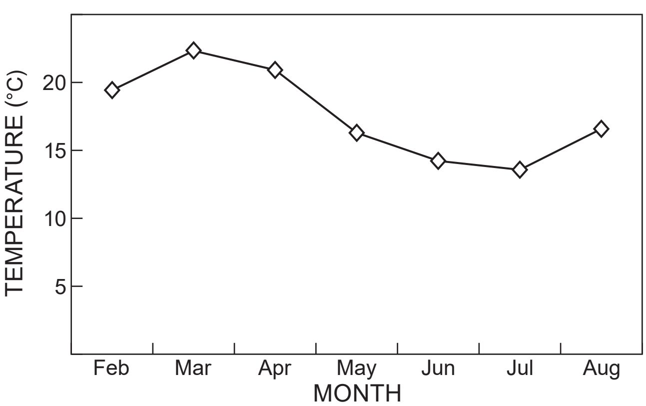

Figure 3: Monthly mean air temperature (°C) recorded during the experimental period

Results and discussion

Growth parameters

Plant height

The effects of N fertiliser levels on height of the two ginger species during the 2015/16 and 2016/17 seasons are presented in Figure 4a and b. The results showed significant differences ($p < 0.05$) between the two species and treatments. Commercial ginger recorded the highest plant height with the application of 1.625 g N plant^{-1} for both cropping seasons. African ginger also exhibited increased plant height with the application rate of 1.625 g N plant^{-1} in both seasons. However, the heights were significantly lower compared with commercial ginger in all treatments. Consistent with the study hypothesis, the control (0 N) exhibited reduced plant height for both species and cropping seasons (Figure 4a and b). Decreased plant biomass in response to 0 N was previously reported for *Pelargonium sidoides* (Mofokeng et al. 2015). Across the treatments in this study, N addition showed no significant effect on plant height for the two years, indicating that season would not be the primary limiting factor for N application to the two species.

The increased plant height with increase in N application may be attributed to the use of N by plants for cell elongation during active cell division. In addition, N increases the frequency of division of meristematic cells and thus increases plant height (Kavanova et al. 2008). Attoe and Osodeke (2009) attributed the increase in height of

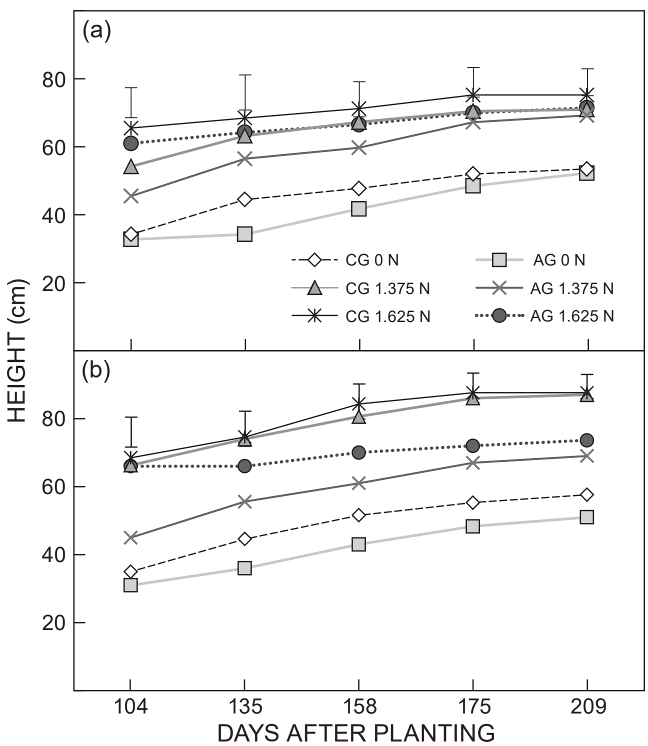

Figure 4: Plant height in response to different nitrogen (N) application rates in two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during the (a) 2015/16 and (b) 2016/17 cropping seasons. Error bars indicate LSD values. CG = commercial ginger, AG = African ginger

Z. officinale to increased N application. The increase in plant height from 104 to 209 d after planting with N application at different rates may be associated with the accumulation of nitrate and proteins as the main forms of N used in vegetative plant tissues.

Number of tillers per plant

One-way ANOVA showed that the number of tillers per treatment for the two species was significantly affected by N level during the two cropping seasons (Figure 5a and b). Commercial ginger exhibited the highest tiller number with application of 1.375 and 1.625 g N plant^{-1} for both cropping seasons. In comparison with the 0 N treatment, the number of tillers per plant was lower from 104 to 209 d after planting for African ginger during both cropping seasons. Likewise in both cropping seasons, 0 N application reduced the number of tillers per plant for commercial ginger compared with that of treated plants *(*Figure 5a and b). The results show that N application improved number of tillers per planting from 135 to 209 d after planting for both ginger species and cropping seasons. The differences in number of tillers between the two species gradually increased with increasing N application rate (Figure 5a and b). Application of the highest levels of N also increases the number of tillers for various plant species (Crook and Ennos 1995). The promotive effect of N on the number of tillers can be explained on the basis of N supply increasing the number of meristematic cells and their growth leading to the formation of shoots (tillers) in addition to leaf expansion and number (Lawlor 2002). Asafa and Akanbi (2018) attributed the increase in growth parameters of *Z. officinale* to the application of N fertiliser. However, previous reports have shown that in terms of tillers, other factors also contribute to productivity (James 2008). Furthermore, different conclusions on yield-related parameters (number of leaves and propagule weight) have been reported in which these parameters did not increase or decrease following the application of N fertiliser (Asafa and Akanbi 2018).

Leaf number

The number of leaves per plant were influenced by N fertilisation rates ($p < 0.05$). It was observed that the application of 1.625 and 1.375 g N plant^{-1} increased the production of leaf number in commercial ginger for the two cropping seasons (Figure 6a and b). The number of leaves was also enhanced for African ginger with the application of 1.625 g N plant^{-1}, although the number of leaves was lower (Figure 6a and b). It should be considered that the absorption and assimilation of N by the two ginger species might vary according to genotypic factors. The contribution of leaf N remobilisation to various plants is reported to be cultivar dependent, varying from 50% to 90% (Masclaux-Daubresse et al. 2008).

The present results showed an increasing trend in the number of leaves per plant with days after planting. The data showed an enormous increase from 158 to 209 d after planting with the application of 1.375 and 1.625 g N plant^{-1} for commercial ginger during the 2016/17 cropping

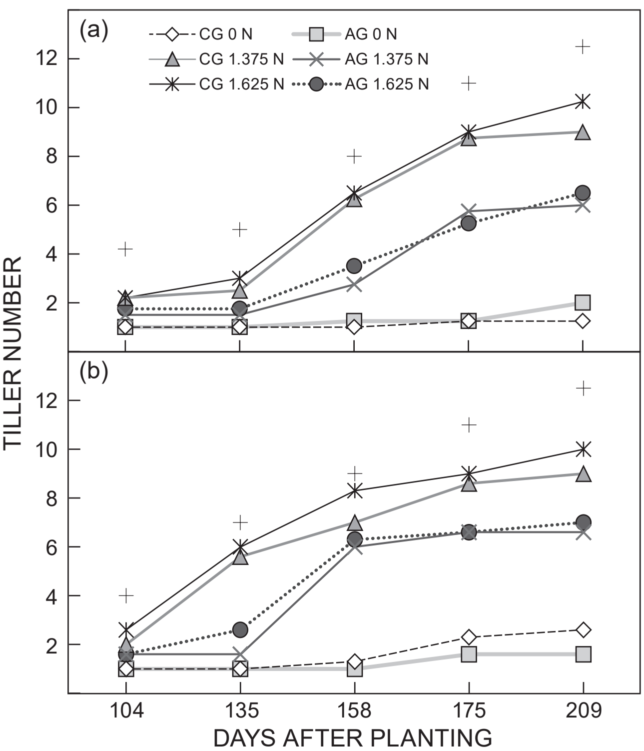

Figure 5: Number of tillers in response to different nitrogen (N) application rates for two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during the (a) 2015/16 and (b) 2016/17 cropping seasons. CG = commercial ginger, AG = African ginger. Crosses (+) indicate LSD values

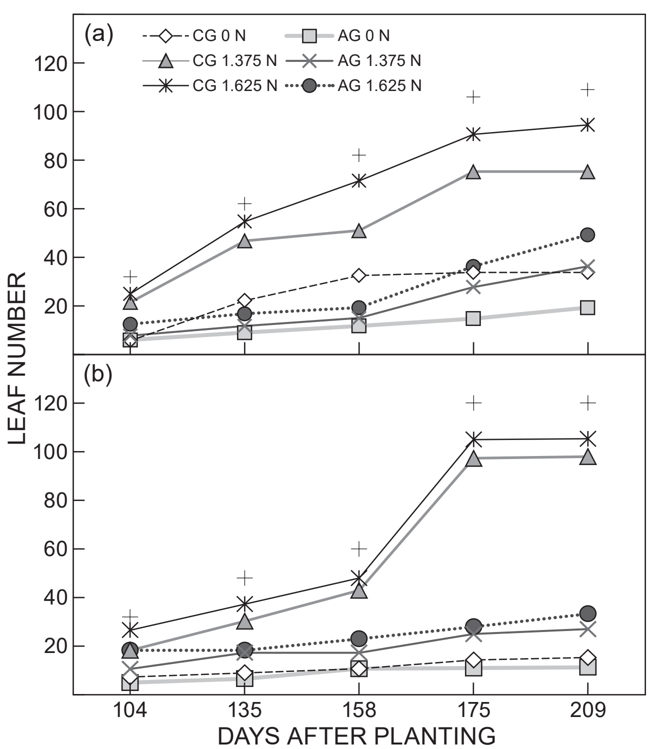

Figure 6: Leaf number in response to different nitrogen (N) application rates for two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during the (a) 2015/16 and (b) 2016/17 cropping seasons. CG = commercial ginger, AG = African ginger. Crosses (+) indicate LSD values

season (Figure 6b). Dohleman et al. (2012) observed higher aboveground plant biomass in response to greater N concentrations. In contrast, the addition of N did not evidently promote mass accumulation and leaf longevity in *Eucalyptus* plants compared with those in response to P fertilisation (Laclau et al. 2009). Murillo-Amador et al. (2006) attributed the decrease in growth and leaf biomass to competitive inhibition between ions of the fertiliser applied. Jaćimović et al. (2010) observed no effect of increase in N dose on shoot and leaf dry mass of sweet basil.

Chlorophyll content

The chlorophyll content differed significantly ($p < 0.05$) among the two species and N application rates. Maximum chlorophyll content was observed with the application of 1.625 g N plant^{-1} for African ginger during the 2016/17 cropping season (Figure 7b). For both African ginger and commercial ginger, SPAD leaf readings at different days after planting showed lower values for non-N-treated plants during both cropping seasons. Differences between ginger species for SPAD readings were notable (Figure 7a and b). The results showed that it should be possible to select for species differences in levels of chlorophyll content per unit leaf area at different planting dates using a SPAD 502 meter. This is in agreement with the observation that SPAD chlorophyll measurements have intermediate productivity and are of use in selection (Gutiérrez-Rodriguez et al. 2000). Previous studies have reported significant correlation at different harvesting times between SPAD readings and N fertiliser rate (Debaeke et al. 2006). According to Cartelat et al. (2005), chlorophyll readings can be useful in detecting N deficiencies in growing crops. However, the SPAD meter cannot be used to make accurate predictions of the amount of N to apply to a crop during the growing season. A positive correlation was also observed between chlorophyll meter readings and leaf N in dryland and irrigated pumpkins (Swiader and Moore 2002). Argenta et al. (2004) indicated that a portable chlorophyll meter (SPAD 502) is an instantaneous tool to assess plant N status. Swiader and Moore (2002) also reported on the potential usefulness of the SPAD 502 chlorophyll meter as an N management tool in estimating plant N status. However, the SPAD values in response to N requirements could vary with plant species and growing conditions.

Open stomatal pores

The stomatal patterns of African ginger and commercial ginger leaves in response to fertiliser N application rates were evaluated by SEM (Table 2; Figures 8a–f and 9a–f) in both cropping seasons. African ginger recorded a higher number of open stomatal pores compared with that of commercial ginger in both seasons. The results indicated higher open stomatal pores under higher N conditions compared with the plants supplied with less N (Figures 8a–f and 9a–f) for both cropping seasons. In agreement with the present observations, high N fertilisation was also described to increase open stomatal pores in a potato crop by Yan et al. (2012). Average percentage open stomata ranged from 37% to 60% for commercial ginger, whereas for African ginger it ranged from 50% to 68.2% for the first season. It was shown that African ginger had a higher percentage of open stomata compared with commercial ginger at 0 and 1.602 g N plant^{-1}.

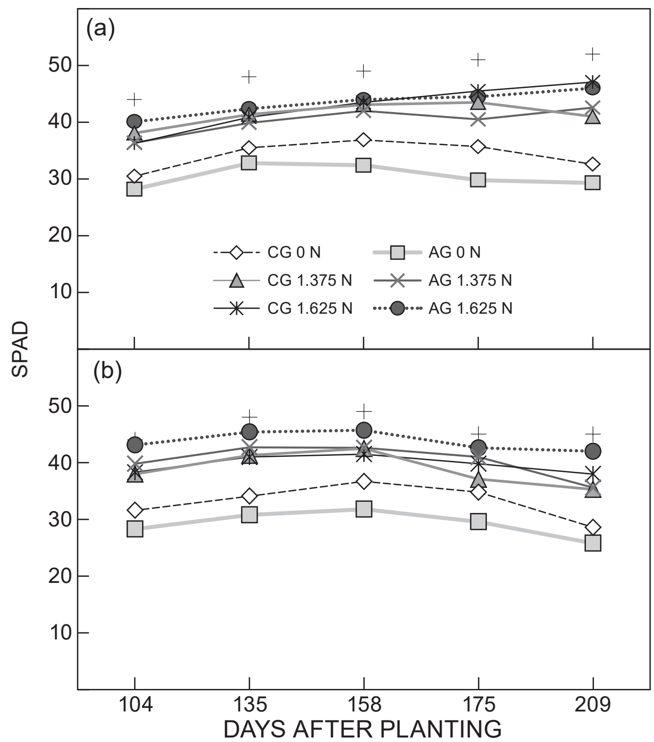

Figure 7: Chlorophyll content in response to different nitrogen (N) application rates on two ginger species (*Siphonochilus aethiopicus* and *Zingiber officinale*) during the (a) 2015/16 and (b) 2016/17 cropping seasons. CG = commercial ginger, AG = African ginger. Crosses (+) indicate LSD values

Table 2: Total stomata and open stomata percentage of commercial ginger (CG) and African ginger (AG) in response to nitrogen (N) fertiliser rates in the 2015/16 and 2016/17 cropping seasons

Species	Treatment	2015/16			2016/17		
		Total stomata	Open stomata	Open stomata (%)	Total stomata	Open stomata	Open stomata (%)
CG	0 N	25	12	48.0	47	15	31.9
	1.375 N	43	16	37.2	46	28	60.8
	1.625 N	46	29	63.0	47	34	72.3
AG	0 N	46	23	50.0	47	27	57.4
	1.375 N	41	28	68.2	47	28	59.5
	1.625 N	45	30	66.6	46	41	89.1

Data for the open stomata number for the two species (i.e. African ginger and commercial ginger) differed significantly, indicating that the effect of N fertiliser rates was more prominent in the 2016/17 growing season. The mechanism and role of stomata in protecting plants from damage has been described by Raven (2014). Furthermore, Ghasemzadeh et al. (2010) indicated that stomatal opening and regulation plays a significant role in the control of photosynthesis rate.

In African ginger leaves, a high rate of N fertilisation promoted a two-fold increase in stomatal pores. The increased number of open stomata pores in African ginger indicated its adaptive capability to varying environmental conditions. Lawson and Blatt (2014) attribute increased stomatal opening to an increase in photosynthetic rate and activity.

Yield parameters

Fresh and dry rhizome mass

The results for fresh and dry rhizome mass revealed significant differences between African ginger and commercial ginger in response to varying N fertiliser application rates (Figure 10a and b). Commercial ginger showed increased rhizome fresh mass for both cropping seasons in response to

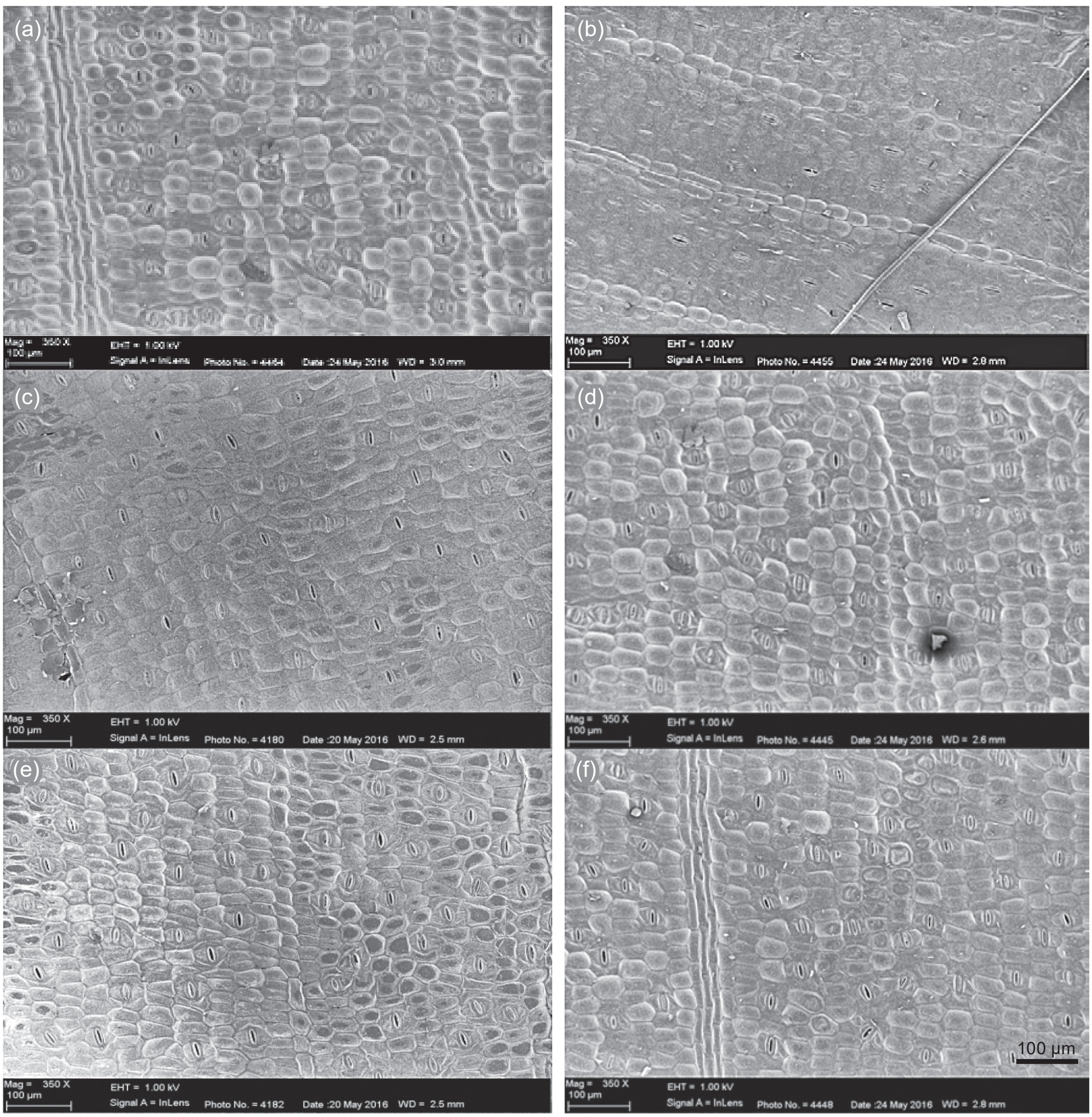

Figure 8: Total stomata and open stomata of *Siphonochilus aethiopicus* (AG) and *Zingiber officinale* (CG) leaves in response to different nitrogen (N) application rates during the 2015/16 cropping season. Images represent the following: (a) AG–0 N, (b) AG–1.375 g N plant^{-1}, (c) AG–1.625 g N plant^{-1}, (d) CG–0 N, (e) CG–1.375 g N plant^{-1} and (f) CG–1.625 g N plant^{-1}

all fertiliser N application rates compared with that of African ginger. The increase in rhizome fresh mass was observed when commercial ginger received 1.625 g N plant^{-1} for the two growing seasons. Application of 1.375 g N plant^{-1} proved optimum and elevated the fresh rhizome mass in the 2015/16 growing season (Figure 10a and b). Akinyemi et al. (2014) reported on the improved growth and yield in response to fertiliser application of ginger. Correspondingly, an enhancement in rhizome dry weight was recorded for commercial ginger treatments for both cropping seasons (Figure 10a and b). The results were consistent with the findings of Rathke et al. (2006), who reported that increase in N application could enhance root yield. The effect of N on plant growth may improve the biochemistry and physiology of plants and these could impact on yield (Lawlor 2002). Nitrogen acts as a key component and has a significant effect on various attributes studied at different growth stages (Xu et al. 2012). This showed that the increased rates of N fertilisers contributed much to the growth of the two species.

Nitrogen-use efficiency of rhizome fresh and dry mass

Nitrogen-use efficiency was influenced by N application rates for rhizome fresh and dry mass (Figure 11a–d). Nitrogen-use efficiency in a crop plays a significant role by increasing crop

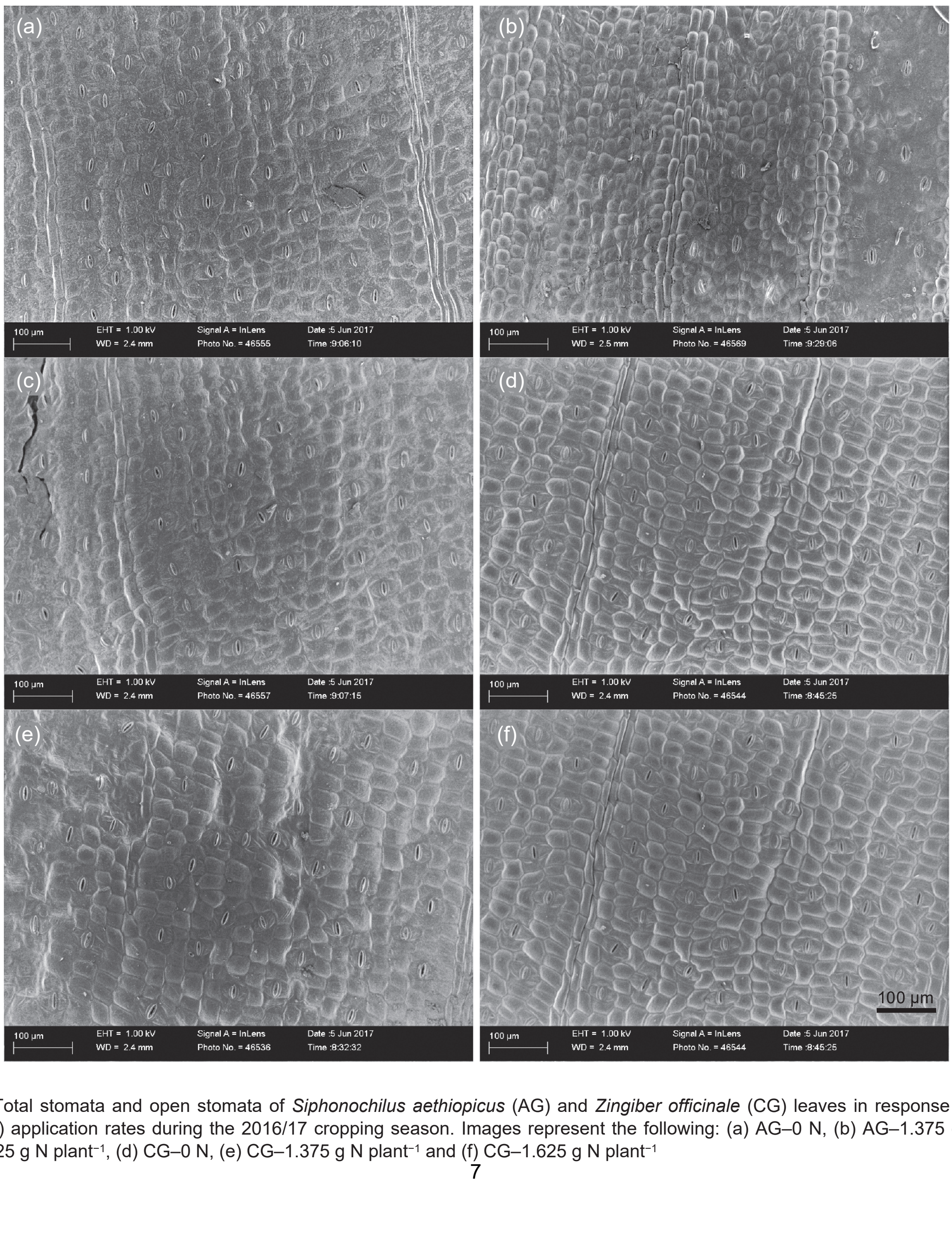

Figure 9: Total stomata and open stomata of *Siphonochilus aethiopicus* (AG) and *Zingiber officinale* (CG) leaves in response to different nitrogen (N) application rates during the 2016/17 cropping season. Images represent the following: (a) AG–0 N, (b) AG–1.375 g N plant^{-1}, (c) AG–1.625 g N plant^{-1}, (d) CG–0 N, (e) CG–1.375 g N plant^{-1} and (f) CG–1.625 g N plant^{-1}

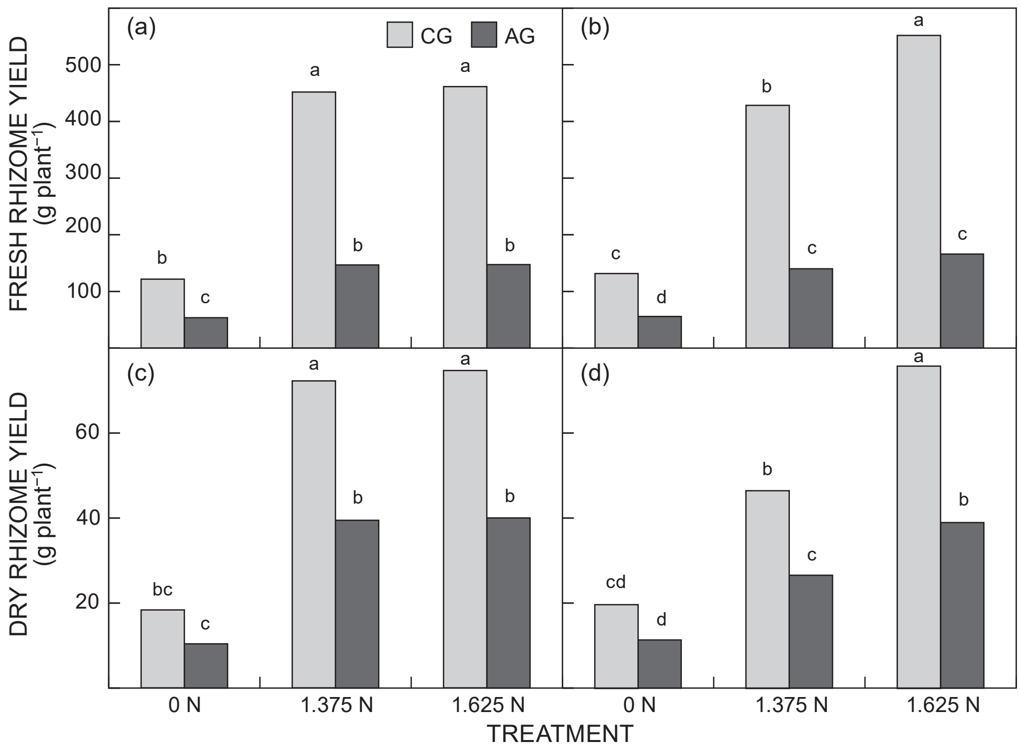

Figure 10: Fresh and dry rhizome mass of *Siphonochilus aethiopicus* and *Zingiber officinale* in response to different nitrogen (N) application rates during the (a and c) 2015/16 and (b and d) 2016/17 cropping seasons. Different lower-case letters above bars indicate a significant difference ($p < 0.05$). CG = commercial ginger, AG = African ginger

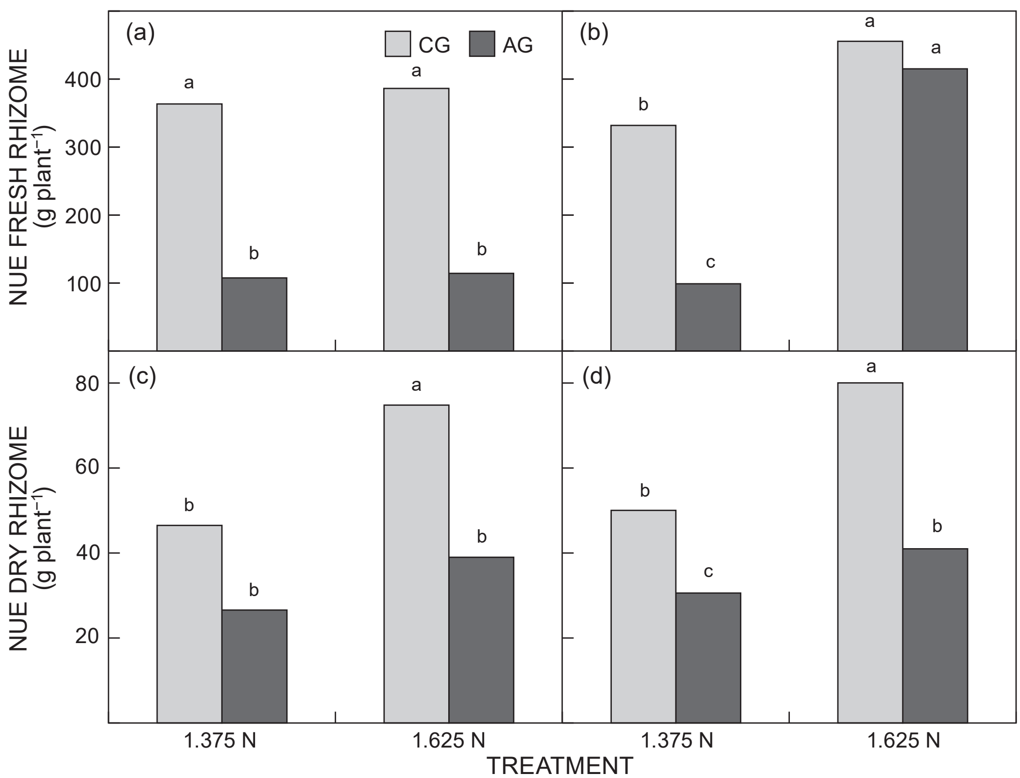

Figure 11: Nitrogen-use efficiency (NUE) of fresh and dry rhizomes of *Siphonochilus aethiopicus* and *Zingiber officinale* in response to different nitrogen (N) application rates during the (a and c) 2015/16 and (b and d) 2016/17 cropping seasons. Different lower-case letters above bars indicate a significant difference ($p < 0.05$). CG = commercial ginger, AG = African ginger

yield due to regulation of crop metabolic processes (Xu et al. 2012). The highest NUE ranged from 386.3 to 455.5 g plant^{-1} for fresh rhizomes during both the 2015/16 and 2016/17 cropping seasons. Similar increases in NUE were observed for dry rhizome for commercial ginger for all fertiliser N treatments during both cropping seasons (Figure 11c and d). The findings by Dordas (2015) provided new information on the relationship between NUE, N application, chlorophyll meter readings and N leaf concentration of oregano. A previous report by Fageria and Baligar (2005) supported the findings of the present study, indicating that the application of N improved yield and NUE.

The lower NUE at 1.375 and 1.625 N g plant^{-1} for African ginger during the 2015/16 cropping season may be related to saturation of N absorption capacity by plants at a higher N rate (Fageria and Baligar 2003). In addition, the findings on nutritional variability demonstrated that plants grown at the lowest nutrient concentrations will inevitably have the highest utilisation measure because of dilution effects (Fageria et al. 2013). According to Baligar et al. (2001), reduced NUE with N application could be a result of loss of mechanisms such as volatilisation, denitrification and leaching, or temporary unavailability.

Conclusion

The present study demonstrates that growth parameters (height, tiller and leaf number) of *Z. officinale* and *S. aethiopicus* were significantly affected by N fertilisation rates. *Zingiber officinale* exhibited higher measures of plant height, number of tillers, chlorophyll content and leaf number. Scanning electron microscopy analyses of open stomata revealed that a higher N level increased open stomata in both ginger species and both cropping seasons. African ginger showed higher percentage of open stomata compared with that of commercial ginger, indicating that African ginger was more resistant to N deficiency than commercial ginger. In addition, fresh and dry mass of commercial ginger was higher in both seasons compared with those of African ginger. In general, the results showed that commercial ginger exhibited higher fresh and dry mass plus NUE than African ginger in both seasons. The results of the present study suggest that optimal levels of N can maximise the production of ginger species. The results are useful to elucidate the use of N fertiliser levels on yield of both commercial and African ginger.

Geolocation information

Experimental Farm, University of Pretoria: 25°45′S , 28°16′ E.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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