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Responses of *Moringa oleifera* root growth to container size during overwintering in temperate regions

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Due to its fast growth rates, *Moringa oleifera* is being grown as an annual crop in temperate areas with freezing winter temperatures. Seedlings are raised under greenhouse conditions for overwintering and then transplanted outside at the beginning of spring, which allows for several cuttings prior to onset of the subsequent winter. However, there is limited information on container-size for overwintering of *M. oleifera* seedlings under greenhouse conditions. The objective of this study was to determine the responses of *M. oleifera* root growth to different container-sizes during overwintering in temperate regions under greenhouse conditions. Uniform two-month old seedlings were hardened-off and transplanted into five different container-sizes, measured in volume. Seedlings were fertilised once at transplanting and irrigated through scheduling with moisture meter. Six months after the treatment, container-size had highly significant effects on root length and crown girth, contributing 91 and 60% to total treatment variation of the respective plant variables. Root length and container-size exhibited quadratic relations, with optimum container-size computed to be 300 ml. In contrast, crown girth and container-size exhibited linear relations. In conclusion, the findings in this study suggested that a much smaller container (300 ml) than the one currently used (750 ml) would allow optimum overwintering of *M. oleifera* seedlings, thereby reducing production costs.

Key words: Container-cost, crown girth, labour-cost, *Moringa* species, shoot dormancy, root development.

INTRODUCTION

Moringa oleifera has attained the status of a 'developmental tree' of choice in sub-Sahara Africa (Leone et al., 2015). 'Developmental trees' are those used by governments to intervene in various socio-economic challenges. Due to its demand-driven attributes in improving socio-economic challenges and its most promising status for nutraceutical bioactive and industrial products (Anwar et al., 2007; Agyepong, 2009; Hassan and Ibrahim, 2013; Leone et al., 2015), *M. oleifera* is viewed as the 'miracle tree' (Fuglie, 2001). The tree is grown under a wide range of marginal environments, with extreme seasonal changes such as drought and frost (Leone et al., 2015).

Moringa species originated in tropical regions (Leone et al., 2015) and is adapted to warm climatic regions (Palada and Chang, 2003). In temperate regions with limited freezing winters, moringa trees enter dormancy for overwintering (Palada and Chang, 2003). Also, the plant

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has the ability to tolerate limited frost and drought (Leone et al., 2015), particularly during overwintering. Generally, plant species respond to their environments in a manner that optimises their resource use (Ågren and Franklin, 2003). When soil and aboveground conditions are optimum, root and shoot flushes alternate (McMahon et al., 2005), with more resources being shunted to the organ where growth is taking place (Andrews et al., 1999).

In temperate areas of South Africa with limited frost, moringa seedlings are raised in greenhouses for overwintering and transplanted soon after winter (Muhl 2009). Apparently, moringa enters dormancy in response to day-length since the phenomenon could not be prevented by increasing greenhouse temperatures, but could be prevented through increasing photoperiod at 28°C (unpublished data). During the warm growing seasons, *M. oleifera* seedlings in the greenhouse are raised for transplanting in 750 ml plastic containers. Due to limited information on container-size and the behaviour of moringa roots during overwintering, the same containers (750 ml) as used during the normal growing season are used for overwintering purposes. The overwintering process is initiated by sowing seeds at the beginning of winter (April-June) to mid spring (July-September), when seedlings are hardened-off and transplanted in open fields for harvesting prior to setting in of the next winter. The objective of this study was to determine the responses of *M. oleifera* root growth to different container-sizes during overwintering in temperate regions under greenhouse conditions.

MATERIALS AND METHODS

Study location/area

The study was conducted at the Greenhouse, Green Technologies Research Centre, University of Limpopo, Limpopo Province, South Africa (23°53'10"S, 29°44'15"E). Ambient day/night temperatures averaged 28/5°C, with maximum temperatures controlled using thermostatically-activated fans. The trial was conducted during winter (April-June) to mid spring (July-September) in 2015 and validated in 2016.

Procedure, treatments and experimental design

Seeds of *M. oleifera* were sown on Hygromix-T (Hygrotect, Pretoria North, South Africa) in 160-hole polystyrene seedling trays under greenhouse conditions. Two-month-old uniform seedlings were hardened-off outside the greenhouse for two weeks and transplanted in 250, 500, 750, 1000 and 1250 ml plastic bags filled with steam-pasteurised (300°C for 1 h) loam and river sand, mixed with Hygromix-T at 3:2:1 (v/v) ratio. Seedlings were allowed to establish until leaf abscission sets in and plants of uniform height were arranged in a randomised complete block design, with seven and eight replications in 2015 and 2016 trial seasons, respectively. Treatments were blocked for wind speed from the cooling fans, which create heterogeneous conditions in the greenhouse. Seedlings were placed on the greenhouse benches at 20-cm inter-row and 20-cm intra-row spacing.

Cultural practices

Each container was originally irrigated to field capacity and thereafter, five Hadeco Moisture meters (Hadeco Magic^{R}, RSA) were randomly installed each in a different container-size and plants irrigated with 250 ml tapwater when readings averaged below 2 units. At transplanting, seedlings were fertilized with 3 g 2:1:2 (43) NPK fertiliser mixture to provide 0.70 mg N, 0.64 mg K, 0.64 mg P, 1.8 mg Mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tap water and 2 g 2:3:2 (22) NPK fertilizer mixture with 5% Ca.

Data collection

At six months after initiating the treatment, plant height was measured. Plants were taken out of the containers and soil particles rinsed in a 20-L container half-filled with water. Roots were pressed between two pieces of paper towel to remove excess water. Diameter of crown girth was measured at the soil line using a digital Vernier caliper. Roots were separated from shoots and length of taproot and lateral roots per treatment were measured and combined. Roots and shoots were dried in air-forced ovens at 70°C for 72 h and the mass measurements used to calculate the root/shoot ratios.

Data analysis

Root length, crown girth, plant height, dry root mass, dry shoot mass and root/shoot ratio were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2015). The degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation (TTV) for variables measured. Mean separation was achieved through Fisher's least significant difference test at 5% level of probability. Unless otherwise stated, results were discussed at 5% level of probability.

RESULTS

The seasonal interactions for 2015 and 2016 for plant variables were not significant and data were pooled (n = 75) and subjected to ANOVA. The effects of container-size were highly significant on root length and crown girth, contributing 91 and 60% in TTV of the respective variables (Table 1). However, the treatments had no effects on plant height, dry root mass, dry shoot mass and root/shoot ratio. The longest root length was in the 1250 ml container, followed by the 1000 ml container, whereas those in the standard and the smaller containers did not differ (Table 2). Relative to the 750 ml standard container, root length was significantly reduced by 18-28% in the smaller containers, but increased by 28% in the largest container (Table 2). In contrast, crown girth was increased by 11% in the smallest container.

The largest crown girth occurred in the 250 ml container, which was much smaller than the 750 ml standard, whereas the smallest was in the largest 1250 ml container (Table 2). Growth of root length and container-size during overwintering exhibited quadratic relations, with the relationship being explained by 94% of the model (Table 2). Using the relation $x = -b_1/2b_2$ from

Table 1. Partitioning sum of squares for root length and grown diameter of *Moringa oleifera* seedlings in five different plastic bag sizes at 65 days after transplanting (n = 75).

Source	DF	Root length		Crown diameter		Root/shoot ratio	
		MS	TTV (%)[z]	MS	TTV (%)	MS	TTV (%)
Replication	14	439.013	5	9.048	25	32.116	52
Treatment	4	8396.513	91**	21.530	60**	16.926	27[ns]
Error	56	372.517	4	5.436	15	12.952	21
Total	74	9208.021	100	36.014	100	61.994	100

[z]TTV = Total treatment variation; ***highly significant at P ≤ 0.01, [ns]not significant at P ≤ 0.05.

Table 2. Responses of root length and crown diameter in *M. oleifera* seedlings to plastic container-size during winter under greenhouse conditions at 60 days after transplanting (n = 75).

Container size (ml)	Root length (cm)		Crown girth (mm)	
	Variable[x]	RI (%)[y]	Variable	RI (%)
750[z]	107.47[b]± 4.2	-	17.31[bc]±0.7	-
250	77.87[c]±3.5	-28	19.21[a]±0.4	11
500	83.40[c]±4.3	-18	18.05[ab]±0.8	4
1000	108.80[b]±5.1	1	17.31[bc]±0.6	0
1250	137.20[a]±7.4	28	15.92[c]±0.6	-8
Relation	Quadratic		Linear	
R^2	0.94		0.92	

[x]Column means ± SE followed by the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test. [y]Relative impact (RI) = [(treatment/control) – 1) × 100. [z]standard plastic bag size for *M. oleifera* seedlings.

the quadratic equation, $Y = -0.00003x^2 + 0.0182x + 71.23$ (Gomez and Gomez, 1984), the optimum container-size for root length was 300 ml. In contrast, crown girth and container-size exhibited linear relation (Table 2), which was explained by 92% of the model.

DISCUSSION

In the current study, as shown by root length and crown girth, physiological activities continued in moringa seedlings during overwintering under greenhouse conditions. The overwintering process is generally gradual, thereby allowing carbohydrates and various mobile essential nutrient elements in affected plants to be partitioned in root systems (McMahon et al., 2005). Limited information is available on how much of the partitioned materials are stored in root systems of moringa seedlings during overwintering, since most of the focus had previously been on chemical composition and nutrient content of leaves (Leone et al., 2015). The partitioned and stored materials in roots are essential since they are indispensable for flowering and foliation in early spring for most plant species that have overwintering capabilities (McMahon et al., 2005). The latter capabilities are conspicuous in temperate plant species (McMahon et al., 2005).

The observed positive curvilinear quadratic relations between root length and container size is indicative that the relation follows the density-dependent growth (DDG) patterns (Salisbury and Ross, 2005). The DDG patterns are observed when organisms are subjected to increasing abiotic and biotic factors (Salisbury and Ross, 2005) and are generally characterised by three phases, namely, stimulation, neutral and inhibition phases (Mashela et al., 2015). In the current study, smaller containers stimulated the generation of lateral roots and thereby improve overall root length, whereas the largest containers had the opposite effects. Similar stimulation effects were reported when plants were subjected to increasing concentrations of allelochemicals (Liu et al., 2003) and phytonematicides (Mashela et al., 2015).

In the current study root length was the sum of the length of the taproot and the lateral roots. Apparently, as elongation of the taproot is limited by the depth of the container, more lateral roots are generated (Richards and Rowe, 1977), thereby increasing the collective overall root length. The importance of the observed positive curvilinear quadratic relation was that the generated quadratic equation allowed the computation of the optimum container-size using the $x = -b_1/2b_2$ relation (Gomez and Gomez, 1984). Similarly, the opposite argument could be advanced for the increased crown diameter in smaller containers as was previously reported under root restrictions in peach seedlings (Richards and Rowe, 1977).

In the current study, the optimum container-size for overwintering of *M. oleifera* seedlings under greenhouse was approximately 300 ml, which was more than twice smaller than the standard container used in the study. At the proposed optimum container-size, the crown diameter would not be limited since the variable and the container-size exhibited a linear relationship. Raising moringa seedlings for overwintering in containers larger than the optimum would increase overall production costs, which include container-, growing medium-, water-, electricity-, fertiliser-, greenhouse space- and labour-costs. In contrast, raising moringa seedlings for overwintering in containers smaller than the optimum is not recommended since root growth would be much restricted.

Conclusions

The results of this study suggested that the smaller containers (300 ml) than those (750 ml) currently used would be suitable for overwintering of *M. oleifera* seedlings in regions with frosts, where this high-nutrition value crop is grown as an annual vegetable. These containers would reduce the production costs for overwintering seedlings.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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