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Simultaneous Effects of Water and Nitrogen Stress on the Vegetative and Yield Parameters of Choy Sum (*Brassica chinensis* var. *parachinensis*)

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ABSTRACT

Hypothetically, leafy vegetables need water and nitrogen (N) simultaneously in their applications for good growth. Therefore, this study was conducted to determine the effects of four watering frequencies (watering once a day, once a week and once in every two weeks and no watering) and five levels of nitrogen (0, 34, 68, 136 and 272 kg N ha^{-1}) on the vegetative and yield parameters of choy sum over a period of four weeks. The choy sum was grown in polyethylene bags under a rain shelter. The experimental design was a split-split plot with four replications. Plant vegetative and yield parameters measured weekly were plant height, leaf number, total leaf area, maximum root length, the various plant part weights and total tissue nitrogen. Water stress detrimentally affected choy sum's leaf growth more than root growth and the effect of water stress was more than nitrogen stress. Higher nitrogen rates in water-stressed condition increasingly reduced the number of leaves and height of the choy sum. Choy sum grown under once-a-day watering and once-a-week treatments did not experience water stress. The optimal soil water content and nitrogen application rate were 0.4 m^3 m^{-3} and 30 to 40 kg N ha^{-1}, respectively. The rate of 34 kg N ha^{-1} and once-a-week watering treatment generally gave the highest values for all the measured vegetative and yield parameters. Under lower and point of sufficient nitrogen rates, nitrogen was used for leaf thickness and weight rather than for intercepting light via leaf area expansion.

Keywords: Brassica, choy sum, drought, nitrogen fertiliser, water deficit, water stress

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INTRODUCTION

Choy sum (*Brassica chinensis* var. *parachinensis*) is one of the main leafy vegetable crops grown in Asia, including Malaysia (Tin *et al*., 2000). This vegetable is rich in vitamins and fibre, and it has a short life cycle that allows it to be harvested in a month (Chin, 1999). This crop grows best in conditions of adequate water (10 to 12 mm of water per day), nitrogen (N) requirement of 68 kg N ha^{-1} and air temperature between 23 and 35 ^{o}C (Vimala & Chan, 2000).

Chin (1999) remarked that choy sum is able to tolerate mild water deficit better than waterlogged conditions. Furthermore, excessive rainfall (>300 mm per month) could damage the leaves of the choy sum and in turn reduce the yield quality (DOA, 1998), but this problem can be mitigated by growing choy sum under a rain shelter. Different Brassica species respond differently to water stress. Caisin (*Brassica rapa* subsp. *parachinensis*), for instance, was observed to be more tolerant of waterlogging and water deficit than Chinese kale (*Brassica oleracea* var. *alboglabra*) (Issarakraisila *et al*., 2007). Nonetheless, both these Brassica species still experienced reduced total leaf area and leaf weight, delayed flowering and increased tissue nitrogen concentration under 14 days of water deficit conditions.

Adequate water and nitrogen supply are important for plant growth and maximum yield (Gutierrez & Whitford, 1987; Laurie *et al*., 2009). Too little or too much water and/or nitrogen can have negative effects on plant growth, which leads to lower yield. The yield of kale, for instance, had increased by 71% when 100 kg N ha^{-1} was applied and the yield declined after 200 kg N ha^{-1} (Hill, 1990).

Nitrogen is important because it is one of the components of chlorophyll and it plays a role in protoplasm formation as it is also one of the building blocks of amino acids (Campbell & Reece, 2002). According to Russell (1988), both soluble amino compounds and protein increase as nitrogen content increases. Nitrogen deficiency occurs when there is a lack of nitrogen to manufacture adequate structural and genetic materials, which ultimately causes stunted plant growth (Laurie *et al*., 2009). Excessive nitrogen, instead, increases the demand for carbon (C), leading to a decrease in the proportion of carbohydrate available for cell-wall material (Russell, 1988). Excessive nitrogen can also cause the plant to be more susceptible to diseases such as soft rot damage on pak choy (*Brassica campestris* var. *chinensis*) (Hill, 1990) and head rot on broccoli (*Brassica oleracea* var. *italic*) (Everaarts, 1994).

Availability of water and nitrogen affect plant growth and yield differently. Pandey *et al*. (2000) indicated that the effect of nitrogen was highly significant under non-limiting water conditions, but nitrogen gave no significant effect when the plant was water-stressed. They also reported that the yield reduction under water deficit conditions was much more severe when nitrogen was applied at high rates. Barraclough *et al*. (1989) found that the grain yield of winter wheat under drought with low nitrogen supply showed the lowest yield. Wu *et al*. (2008) showed that leaf area ratio, roots-to-shoot ratio and relative water content of pagoda shrub (*Sophora davidii*) increased under severe drought (20% of field capacity) and with low nitrogen (92 mg N kg^{-1} soil). Under the same severe drought stress condition, the height, leaf number, leaf area and biomass of pagoda seedlings decreased with high nitrogen (184 mg N kg^{-1} soil). Ahmadi and Bahrani (2009) reported that the highest nitrogen application rate with adequate irrigation gave the highest value in plant height, number of branches, pods and seeds and oil yield for rapeseed (*Brassica napus* L.).

Although the response of many crops to water and nitrogen stresses have been widely reported, studies specifically on choy sum's tolerance to these two stresses, especially the simultaneous effects of these two stresses on choy sum, remain rare. The Malaysian Research and Development Institute (MARDI) indicated that under Malaysia's growing conditions, the nitrogen requirement for choy sum is 68 kg N ha^{-1}. Vimala and Chan (2000) showed that choy sum can still grow well in the dry season provided irrigation is supplied. However, the degree to which choy sum can tolerate water stress remains uncertain. Hence, this study was carried out to evaluate the simultaneous effects of several watering frequencies and nitrogen rates on the vegetative and yield parameters of choy sum. We hypothesised that water stress would have a larger effect than nitrogen stress on choy sum vegetative and yield parameters and that choy sum's vegetative growth and yield would be detrimentally affected by water stress when the soil water content falls below the critical level.

MATERIALS AND METHODS

Experimental Setup

The experiment was set up as a split-split plot design with four watering frequencies: S1 (once a day), S7 (once a week), S14 (once every two weeks) and SX (without watering) as whole plots; five nitrogen (N) application rate ratios: N0 (0xR), N0.5 (0.5xR), N1 (1xR), N2 (2xR) and N4 (4xR), where R is the recommended rate (68 kg N ha^{-1}) by MARDI (Vimala & Chan, 2000), as sub plots; and five growth stages (time): 0, 7, 14, 21 and 28 days after transplanting (DAT) as sub-sub plots, with four replications.

This experiment was conducted under a rain shelter at Agronomy Research Farm (2° 59.47' N and 101° 42.882' E), Universiti Putra Malaysia, Serdang, Selangor. The experiment started on March 25, 2011 and ended on April 21, 2011.

The soil (Munchong series – Typic Hapludox), taken from the field at soil depth 0-150 mm, was air dried and sieved (2 mm) before being placed into polyethylene bags (5 kg of soil per bag). The soil pH (1:2.5) (Meter-827 pH Lab), electrical conductivity (EC Meter-Lab 960), total carbon (combustion method; LECO-CR 412 Carbon Analyser), total nitrogen (Kjeldahl method; Jones, 1991), particle size analysis (pipette method; Gee & Bauder, 1986), bulk density (core ring method; Blake & Hartge, 1986) and water retention (membrane plate method; Richards, 1947) were analysed and are summarised in Table 1.

TABLE 1
Soil properties used in this study

Parameters	Value
Soil series	Munchong (Typic Hapludox)
pH	6.8
EC (dS m^{-1})	0.62
Particle size distribution (%)	
Clay (2-50µm)	65.41
Silt (< 2µm)	7.63
Sand (> 50 µm)	26.74
Texture class (USDA)	Clay
Total carbon (%)	0.99
Total nitrogen (%)	0.15
Bulk density (Mg m^{-3})	1.08
Volumetric soil water content (%)	
Saturation	74.97
Field capacity	44.55
Permanent wilting point	25.32

The NPK fertilisers were applied manually with a rate of 68 kg N ha^{-1}, 10 kg P ha^{-1}, and 96 kg K ha^{-1} (Vimala & Chan, 2000), respectively, using straight fertilisers i.e. urea (46% N), triple superphosphate (20% P) and muriate of potash (50% K). The fertilisers were applied only once every two days before transplanting. The choy sum seedlings were transplanted manually after 14 days in the nursery so that each polyethylene bag had four seedlings. Each experimental unit comprised 20 polyethylene bags. All seedlings were watered only in the mornings with 1 L of water per polyethylene bag or 10 mm per poly bag. One polyethylene bag was randomly selected from every experimental unit, and all plants in the selected polyethylene bag were sampled (destructive sampling) for plant analyses.

Plant Parameters

The plant samples were measured for plant height, number of leaves, leaf area (LI-3100 Area Meter), maximum root length and weights of plant part (leaves including petioles, stem and roots). Specific leaf area (SLA) and shoot-to-roots ratio were also calculated. The total nitrogen (wet ashing method: Jones, 1991; Auto-Analyzer, 2000 Series) for tissues sample was also determined.

The analysis of variance (ANOVA) was done by using a package of Statistical Analysis System, SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). No data was transformed prior to ANOVA as the distribution of data did not violate any of the ANOVA assumptions. The mean separations were analysed by Student-Newman-Keuls (SNK) at 5% of significance level.

Soil Water Content

Daily soil volumetric water content (VWC) throughout the experiment is shown in Fig.1. The soil VWC at field capacity (FC) and permanent wilting point (PWP) (determined using the ceramic plate method; Richard, 1947) were 44.6% and 25.3%, respectively. The mean soil volumetric water content (± standard error) under the S1, S7 and S14 watering treatments were 42% (±0.34), 35% (±0.76) and 29% (±1.15), respectively. The mean soil volumetric water content (VWC) under the S14 treatment (watering once every two weeks) was close to PWP, and the

S1 treatment (watering once a day) close to FC. The mean soil VWC under S1 was 17% and 31% more than that under S7 and S14, respectively. The SX treatment (without watering) contained only 3% (±0.30) mean soil VWC (data not shown) that was 76% lower than PWP, while the water level was far too low and held on too tightly by the soil for plant survival.

RESULTS AND DISCUSSION

The choy sum seedlings in the SX treatment (without watering) died soon after transplanting due to severe water stress. Therefore, the ANOVA was based on the three watering treatments (S1, S7 and S14) and the five application levels of nitrogen. The ANOVA results (Table 2) reveal that the significant SxNxT interaction was not observed in most of the measured parameters except for the leaf number and plant height. The NxT interaction was significant on the root length, total leaf area and SLA, while the effect of the SxT interaction was seen on the total dry weight, the individual dry weights of leaves and shoot and total leaf area.

Water stress (S) detrimentally affects leaf growth more than root growth as in maize (Davies, 2006) and oil palm (Sun *et al*., 2011), giving a lower shoot-to-roots ratio in water-stressed conditions. This trend was observed in this study (Table 2 and Fig.2). Also observed in this study was that the shoot dry weight but not the root dry weight was affected by the water stress (S) (Table 2).

The results of this study show that the choy sum's growth was more affected by water stress than by nitrogen stress. The shoot-to-roots ratio was significantly affected by water stress (S) but not by nitrogen stress (N) (Table 2). The S1 and

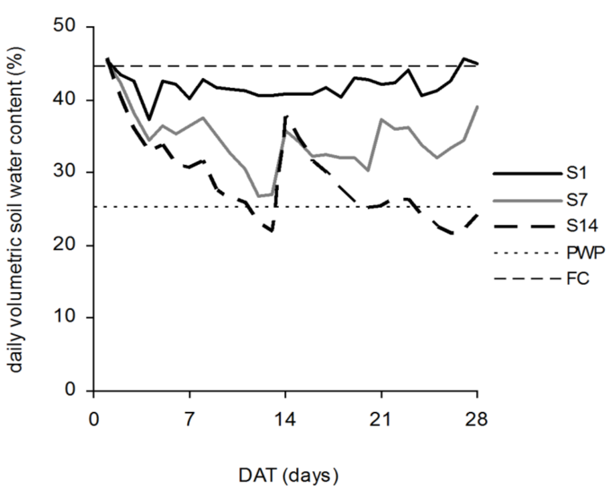

Fig.1: Daily volumetric soil water content for watering frequencies: S1 (once a day), S7 (once a week) and S14 (once every two weeks). Note: FC (field capacity), PWP (permanent wilting point) and DAT (days after transplanting).

S7 watering treatments gave the highest shoot-to-roots ratio, whereas S14 was the lowest (Fig.2). Sun *et al*. (2011) and Yin *et al*. (2009) reported that the plant growth in their studies was more affected by water availability than by nutrient (nitrogen and potassium) availability. Water stress has a larger effect than nitrogen stress on plants because highly water-stressed plants lead to higher plant osmotic stress, and this

TABLE 2
Summary of analysis of variance (ANOVA) indicating Pr > F for Choy Sum's vegetative and yield parameters under effects of watering frequencies (S), nitrogen rate (N) and time (T)

Parameters	S	N	T	SxN	SxT	NxT	SxNxT
Leaf number	0.048*	0.038*	<0.001**	0.211	<0.001**	<0.001**	0.048*
Height	0.011*	0.024*	<0.001**	0.192	<0.001**	<0.001**	<0.001**
Maximum root length	0.836^{ns}	0.364	<0.001**	0.966^{ns}	0.266^{ns}	0.007**	0.592^{ns}
Leaves (dry weight)	0.183	0.096^{ns}	<0.001**	0.380	0.014*	0.137^{ns}	0.527^{ns}
Stem (dry weight)	0.451^{ns}	0.488^{ns}	<0.001**	0.935^{ns}	0.241^{ns}	0.189^{ns}	0.996^{ns}
Roots (dry weight)	0.235^{ns}	0.460^{ns}	<0.001**	0.999^{ns}	0.115^{ns}	0.938^{ns}	0.999^{ns}
Shoot (dry weight)	0.138	0.076^{ns}	<0.001**	0.382^{ns}	0.002**	0.051^{ns}	0.562^{ns}
Total (dry weight)	0.146	0.079^{ns}	<0.001**	0.492	0.002**	0.057^{ns}	0.765^{ns}
Shoot-to-roots ratio	0.030*	0.305^{ns}	<0.001**	0.408^{ns}	0.527^{ns}	0.433^{ns}	0.837^{ns}
Total leaf area	0.201	0.024*	<0.001**	0.283	0.005**	<0.001**	0.470^{ns}
Specific leaf area	0.511^{ns}	0.275	<0.001**	0.357^{ns}	0.165^{ns}	0.049*	0.889^{ns}

Note: ^{ns} not significant; * $p \leq 0.05$; ** $p \leq 0.01$; ns, *, and ** show the applicability of p level.

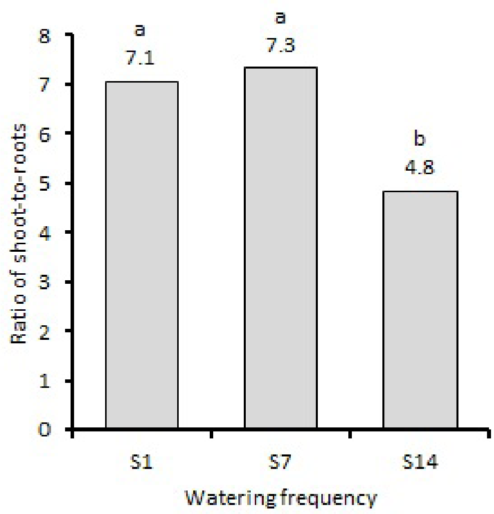

Fig.2: Shoot-to-roots ratio as affected by watering frequencies: S1 (once a day), S7 (once a week) and S14 (once every two weeks). Means with the same letter are not significantly different based on SNK test at p=5% level.

results in increasingly lower nutrient uptake (Mustafa & Abedelmagid, 1982; Pala *et al*., 1996).

Pandey *et al*. (2000) and van den Driessche *et al*. (2003) observed that nitrogen fertilisation under water-stressed conditions would exacerbate the stress experienced by plants, in particular when the nitrogen rate applied is also high. Consequently, in this study, the combination of S14+N4 treatments (highest water stress with highest nitrogen level) gave the lowest leaf number and plant height (Fig.3). In contrast, the S7+N0.5 gave the highest values for these two parameters. Fig.4 additionally shows that the total nitrogen content of the whole plant tissues was the lowest for treatments experiencing the highest water stress level

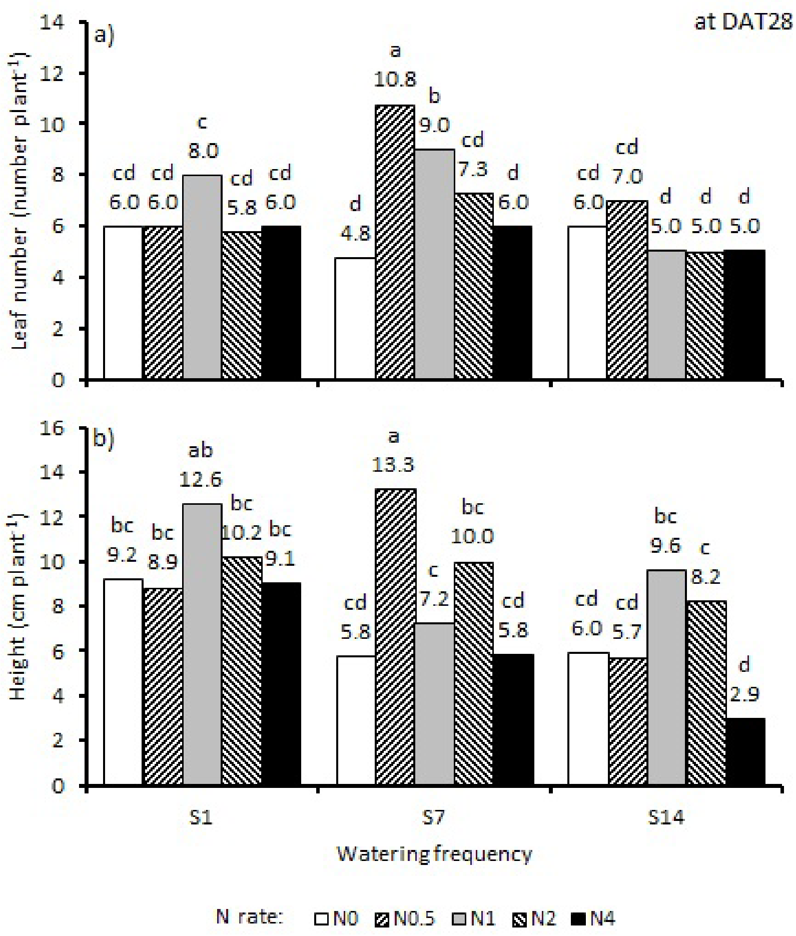

Fig.3: Effect of watering frequencies and N rates on: (a) leaf number and (b) plant height. Means with the same letter are not significantly different based on SNK test at p=5% level (means separations over all treatment combinations). The watering frequencies: S1 (once a day), S7 (once a week), and S14 (once every two weeks) and the nitrogen rates: N0 (0xR), N0.5 (0.5xR), N1 (1xR), N2 (2xR) and N4 (4xR) where R is 68 kg N ha^{-1}. Shown here are the leaf number and plant height at only 28 days after transplanting (DAT 28).

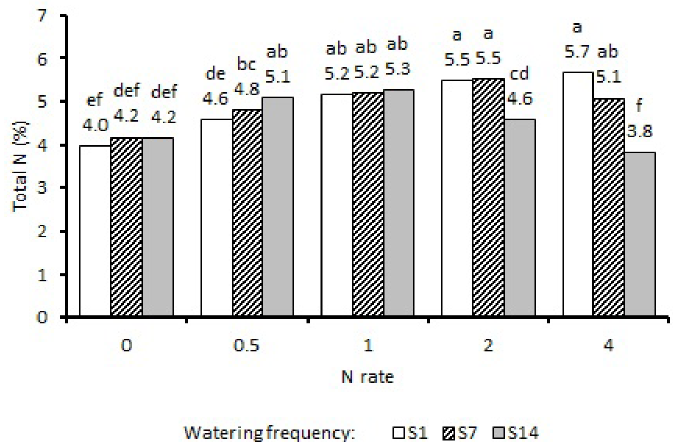

Fig.4: Total N content (at 28 days after transplanting) in choy sum whole plant tissues as affected by watering frequencies: S1 (once a day), S7 (once a week) and S14 (once every two weeks) and nitrogen rates: N0 (0xR), N0.5 (0.5xR), N1 (1xR), N2 (2xR) and N4 (4xR) where R is 68 kg N ha^{-1}. Interaction SxN was highly significant ($F=7.185$; $p<0.01$) and means with the same letter are not significantly different based on SNK test at p=5% level (means separations over all treatment combination).

(S14) and high nitrogen application rates (N2 and N4). This trend is similar to that reported by Sun *et al*. (2011), who observed low nitrogen content in oil palm leaves when the plant experienced high water stress and high nitrogen application rates.

This study showed that the lowest water frequency treatment (S14) had resulted in lower total leaf area by more than 50% at DAT 28 compared to those with more frequent watering treatments of S1 and S7 (Fig.5a and 6a). When the water content in a plant decreases, plant water potential is reduced and the plant cells start to shrink as they lose turgor pressure. The loss of turgor pressure in the cells inhibits turgor-dependent activities such as cell expansion, and this ultimately affects the growth of the whole plant (Campbell & Reece, 2002) such as reduction in plant height, total leaf area, plant mass and yield (Ahmadi & Bahrani, 2009). As a drought tolerance mechanism, plants can lower their total leaf area to reduce their water loss via transpiration (Liu & Stützel, 2004). Fig.6a shows that maximum total leaf area could be achieved at approximately 0.4 m^3 m^{-3} mean soil water content. Note that no function was fitted to this chart as there were only three points. Nonetheless, it is clear that even at a lower mean soil water content of 0.35 m^3 m^{-3}, total leaf area was already close to maximum.

Water stress also caused the total plant dry weight and the individual dry weights of leaves and shoots to decrease as the water stress levels increased, particularly from DAT 14 onwards (Fig.5b-d and 7). The dry weight of choy sum leaves contributed to half of the total plant dry weight. Consequently, any large reduction in the leaves' dry weight, such as due to the S14 treatment, would considerably

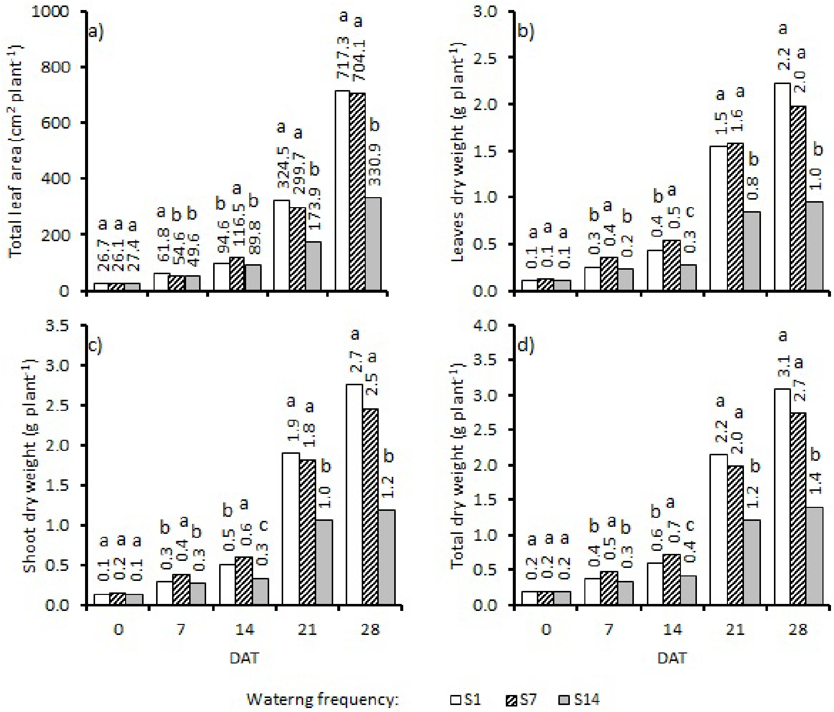

Fig.5: Effect of watering frequencies at each DAT (days after transplanting) on: (a) total leaf area, (b) leaves dry weight, (c) shoot dry weight and (d) total dry weight. At each DAT, means with the same letter are not significantly different based on SNK test at p=5% level. The water frequencies: S1 (once a day), S7 (once a week) and S14 (once every two weeks).

reduce the shoot-to-roots ratio as well (Fig.2). Although no function was fitted to the relationship between shoot dry weight and mean soil water content (Fig.7) for the same reasons as stated earlier, it was clear that maximum shoot dry weight could be achieved at approximately 0.4 m^3 m^{-3} mean soil water content, like the one obtained for the maximum total leaf area (Fig.6a).

The effect of nitrogen on maximum root length was less clear (Fig.8a). This is probably because, as mentioned previously, water stress would affect leaf growth more than root growth and that there was no differences in root dry weight between the treatments (Table 2). A study by Boa *et al.* (2007) also found that the root elongation of *Arabidopsis* (rockcress) was insensitive to increases in nitrogen supply.

From DAT 21 onwards, the total leaf area of choy sum would increase with increasing nitrogen rates until at N0.5, after which the total leaf area would decline with further increases to the nitrogen levels (Fig.8b). Fitting the best function to the relationship between total leaf area and nitrogen rate, it was shown that the maximum total leaf area could be achieved at 28 kg N ha^{-1} (Fig.6b).

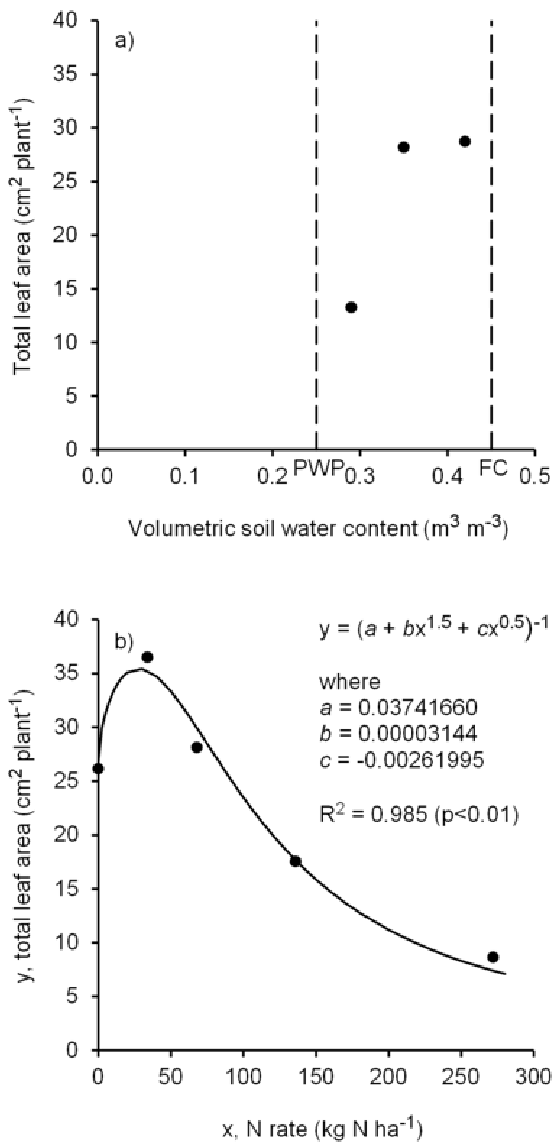

Fig.6: Relationship between choy sum's mean total leaf area at 28 days after transplanting (DAT 28) with (a) mean soil water content and (b) N rates. Note: PWP and FC denote the soil's permanent wilting point and field capacity, respectively. Regression was based on mean values.

These two optimal levels of mean soil water content (0.4 m^3 m^{-3}) and nitrogen rate (28 kg ha^{-1}) were similar to that required for obtaining the maximum number of leaves. Fig.9 shows that the maximum number of leaves can be achieved at approximately 0.35 m^3 m^{-3} mean soil water content and at 30 to 40 kg N ha^{-1}. Consequently, we can

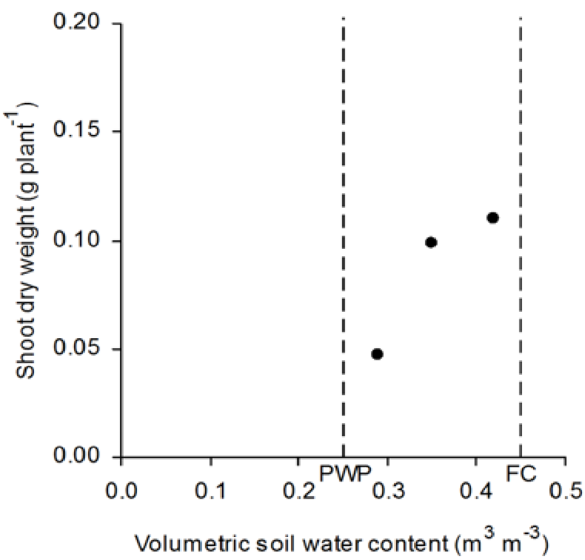

Fig.7: Relationship between choy sum's mean shoot dry weight at 28 days after transplanting (DAT 28) and mean soil water content. Note: PWP and FC denote the soil's permanent wilting point and field capacity, respectively.

generalise that the choy sum grown in this study's conditions would require 0.4 m^3 m^{-3} mean soil water content and 30 to 40 kg N ha^{-1} to obtain maximum yield in terms of the most number of leaves and highest total leaf area and shoot dry weight. The N0.5 treatment in this study had 34 kg N ha^{-1} applied, which is within the optimal 30 to 40 kg N ha^{-1} range.

The optimal 0.4 m^3 m^{-3} soil water content is above the critical water content of the soil used in this study. The critical soil water content (θ_{cr}) is the soil water content below which plants start to experience water stress. It can be determined by Teh's equation (2006):

$$\theta_{cr} = \theta_{PWP} + p\left(\theta_{FC} - \theta_{PWP}\right) \quad [1]$$

where θ_{PWP} and θ_{FC} are the soil's permanent wilting point and field capacity, respectively; and p is typically 0.5 for C3 plants (Doorenbos & Kassam, 1979) such as choy sum. Using Equation [1] and values from Table 1 for θ_{PWP} and θ_{FC} and $p = 0.5$ meant that the critical soil water content θ_{cr} for this study's soil was calculated to be 0.35 m^3 m^{-3}, which was similar to the mean soil water content under the S7 treatment. This shows that the choy sum growing under the S7 watering frequency treatment did not experience (but was close to experiencing) water stress. This explains the general lack of difference in the measured growth and yield parameters between the S1 and S7 treatments.

The SLA, which is the ratio of leaf area to leaf dry weight, indicates leaf thickness as smaller SLA would indicate thicker leaves (Meziane & Shipley, 2001; Teh *et al*., 2004). The linear decline of SLA with increasing

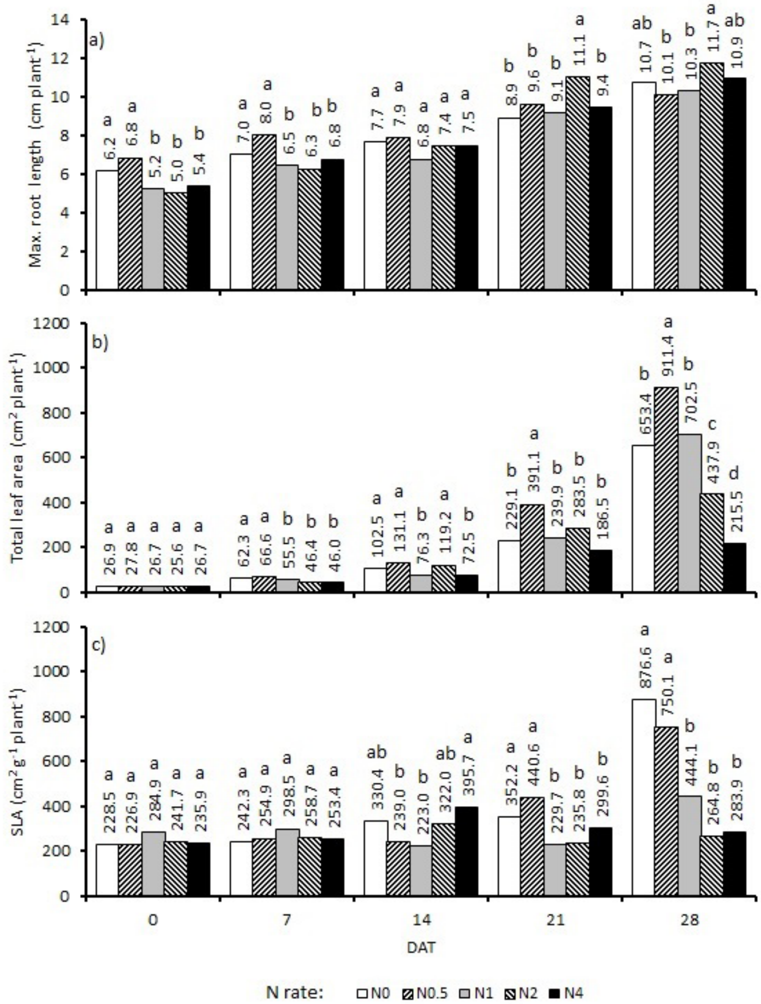

Fig.8: Effects of N rates at each DAT (days after transplanting) on: (a) maximum root length, (b) total leaf area and (c) specific leaf area (SLA). At each DAT, means with the same letter are not significantly different based on SNK test at p=5% level. The N rate levels: N0 (0xR), N0.5 (0.5xR), N1 (1xR), N2 (2xR) and N4 (4xR) where R is 68 kg N ha^{-1}.

nitrogen levels is particularly clear on DAT 28 (Fig.8c). Choy sum treated with N0 level showed the highest SLA, which would decline at a mean rate of 5 cm^2 g^{-1} (kg N $ha^{-1})^{-1}$ (linear regression not shown) until at N2 (136 kg N ha^{-1}), after which the SLA would remain constant. This suggests choy sum invests nitrogen use more for thylakoid stacking in photosynthetic cells and the synthesis of carboxylation enzymes for photosynthesis rather than for increasing the leaf area for light capture (Taiz & Zeiger, 2009). In other words, choy sum's preference is to use nitrogen to increase the weight and thickness of its leaves rather than to expand the area of the leaves. Similarly,

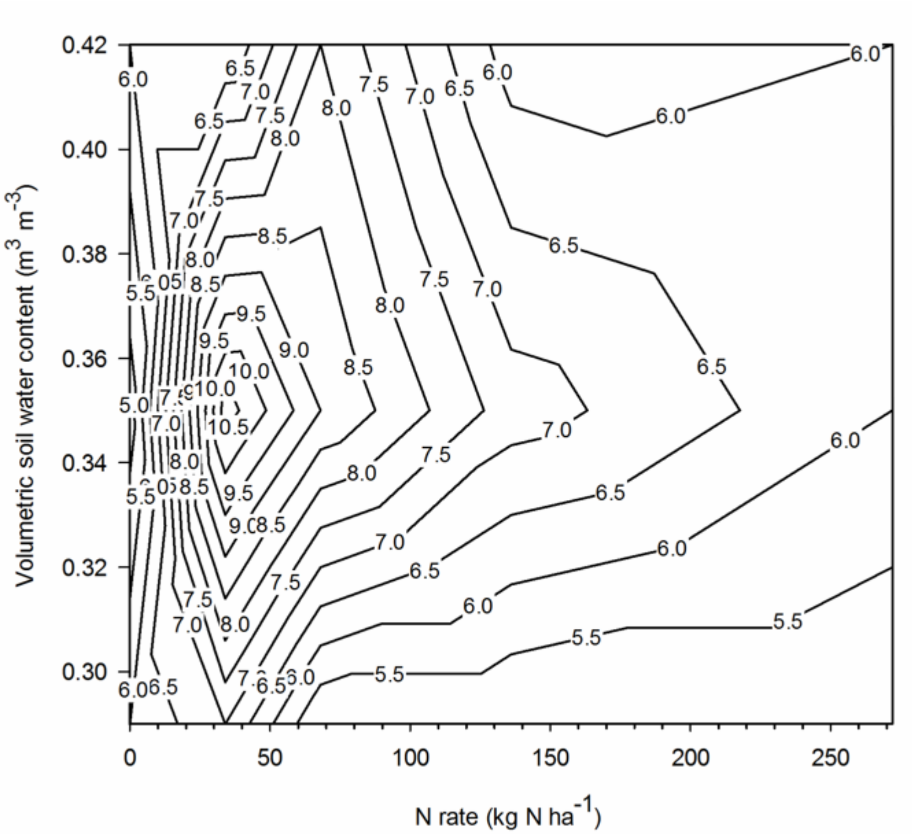

Fig.9: Relationship between choy sum's mean leaf number at 28 days after transplanting (DAT 28) with mean soil water content and N rate applied.

the SLA of Massai grass (*Panicum maximum* cv. *Massai*) also decreased with increasing nitrogen levels (Lopes *et al*., 2011).

CONCLUSION

Water stress detrimentally affected choy sum's leaf growth more than its root growth. Water stress also detrimentally affected choy sum's growth more than nitrogen stress, and increasing nitrogen application rates in water-stressed conditions significantly reduced the leaf number and plant height of choy sum. The S1 (once-a-day) and S7 (once-a-week) watering treatments generally gave a similar effect to the growth and yield of choy sum because choy sum growing under these two watering frequencies did not experience any water stress. Consequently, watering choy sum more than once a week was not needed as long as the soil water content remained above the critical level of 0.35 m^3 m^{-3}. The optimal soil water content and nitrogen application rate were 0.4 m^3 m^{-3} and 30 to 40 kg N ha^{-1}, respectively, for maximum choy sum yield (i.e. most number of leaves and highest total leaf area and shoot dry weight). This optimal nitrogen rate was 40 to 60% of the recommended nitrogen application 68 kg N ha^{-1}. The N0.5 (half the recommended nitrogen rate) treatment used in this study was 34 kg N ha^{-1}, and this nitrogen rate together with the S7 watering treatment (watering once a week) generally gave high values for all the measured vegetative and yield parameters. Under lower and point of sufficient nitrogen rates, nitrogen was used for leaf thickness and weight rather than for intercepting light via leaf area expansion.

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