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Growth Responses of Marama Bean (*Tylosema esculentum)* to Water Deficit Conditions

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Abstract: This study was undertaken in order to evaluate the influences of several water supply regimes on marama growth parameters. Therefore, glasshouse-grown marama plants were subjected to several water treatments by applying different irrigation doses. Measurements of several growth parameters were taken during all the experimental period. Vegetative growth of the more intense water-stressed plants was significantly restricted, while there was a 31-66% reduction of total dry matter production compared to the plants of the rest three water treatments. Irrigation clearly promoted greater biomass allocation to the shoot and leaves and thereby increased the above ground: Below ground dry matter ratio. In conclusion, this study revealed the beneficial effect of an adequate water supply on growth and dry matter production of marama, which seems not to be drought tolerant-as long as it cannot grow well without a good water supply but a rather drought avoiding species, using its tubers as water reservoirs.

Key words: *Tylosema esculentum*, marama, water stress, vegetative growth, tuber

INTRODUCTION

Marama bean (*Tylosema esculentum* (Burch.) A. Schreib) is a perennial field crop, indigenous to the arid and semi-arid grasslands of southern Africa (Bousquet, 1981, 1982). The bean of the plant is highly nutritious, with protein and oil content comparable to soybean (*Glycine max*) and groundnut (*Arachis hypogaea*), respectively (Bower *et al*., 1988). The plant grows rapidly under good conditions and has an open growth habit with runners extending along the ground (Mitchell *et al*., 2005). Because of its great ability to survive under unfavourable conditions, the plant can be considered suitable for cultivation, especially under preventive conditions for other crops (Anonymous, 1979; Keegan and Van Staden, 1981).

Although tropical grain legumes generally have the ability to grow in wide range of environmental conditions, water stress is considered as a major environmental factor limiting their vegetative and reproductive growth and yield (Ramirez Vallejo and Kelly, 1998; Carranca *et al*., 1999). Moreover, it has been largely reported that plant responses to soil drying involve several modifications of morphological and physiological parameters (i.e., a variety of adaptive mechanisms), such as a shift in the allocation of dry matter from shoots to roots, a reduction in leaf expansion, leaflet movements, stomatal closure and osmotic adjustment (Kramer, 1983; Lawlor and Leach, 1985; Vadell *et al*., 1995),

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relatively depending on the climate in the native habitat of the plant species (Bultynck *et al*., 2003). Although the partitioning of biomass between above- and below-ground organs in response to water deficit and the water storage are two of the most important plant traits that has been studied extensively, little information exists on *T. esculentum* particular responses.

The objective of this paper was to evaluate the effects of several water supply regimes on marama growth parameters, i.e., to study the impacts of water relations on vegetative growth and biomass production and partitioning and to examine whether an adequate water supply is really prerequisite for a satisfactory growth of marama, by means of pot experiments.

MATERIALS AND METHODS

Experimental Details

Two pot experiments were conducted in a glasshouse of the Agricultural University of Athens (AUA) during the summers of 2002 and 2003. The experiments were arranged in a randomized complete blocks design with seven replicates and four water treatments. Minimum/maximum air temperature and relative humidity were: 20/40°C and 40/60%, respectively and the plants were subjected to a natural day length ranging between 13-15 h during the experiments.

The *T. esculentum* seeds were collected directly from the wild in Letlhakane, Botswana (21°34′ S; 25°42′ E) and stored at 4°C and 50% RH until their use. All selected seeds were about two years old and their fresh weight was ranged between 2 and 3 g. In order to optimize germination, seeds were scarified with sandpaper according to the method proposed by Travlos *et al*. (2007). One pregerminated seed having a radicle of 2-4 cm length was planted in each plastic pot (15 cm in diameter), filled with 2.4 L mixture of peat and perlite (2:1, v/v). The pH of the mixture was ranged between 4 and 5. At the beginning and until 20 Days After Sowing (DAS) plants were irrigated with ample distilled water (40% of the water-holding capacity) in order to promote plant emergence and first growth. Then, by withholding irrigation for 6 days, soil water content of all pots fell to 25% of the water-holding capacity.

The differentiation of the Water Treatments (WT) started from 26 DAS by applying different irrigation doses at the same times. The following water treatments (irrigation doses) were imposed: WT_1 (50 mL of water per pot), WT_2 (100 mL of water per pot), WT_3 (200 mL of water per pot) and WT_4 (400 mL of water per pot). The irrigations from 26 until 110 DAS (day of harvest) were 20 in total for each water treatment (every 4 to 7 day intervals). The total water quantity from 26 DAS for the plants of each treatment was 1, 2, 4 and 8 L for WT_1, WT_2, WT_3 and WT_4, respectively.

Data Measurements

Measurements of the length of vines (main stems), number of leaves and number of secondary stems per plant were taken on all plants starting from 30 DAS. In total, 20 measurements of each vegetative feature were taken. Plants remained in the vegetative phase throughout the experiments. Soil Water Content (SWC) was also measured gravimetrically at depth of 0-20 cm as recommended by Ritchie *et al*. (1990) at 26, 40, 55, 70, 85, 95 and 105 DAS.

Furthermore, the dry weights (85°C for five days) and the water content (%) of the above and below-ground parts of each plant were determined at 110 DAS (day of harvest). In addition, dry matter partitioning was also calculated after harvest.

Statistical Analysis

Data from both experiments were analysed together and treatment values for all features were expressed as means between the two years. Statistical analysis of the results was performed using one-way ANOVA, while mean comparison was performed using Fisher's Least Signification Different (LSD) test at $p<0.05$ by means of Statistica 6.0® software package (Statsoft., Tulsa, OK, USA).

RESULTS AND DISCUSSION

There were no significant differences between the plants of the two experiments (2002 and 2003) for any measured vegetative parameter. Water availability appeared to promote vegetative growth of *T. esculentum* plants. The time courses of the length of main stem and of the number of leaves per plant exhibited significant differences among water treatments (starting already from 40 DAS). Stem length was consistently highest in the WT_4-plants, reaching a value of 173 cm at the end of the experimental period. Water availability doubled the number of secondary stems per plant at the WT_4-treatment (Table 1).

All measured vegetative features showed an increasing trend during the experimental period for all water treatments except WT_1. Table 1 also gives the seasonal course of stem elongation and branching of WT_1-plants was kept relatively steady (the slight decrease of stem length was due to the desiccation of some stem edges), while the number of leaves per plant exhibited a decreasing trend due to a quite rapid leaf shedding which was occurred from about 65 DAS. The SWC values were ranged between 8-26, 20-25, 25-36 and 24-48% for WT_1, WT_2, WT_3 and WT_4 respectively during the experimental period.

In marama, stem elongation was significantly restricted by water deficits, as in many other grain legumes (Acosta-Gallegos and Shibata, 1989; Nielson and Nelson, 1998), but our results also indicate a significant reduction in the number of leaves and secondary stems per water-stressed plant. Leaf expansion is clearly among the most sensitive of the processes that are affected by water deficit (Alves and Setter, 2004), thus the low number of leaves in stressed *T. esculentum* plants seems quite expected, as long as it is a way of water deficient plants to reduce leaf surface and expansive water loss (Karamanos, 1984). The well-watered marama plants produced significantly more leaves in comparison with all other plants, probably due to the more profuse branching. The significantly higher vegetative growth rate of well-watered marama plants is consistent with the concept that the vegetative growth of grain legumes (including drought-susceptible species) is significantly enhanced by means of the supply of adequate soil moisture (Sangakkara *et al*., 2001). The leaf-fall observed in the WT_1-plants can be attributed to the low substrate (and tuber) water content and is in agreement with the results of a similar research in UK (Mitchell *et al*., 2005). Air temperature is not involved in this leaf shedding, as long as the average daily temperature was always above 25°C, i.e., the critical value for marama leaf drop, as proved by means of our similar field experiments.

Table 1: Vegetative growth of *Tylosema esculentum* plants for the several Water Treatments (WT) throughout the experimental period

		Water treatments			
Parameters	DAS	WT_1	WT_2	WT_3	WT_4
Length of the main stem (cm)	30	47.000 ± 5.2^{b}	50.100 ± 6.2^{ab}	53.100 ± 7.4^{ab}	60.100 ± 7.9^{a}
	50	55.500 ± 7.3^{c}	68.300 ± 8.8^{bc}	80.500 ± 9.2^{b}	105.200 ± 12.6^{a}
	70	54.000 ± 6.9^{d}	72.900 ± 9.2^{c}	92.800 ± 12.4^{b}	135.700 ± 14.1^{a}
	90	52.100 ± 4.2^{d}	78.800 ± 9.3^{c}	104.500 ± 14.4^{b}	152.500 ± 4.6^{a}
	110	51.200 ± 4.4^{d}	87.500 ± 6.5^{c}	118.500 ± 8.5^{b}	172.500 ± 0.5^{a}
No. of leaves/plant	30	6.710 ± 0.91^{b}	7.810 ± 0.89^{ab}	7.890 ± 1.21^{ab}	8.510 ± 0.91^{a}
	50	7.250 ± 0.39^{c}	10.290 ± 0.72^{b}	12.360 ± 0.65^{ab}	14.360 ± 1.15^{a}
	70	6.740 ± 0.51^{c}	12.290 ± 0.71^{b}	14.680 ± 0.48^{b}	22.910 ± 1.21^{a}
	90	5.820 ± 1.23^{c}	13.320 ± 1.06^{b}	16.720 ± 1.31^{b}	27.890 ± 0.61^{a}
	110	5.630 ± 0.92^{c}	15.010 ± 0.22^{b}	18.570 ± 0.37^{b}	30.790 ± 2.50^{a}
No. of secondary stems/plant	30	0.072 ± 0.012^{b}	0.143 ± 0.045^{ab}	0.072 ± 0.024^{b}	0.168 ± 0.031^{a}
	50	0.072 ± 0.023^{c}	0.143 ± 0.031^{bc}	0.215 ± 0.041^{b}	0.785 ± 0.086^{a}
	70	0.108 ± 0.023^{c}	0.196 ± 0.033^{bc}	0.271 ± 0.044^{b}	0.979 ± 0.084^{a}
	90	0.143 ± 0.034^{c}	0.286 ± 0.054^{bc}	0.468 ± 0.073^{b}	1.684 ± 0.124^{a}
	110	0.143 ± 0.028^{c}	0.357 ± 0.042^{bc}	0.786 ± 0.085^{b}	2.012 ± 0.312^{a}

Values are given as mean±SE, Means within the same row followed by the same letter(s) are not significantly different (LSD test at $p<0.05$)

Table 2: Dry weight and water content of *Tylosema esculentum* plants, leaves and stems and tubers for the several Water Treatments (WT) at harvest day (110 DAS)

		Water treatments			
Parameters	Plant part	WT_1	WT_2	WT_3	WT_4
Dry weight (g)	Leaves and stems	0.828±0.221^{c}	1.601±0.047^{bc}	2.253±0.217^{b}	3.464±0.458^{a}
	Tuber	1.424±0.324^{c}	1.951±0.623^{bc}	2.339±0.421^{b}	3.221±0.562^{a}
	Whole plant	2.252±0.745^{c}	3.552±0.679^{bc}	4.592±0.741^{b}	6.685±0.744^{a}
Water content (%)	Leaves and stems	30.363±3.033^{c}	65.876±7.238^{b}	67.576±6.153^{b}	75.746±1.548^{a}
	Tuber	77.534±0.629^{b}	89.952±2.312^{a}	90.529±4.313^{a}	91.153±5.287^{a}
	Whole plant	69.968±2.032^{b}	85.243±4.782^{a}	85.506±3.893^{a}	86.785±4.395^{a}

Values are given as mean±SE, Means within the same row followed by the same letter(s) are not significantly different (LSD test at $p<0.05$)

Table 3: The values of above- to below-ground dry matter ratio for *Tylosema esculentum* plants for the several Water Treatments (WT)

Water treatment	Above-to below-ground dry matter ratio
WT_1	0.581±0.034^{c}
WT_2	0.824±0.048^{b}
WT_3	0.963±0.077^{ab}
WT_4	1.082±0.096^{a}

Values are given as mean±SE, Means within the same row followed by the same letter(s) are not significantly different (LSD test at $p<0.05$)

The fresh and dry weights of above- and below- ground parts (tuber and roots) were associated with the water availability of each treatment. The WT_4-plants produced the significantly highest dry biomass (6.7 g in total) among all the other groups of plants (Table 2). As expected, tuber water content was lowest for the WT_1-plants (78 %) and highest (91 %) for the WT_4-plants, but it has to be noted that it was ranged at relatively high values for all the plants.

Significant differences in dry matter partitioning between water treatments were also detected. Irrigation clearly promoted greater biomass allocation to the shoot and leaves and thereby increased the above- to below-ground dry matter ratio. On the contrary, in the WT_1 and WT_2 plants, tubers and roots had a more important contribution to the total plant dry matter (Table 3). Thus, it is confirmed that the higher part of the total dry matter of intensely water stressed plants was localized in tubers. Similar trends have been commonly reported in a number of plants (Creelman *et al*., 1990; De Costa and Shanmugathasan, 1999), concluding that below-ground part growth is less sensitive to a decrease in soil water potential (is favoured over) than leaves and stems growth (Krizek *et al*., 1985; Steinberg *et al*., 1990; Singh and Singh, 2003). The fact that water stress can reduce not only photosynthesis (biomass production), but it is often accompanied by shifts in photoassimilate partitioning within the plants and usually an increased biomass partitioning to below-ground organs (Bota *et al*., 2004), is related to this study, too.

CONCLUSIONS

Regardless of the origin of marama from arid and semi-arid low rainfall regions of southern Africa, this species seems to require water for a high vegetative growth and dry matter production. *T. esculentum* is not particularly a drought tolerant in the sense of being able to grow undiminished as the soil dries, but a rather drought avoiding species using its tubers as water reservoirs and its leaflet and stomatal movements to save water. The stored water and assimilates in the tuber allow plant survival and then rapid growth under favorable conditions (marama seems to maintain leaf function in a few leaves until an adequate water supply). These results-clearly indicating the significant role of tuber in water economy and the beneficial effect of water on vegetative growth and dry matter production and partitioning of *T. esculentum* plants-have a potential utility and need to be validated by similar field experiments, which are already conducted. The low seed set of this species, combined with the high risk of its over-exploitation and its potential establishment as a crop, confirm the significant role of similar studies, which must be continued in order to optimize marama growth and establishment.

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