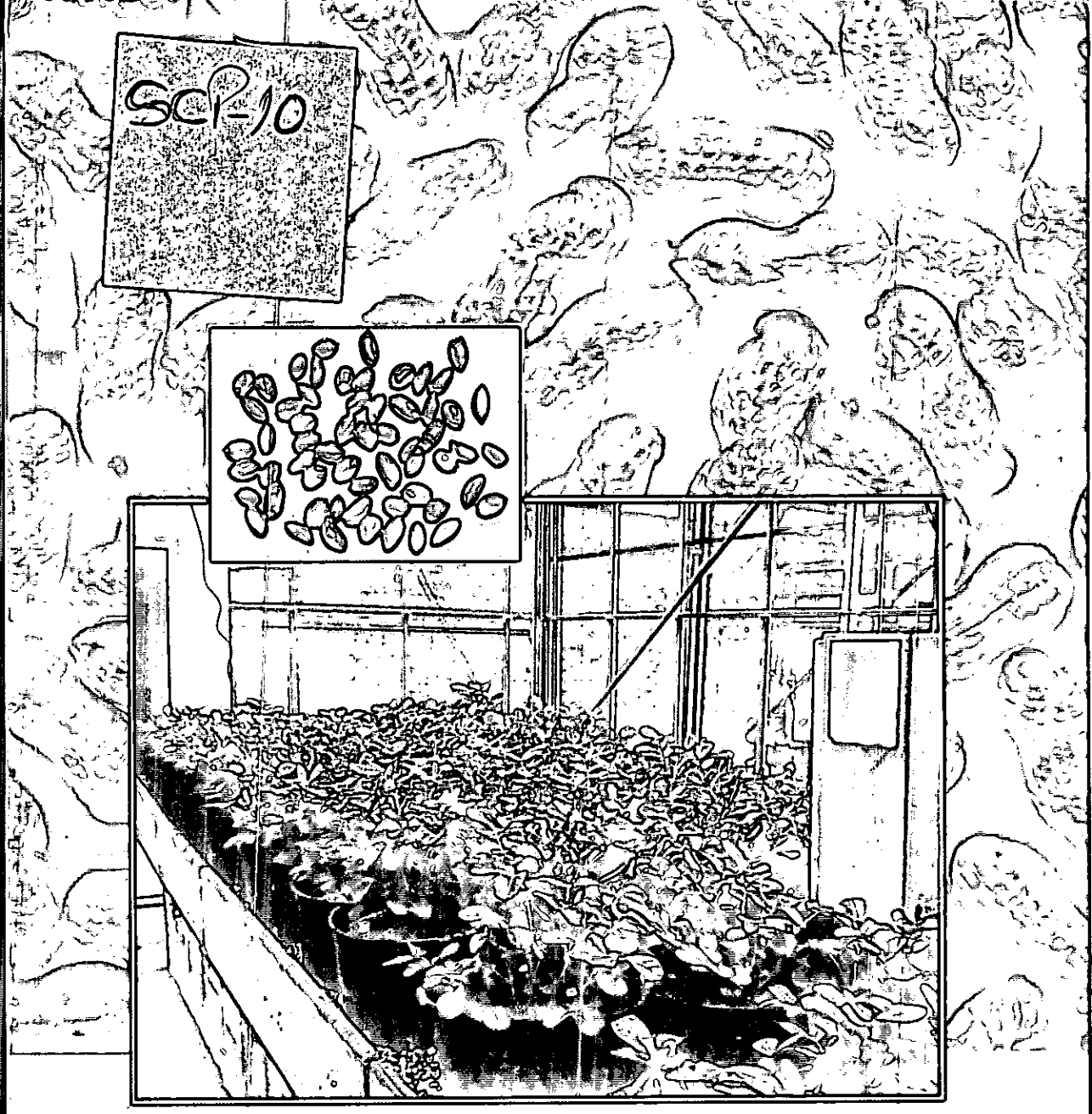

Orlando A. Quilambo

Functioning of peanut (*Arachis hypogaea* L.) under nutrient deficiency and drought stress in relation to symbiotic associations

Functioning of peanut (*Arachis hypogaea* L.) under nutrient deficiency and drought stress in relation to symbiotic associations

Promotor:	Prof. dr. ir. P.J.C. Kuiper
Referenten::	dr. G. Stulen dr. I. Weissenhorn
Beoordelingscommissie:	Prof. dr. W.H.O. Ernst Prof. dr. J. van Andel Prof. dr. H. Doddema

Alegria Cambula, I thank you for having been able to legally and with success-break all the bureaucratic procedures, allowing me to bring and take seeds from Mozambique and Holland, and mainly to having enabled me to transport 10 kg of soil, without undue trouble.
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Finally, I thank my esteemed wife Aidate, who was joined in 1999 by our cherished and little Marcos. Both accepted my " academic exile" with forbearance and love.
My parents Adriana and António, for their conviction, that education is cheaper than a lack of education, my brothers and sisters for their " try you first' recommendation, conviviality and encouragement, thank you.

Dedico esta tese a Aidinha,
Marquinho
e a meus pais.
Namubonga.

List of abbreviations

		Units
ABA	Abscisic acid	
AM	Arbuscular mycorrhiza	
AMF	Arbuscular mycorrhizal fungi	
BB	Peanut cultivar Bebiano Branco	
CMI (S)	Cell membrane integrity (stability)	
Dap	Days after planting	
DSTI	Drought stress tolerance index	$g.g^{-1}$
DW	Dry weight	g
DWFWR	Dry weight fresh weight ratio	$g.g^{-1}$
HI	Harvest index	$g.g^{-1}$
F	Peanut cultivar Falcon	
FW	Fresh weight	g
ISW	Initial seedling weight	g
L	Peanut cultivar Local	
Ln	Leaf number	
LA	Leaf area	cm^2
LAR	Leaf area ratio	$cm^2.g^{-1}$
LDW	Leaf dry weight	g
LWC	Leaf water content	$g.g^{-1}$
LWR	Leaf weight ratio	$g.g^{-1}$
MRL	Maximum root length	cm
MRLAR	Maximum root length to leaf area ratio	$cm.cm^{-2}$
NAR	Net assimilation rate	$g.cm^{-2}.day^{-1}$
NDTI	Nitrogen deficiency tolerance index	$g.g^{-1}$
NC	Peanut cultivar Natal Common	
Nn	Nodule number	
NPK	Nitrogen, Phosphorus and Potassium	
OA	Osmotic adjustment	
PDTI	Phosphorus deficiency tolerance index	$g.g^{-1}$
PEG	Polyethylene glycol	
RLAER	Relative leaf area expansion rate	$cm^2.g^{-1}.day^{-1}$
RDW	Root dry weight	g
RGR	Relative growth rate	$g.g^{-1}.day^{-1}$
RSD	Relative saturation deficit	(%)
R/S	Root shoot ratio	$g.g^{-1}$
RWC	Relative water content	(%)
RWR	Root weight ratio	$g.g^{-1}$
SLA	Specific leaf area	$cm^2.g\text{-}1$
SLW	Specific leaf weight	$g.g^{-1}$
SWR	Shoot weight ratio	$g.g^{-1}$
S/R	Shoot root ratio	$g.g^{-1}$
TI	Tolerance index	
TSN	Total sink number	
TDW	Total dry weight	g
TW	Turgid weight	g
WRC	Water retention capacity	(%)
WSD	Water saturation deficit	(%)

Table of contents

1. THE PEANUT CROP

Peanut (*Arachis hypoagaea* L.) seeds are now the world's fourth important source of edible oil, the third important source of vegetable protein and one of the richest sources of vitamin B1 in plants (Florkowski 1994; Singh 1995). In addition (i) peanut haulms are widely used as good quality animal fodder, (ii) the shells can be used as food for livestock, (iii) burnt as fuel, and (iv) made into particle boards or put to many other uses (Nigam *et al.*, 1991).
In Mozambique peanut is a traditional crop, distributed all over the country, with a concentration in the coastal areas, where it has gained commercial value. The major peanut-growing areas in Mozambique are the provinces of Nampula and Zambezia in the north and Inhambane and Gaza in the south (Malithano *et al.*, 1983). In Mozambique, the production area and average yield decreased in the 70 's and 80 's (Florkowski 1994). Yields are generally low, because of irregular rainfall (Ramanaiah *et al.*, 1989) and deficient soil moisture during the growing season. Other factors are poor agricultural practice by small-scale farmers, lack of good seed quality, the use of unimproved landraces, pests and diseases.

2. EFFECTS OF DROUGHT STRESS ON GROWTH AND YIELD

About 80 % of the world peanut production comes from seasonally rainfed areas in the semi-tropics, where climate is characterised by a low and erratic rainfall (Wright and Nageswara Rao 1994). Drought is recognised as a major constraint, limiting peanut production in these areas (Gibbons 1980). Soil water deficit reduces leaf area and stem growth through impact on plant water status, photosynthesis and leaf expansion (Wright and Nageswara Rao 1994).
A reduction in leaf area development is realised in peanut via a reduction in the rate of leaf initiation (Ong *et al.*, 1985) and in a reduced rate of leaf expansion, through a reduction in relative water content (RWC), or leaf turgor potential (Allen *et al.*, 1976).
Root growth of peanut is promoted by a mild water stress. Several studies have reported a reduced growth of the root on a long-term scale, together with a reduced investment in the leaves (increased root shoot ratio (R/S) or root weight ratio (RWR) (Munns and Cramer 1996). The high abscisic acid (ABA) concentration in the roots has been indicated as promoting root elongation rates (Saab *et al.*, 1990) and high carbohydrate concentration in the roots. According to Saab and Sharp (1989) a reduction in leaf expansion rate by drought occurs before any reduction in photosynthesis, so leaves of droughted plants have a high concentration of carbohydrates, which may be made available for root growth (Farrar 1996).

Nageswara Rao *et al.* (1989) showed that the peanut crop was sensitive to drought under almost all drought patterns, but less sensitive, when drought occurred early in the season. The same authors indicated that a high yield potential was associated with a great drought sensitivity.

Since the 80 's until recently, Mozambique was in a permanent drought situation, with only occasionally rain in some years, causing losses in many crops, mainly seed crops. Drought stress or water deficit induces changes in metabolic processes, as shown in Table 1.1.

These changes concern cell division and expansion, various factors in carbon and nitrogen metabolism and changes in activity of phytohormones. Sensitivity to drought also varies between the above parameters.

ABA has been universally indicated as the phytohormone whose levels increase during abiotic stresses (drought, salt, flooding, cold, chilling, low nitrogen/phosphorus and shading), while levels of cytokinins decrease (no data available for cold and chilling) (Morgan 1990). The same author indicated that drought, flooding and cold acclimation are conducive to decrease in gibberellins. ABA is a signal common to the initiation of many environmental responses, that ultimately result in the accumulation of various osmoprotectants (Ishitani *et al.*, 1995). The rapid induction of its accumulation in plants loosing turgor is a well known

Table 1.1 Relative sensitivity of various plant processes to water stress.

	Relative sensitivity		
Plant process	very sensitive	moderately sensitive	insensitive
Growth			
Cell division			
Cell expansion			
Carbon metabolism			
Stomatal opening			
CO_2 assimilation			
Sugar accumulation			
Protochlorophyll formation			
Nitrogen metabolism			
NO^-_3 reduction			
N_2 fixation			
Proline accumulation			
Protein synthesis			
Protein hydrolysis			
Hormonal activity			
Indole acetic acid			
Gibberellin			
Cytokinin			
Abscisic acid			
Ethylene			

Modified and adapted from: Hsaio (1973); Krieg (1993).

under controlled conditions in order to select cultivars, which show differences in growth parameters that may enable them to avoid drought and nutrient deficiency (Chapter 2).
The second aim focuses on growth responses of the selected cultivars to deficiency in phosphorus and nitrogen, emphasising again on root and leaf growth and carbon allocation patterns, under controlled environmental conditions (Chapters 3 and 4).
The third aim is to examine the sensitivity of the drought-avoiding parameters in the selected cultivars, emphasising differences in root and leaf growth and differences in the carbon allocation pattern to roots, leaves and generative parts. The differences between the cultivars are further explored, regarding cell membrane integrity, water retention capability, leaf water status and accumulation of proline, searching for other parameters, such as osmotic adjustment to drought (Chapters 5 and 6).
Finally, the influence of AM-colonisation on plant growth and alleviation of drought stress is investigated (Chapters 7 and 8).

2 Analysis of growth parameters in four peanut cultivars

SUMMARY

Four peanut cultivars from Mozambique, Natal Common (NC) originating from South Africa, Mozambican landraces Bebiano Branco (BB) and Local (L) and Falcon originating from Zimbabwe (F), were grown in plastic pots for 13 weeks. All cultivars have a short cycle, NC and F being high-yield crops and bred for high-input agriculture, while BB and L, are used for low-input agriculture. The pots were filled with washed sand/vermiculite mixture, supplied with a slow release fertiliser osmocote 13:13:13 NPK. The relative growth rate (RGR) and various other growth parameters were studied in detail. The small-seeded cultivars NC and F showed a higher RGR compared to the large seeded cultivars BB and L.

The natural logarithm of the initial seedling weight (ISW) showed a negative correlation with the RGR and the final mean number of pods/plant and a positive correlation with the root weight ratio (RWR), whereas the RGR showed a positive correlation with the mean number of pods/plant and a negative correlation with RWR. The significant and negative correlation between ln ISW and RGR resulted mainly from a negative correlation with leaf area ratio (LAR), via the morphogenetic effects of the specific leaf area (SLA) and leaf weight ratio (LWR).

The cultivar Falcon allocated more dry matter to the pegs and pods, while the cultivar Local allocated more dry matter to the leaves and roots. A large root system in the cultivar Local may provide an advantage under limited soil moisture and nutrient resources.

1. INTRODUCTION

Peanut is the world's fourth important source of edible oil, third important source of vegetable protein and one the richest sources of thiamine (B1) (Florkowski 1994; Singh 1995). In Mozambique yields of peanut are generally low and average about 500kg/ha.

The main constraints to the potential for peanut production in Mozambique, (Malithano *et al.*, 1984a) both as a rainfed and as an irrigated crop, are: (i) poor agricultural practices, (ii) lack of good seed quality, (iii) irregular rainfall, (iv) growing of unimproved landraces, (v) low soil fertility, (vi) pests, diseases and weeds. Coping with irregular rainfall, pests, diseases and weeds and with low soil fertility requires a certain degree of inherent specialisation to these adverse conditions. Peanut is drought tolerant and can withstand a low water potential (Wright *et al.*, 1994; Collinson *et al.*, 1997).

Using a linear regression method for screening peanut growth performance, Freire and Botão (1994) suggested that the peanut cultivars Bebiano Branco (BB) and Natal Common (NC) should be used as controls in peanut improvement programs in southern Mozambique, due to a good adaptation of both cultivars to the local conditions. The cultivar Bebiano Branco is a selection of a Mozambican "land race", while the cultivar Natal Common originates from South Africa and shows a high yield and stability under varying conditions.

The cultivar Falcon (F) was bred in Zimbabwe and released in 1990. It has the highest yield potential of the short season cultivars grown so far and a low development of leaf diseases. The cultivar Local (L) is a Mozambican land race with a very high genetic variability, normally with a high yield (Malithano *et al.*, 1984b).

However, the real advantage of a specific cultivar over others, especially under water or nutrient limited conditions, can only be explained after a detailed analysis of the relative growth rate (RGR), since the time course of soil depletion of water and nutrients depends on a variety of factors such as evaporation, leaf area and soil characteristics (Tardieu 1996). For example, if two cultivars have different leaf expansion rates, the cultivar with the higher leaf area will deplete the soil more rapidly and, consequently, will experience drier soil after several days without irrigation.

Ketring *et al.* (1982) reported that peanut cultivars significantly differ in duration of the vegetative growth period, canopy structure and root growth and in their response to adverse conditions.

A correlation between transpiration, water use efficiency (WUE) and specific leaf area (SLA) has been reported (Wright *et al.*, 1994a), and genotypes with a greater WUE had a lower SLA.

Variations in plant growth rate cannot be understood using data from photosyn-

thesis and crop yield alone, since photosynthesis is part of the carbon economy and differences in final plant weight are not just determined by the daily carbon budget, but also by possible variation in seed weight, germination time and duration of growth (Poorter *et al.*, 1990).

The peanut seed shows a large variation in size, shape and colour. These variations, which may be present even within a genotype or plant species, may result from differences in maturation at harvest (Williams *et al.*, 1987), environmental conditions to which the mother plant is exposed such as insufficient Ca^{2+}-concentration (Cox *et al.*, 1976) and drought stress (Pallas *et al.*, 1977). The peanut seed size is an important characteristic that determines both seed quality and value of a cultivar (Knauft *et al.*, 1991).

A significant and negative relationship between RGR and seed size has been established for eight legumes (Stebbins 1976) and for *Vigna subterranea* (L.) Verdc. (Onyekwelu 1991).

Data on other growth parameters are less conclusive. For instance, large roots have been related to large seeds, although Fenner (1983) found that embryos of large seeds produced seedlings with relatively small roots and Jurado and Westoby (1992) found no tendency for this relation at all.

The objective of this study was to perform a detailed growth analysis of 4 peanut cultivars grown in Mozambique: Natal Common (NC), Bebiano Branco (BB), Local (L) and Falcon (F), under non limiting nutrient conditions, searching for morphological and physiological traits that can contribute to drought stress and nutrient deficiency tolerance.

2. MATERIAL AND METHODS

2.1. Plant material

Four peanut (*Arachis hypogaea* L.) cultivars, Natal Common (NC), Bebiano Branco (BB), Local (L) and Falcon (F) (Table 2.1), were grown in 1.5 l plastic pots (for the harvest in week 1 to week 3), 5 l pots (for harvests in week 4 to week 9) and 12 l pots (for harvests in week 10 to week 13). The characteristics of the cultivars are given in Table 2.1.

The plants were grown from pre-germinated seeds in filter paper moistened with distilled water, for 72 hours. Pre-germinated seeds with a radicle of 10 mm length, where planted a single plant per pot.

2.2. Growth conditions

The plants were grown from July to October 1997, in a glasshouse, in Haren, The Netherlands. The temperature regime was 25/20 °C day and night, respectively,

Table 2.1 Characteristics of the peanut cultivars used. ISW, is initial seedling weight.

Cultivar		Origin	Average time to maturity (days)*	Growth habit	ISW (g)**
Natal Common	NC	South Africa	112	Erect	0.50±0.07a
Bebiano Branco	BB	Mozambique	112	Erect	0.55±0.04a
Local	L	Mozambique	110-112	Erect	0.61±0.09b
Falcon	F	Zimbabwe	110	Erect	0.69±0.13b

*Adapted from Malithano et al. (1984b) and ** determined in the present study.

70 % relative humidity, 12 h light intensity regime, supplied by sun and additional lighting by lamps (Philips HPI-T 400W), resulting in an average photon flux density at the canopy level of 247 ± 10 $\mu mol.m^{-2}.s^{-1}$ in the morning, 378 ± 16 $\mu mol.m^{-2}.s^{-1}$ at midday and 380 ± 19 $\mu mol.m^{-2}.s^{-1}$ in the afternoon. Light intensity was measured with a quantum sensor (SKP215, Skye, Llandrindod Wells, UK).
The pots were filled with a 1:1 (v/v) washed sand /vermiculite mixture, supplied with a slow release fertiliser osmocote 13:13:13 NPK (Scotts Europe, NL) at a ratio of $3.6.g.l^{-1}$ soil substrate (adapted from Pell *et al.*, 1990). The mineral composition of the osmocote was as follows: 5.8 % nitrogen as ammonium and 6.2 % as nitrate; phosphorus as phosphoric acid and potasium as potassium oxide. The pots were kept near the field capacity by weighing twice a week and adding water when necessary.

2.3. Growth analysis

Plants were harvested each week, counted from the day of planting, with n=8 plants per harvest. At each harvest the number of leaves was counted, as well as the maximum root length (MRL) (Hendrix *et al.*, 1991). The root volume was measured as the increase in water volume, between an initial volume without roots and a final volume with roots, using a measuring cylinder. The plants were separated into roots, cotyledons, stems, leaves, pegs and pods. The pegs and pods were counted, when more than 10 mm long, and their fresh weights were determined.
The dry weights were determined after 48 hours at 80 °C in a drying oven. The leaf water content (LWC) was calculated according to Garnier and Laurent (1994). The relative growth rates (RGR) of the total plant, the shoots and the roots were calculated on a dry weight basis, according to Poorter (1989). The period of exponential growth (0-91 days) was used for curve-fitting, in order to avoid the phase of declining growth.
Leaf area was measured, using the gravimetric method for the first 6 weeks of

growth, and using a leaf area meter (Model 3100 LI-Cor Inc., Lincoln, NE, USA) for the rest of the experiment. The specific leaf area (SLA = plant leaf area divided by total leaf weight) was calculated according to Hunt (1982) and the net assimilation rate (NAR), was derived from the formula RGR = LAR x NAR (Hunt 1982), where LAR is the leaf area ratio (= plant leaf area divided by total plant weight).
The weight ratios (plant part weight/total plant weight) were calculated on a fresh and dry weight basis.

2.4. Data analysis

Data were analysed with the SPSS/PC statistical package, version 4.0.1.
Differences in growth parameters between the cultivars were analysed by one way ANOVA. Trends of RGR, SLA, LAR, RWR and NAR were analysed by linear regression, using a GraphPad Prism package, version 2.01.

3. RESULTS

3.1. Phenological development

The cultivars Bebiano Branco, Falcon and Local showed a similar occurrence of pento-foliate leaves and a similar number of days to 50 % flowering (Table 2.2). This latter parameter was determined by counting the number of flowers/cultivar from the first day of flower appearance to the day when 50 % of the plants showed open flowers. Natal Common and Falcon showed a lower seed weight and initial seedling weight (ISW; Table 2.2).

Table 2.2 Characterisation of the peanut cultivars used in the growth analysis. 50% flowering was determined as described above and the other parameters were at harvest (91 days after planting). Values are means of 8 plants per cultivar, except pods/plant.

Cultivar	Days to 50% flowering	Maximum pods/plant	Seeds/pod	Occurrence of penta and hexafoliates(%)*	
				penta	hexa
NC	37	37	1-3	7.5	2.0
F	35	25	1-3	12.3	2.1
BB	34	37	1-3	15.5	2.8
L	34	30	1-2	14.4	7.0

* The remaining percentage is from tetrafoliate leaves.

3.2. Leaf and root growth

Despite similarities in some primary components of seed yield, such as maximum pods/plant and seeds/pod of the cultivars Natal Common, Bebiano Branco and Local, they showed differences, though not always statistically significant, in seed weight, initial seedlings weight (ISW) and relative growth rate (RGR) (Table 2.3)
The small-seeded cultivars, Natal Common and Falcon, showed a slightly but not statistically significant higher RGR, compared to the large-seeded Bebiano Branco and Local, which showed similar values in all cases (Table 2.3).
The growth of the 4 cultivars showed a similar pattern (Figures 2.1a and 2.1b).
As with total dry weight and shoot dry weight, no significant differences were found in leaf area development (Figure 2.2).
The cultivars Bebiano Branco and Local tended to have the highest leaf area values, particularly at the end of the experiment.

Table 2.3 Weight of 100 seeds (ISW, average of 10 recently germinated seeds) and RGR of the whole plant, of the shoots and the roots. The RGR was determined as described before, on a dry weight basis. Values are means of 8 plants (± SD). Within columns, values followed by the same letter, are not significantly different at the $P<0.05$ level, using the Student t-test.

Cultivar	Weight of 100 seeds(g)	ISW(g)	RGR($g.g^{-1}.day^{-1}$)		
			Whole plant	Shoots	Roots
NC	22.5	0.50±0.07a	0.04±0.002a	0.04±0.002a	0.03±0.003a
F	33.3	0.55±0.04a	0.03±0.001a	0.04±0.002a	0.03±0.002a
BB	34.3	0.61±0.09b	0.03±0.002a	0.03±0.002a	0.02±0.003a
L	36.5	0.69±0.13b	0.03±0.009a	0.03±0.001a	0.02±0.002a

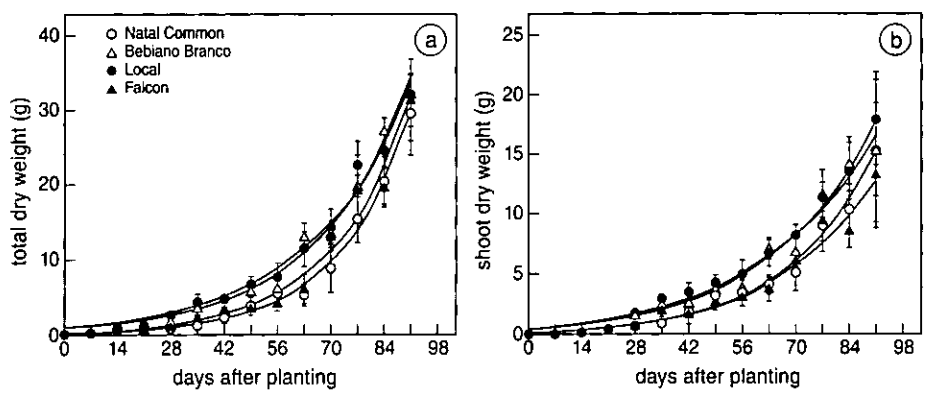

Figure 2.1 Growth of the four cultivars on a dry weight basis of the whole plant (a) and shoot (b). Data represent mean of 8 plants (± SD).

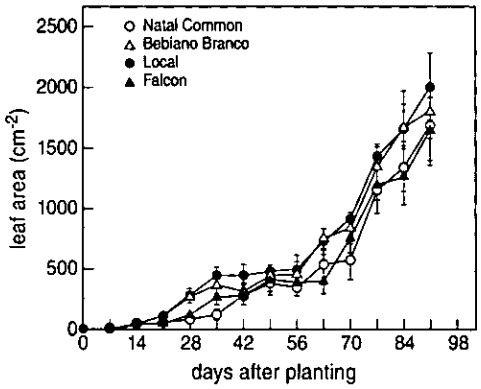

Figure 2.2 Leaf area development of the four peanut cultivars. Data represent mean of 8 plants (± SD).

A significant and negative correlation (r = 0.97 at $P<0.05$) was found between the ln ISW and RGR, and between the ln ISW and final mean pods/plant (r = 0.98 at $P<0.01$ Figures 2.3a and b), whereas the values of RGR and the final mean pods/plant showed a linear and positive correlation (r = 0.94 at $P<0.05$), as shown in Figure 2.3c.

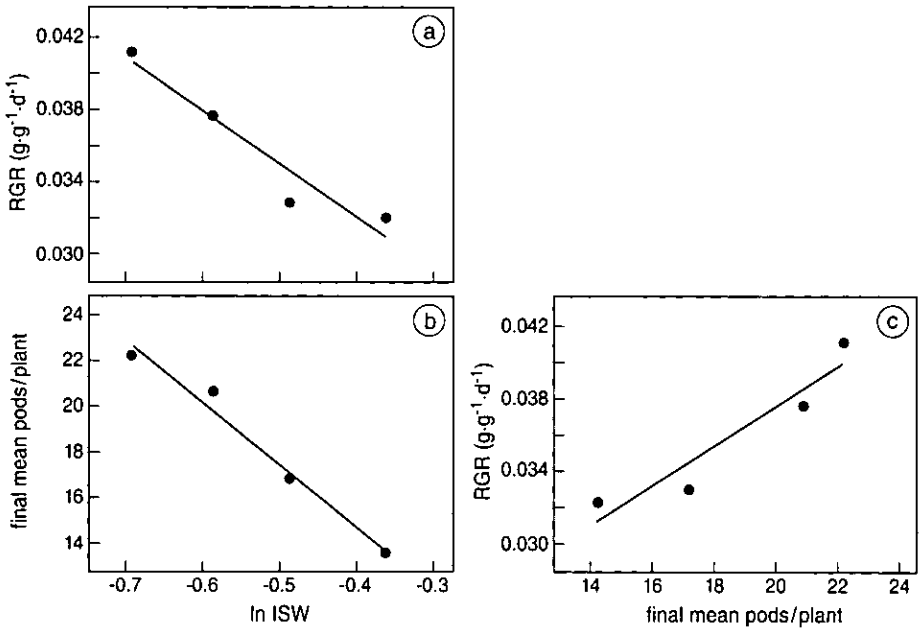

Figure 2.3 Correlation between ln ISW and RGR (a), ln ISW and final mean pods/plant (b) and RGR and final mean pods/plant (c).

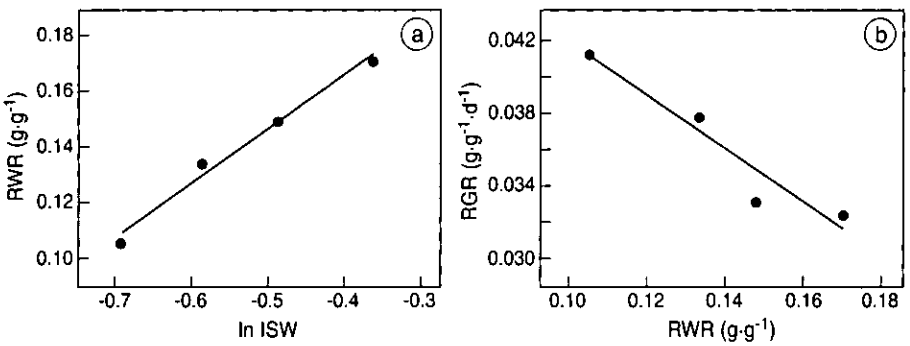

Figure 2.4 Correlation between RWR and ln ISW (a) and RGR and RWR (b).

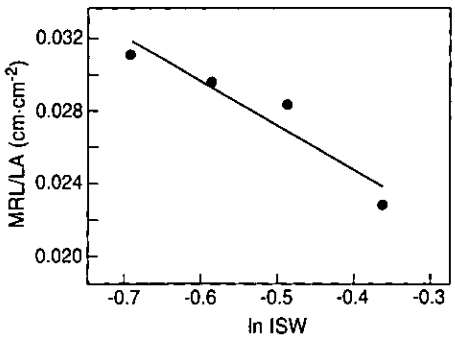

Figure 2.5 Correlation between maximum root length to leaf area ratio (MRL/LA) and ln ISW.

The observed high growth rate of the small-seeded cultivars seems to result from a higher LAR as a result of high SLA and not from a higher NAR.
However, at the beginning of the experiment the positive linear correlation between NAR and RGR was slightly closer than between RGR and LAR ($r = 0.86$, and $r = 0.84$ at $P<0.05$), but during the development of the crop plant LAR became more important. The only significant linear correlation was observed between SLA and RGR at week 1 after planting, ($r = 0.97$ at $P<0.05$, data not shown).
The RWR values were significantly and positively correlated with ln ISW ($r=0.99$ at $P<0.01$).
The maximum root length/leaf area ratio (MRLAR), is a characteristic, which is potentially important for the ability of seedlings to withstand drought (Hendrix *et al.*, 1991). MRLAR was negatively correlated with ln ISW ($r = 0.94$ at $P<0.05$) (Figure 2.5), while other parameters like leaf water content (LWC), shoot weight ratio (S/R), did not differ among the cultivars. The latter parameters did neither show a clear relationship with other growth parameters nor with seed yield.

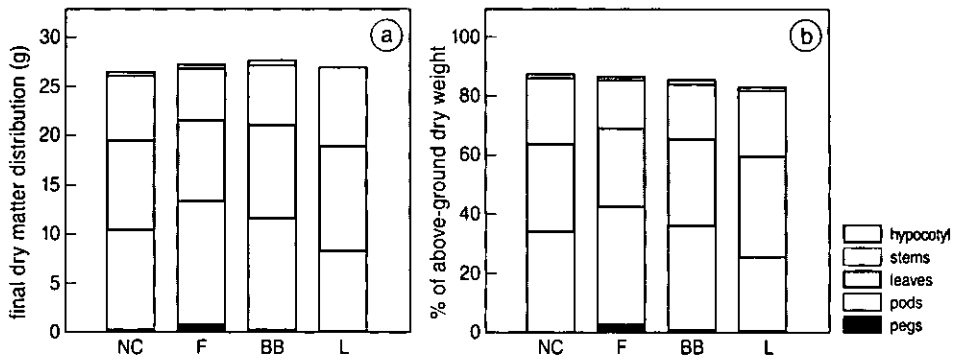

Figure 2.6 Percentage aboveground dry matter allocation in the four peanut cultivars at harvest. Data represent the mean of 8 plants.

Allocation of dry matter showed differences between the cultivars Falcon and Local: Falcon allocated more dry matter to the pods (39.8 %) and pegs (2.5 %) and less to the leaves (26.6 %) and roots (13.6 %). The cultivar Local allocated more dry matter to the leaves (34.2%) and roots (17.1%) and less to the pods (24.7 %) and pegs (0.63 %), as shown in Figure 2.6. The cultivars Natal Common and Bebiano Branco showed intermediate values, allocating more to the pegs (1.8 and 1.14 %) and pods (33.9 and 33.5 %, respectively), compared to the cultivar Local, and more to the leaves, when compared with the cultivar Falcon (30.7 and 29.7 %, respectively).

The cultivar Natal Common showed the highest value of leaf/pod ratio and shoot/pod ratio, particularly at the beginning of the pod formation, 70 and 77 days after planting (data not shown). However, the final values of these parameters were similar for all the cultivars. The cultivar Falcon showed the lowest values, as result of a high investment in the reproductive organs, pegs and pods.

4. DISCUSSION

4.1. Relative growth rate (RGR), growth parameters and seed size

In the four peanut cultivars, used in the present experiment Natal Common (NC), Bebiano Branco (BB), Local (L) and Falcon (F), RGR was negatively correlated with ln initial seedling weight (ISW, Figure 2.3a), indicating that small seeded cultivars have a high RGR. Similar results have been reported by Maranon (1988) and Maranon and Grubb (1993) for Mediterranean annuals. The cause seems to be that the small-seeded cultivars show a high specific leaf area (SLA). Since a high SLA value was found to be negatively correlated with water use efficiency

(WUE) in peanut (Wright *et al.*, 1994ba), the small-seeded cultivars Falcon and Natal Common, may show a low resistance to drought.
Contrary to these findings, the small-seeded cultivars Falcon and Natal Common, exhibited a higher maximum root length to leaf area ratio (MRLAR) than the large-seeded cultivars Bebiano Branco and Local. A reduced leaf area is an advantage under drought conditions, since it allows the plants to reduce the transpiring area, while accessing simultaneously deeper sources of water due to the high root length. A high MRLAR is a characteristic potentially important for withstanding drought which was strongly and negatively correlated with seed biomass (Figure 2.5). From the present results, it may be assumed that the peanut cultivars Natal Common and Falcon have seed and growth characteristics, which enable them to withstand short-term drought periods.
However, also large-seeded characteristics, may be beneficial under drought conditions. In fact, Manga and Yadav (1995) demonstrated that a large seed size of pearl millet led to vigorous seedlings and taller plants with increased tillering and drought tolerance.
Throughout the experiment a significant and positive correlation of RWR and ln ISW was observed (Figure 2.4a), a result confirming the argument of Baker (1972), that large seed weight in different plants species enables a seedling to allocate proportionately more carbon to root development and so is able to produce an extensive root system in a short time. There are also results which indicate that there is no significant relationship between ISW and RWR (Maranon and Grubb 1993) in water-washed sand. They suggested that a negative correlation may be a result of the growth medium, particularly if distilled water is used as growth medium. Our plants were grown in a balanced and solid nutrient medium. Therefore, the explanation of the differences in the results between the cultivars cannot be found in the nutrient supply, but have to be found in the characteristics of the plants. While the large-seeded cultivars showed an ability of developing quickly an extensive root system, the small-seeded cultivars showed a tendency of having a high ratio of the maximum root length over leaf area (Figure 2.5). Small-seeded cultivars tended to have greater values of this ratio than large-seeded ones; i.e. they can reach a greater depth, relative to leaf area than large seeded culivars. This tendency which is associated with a high value of SLA is presumably of value in reducing mortality when drought follows establishment (Maranon and Grubb 1993).
Both strategies, high RWR and MRLAR may enable the plants to cope with adverse environmental conditions. Furthermore, the significant and positive correlation between ln ISW and RWR observed in the present experiment (Figure 2.4a) is not reported in many studies; but it may be an advantage for establishing root symbiosis (Janos 1980). In fact, it has been found that sporulation of the arbuscular

mycorrhizal fungi (AMF) (i) in *Pennisetum pediculatum* (Singh 1992) and the percentage of AM-colonisation and phosphorus (P) uptake in white clover (Blair and Godwin 1991), were favoured by root volume. Deep and extensive roots have been found as contributing to drought resistance and P efficiency in plants (Al-Karaki *et al.*, 1995). The large seeded cultivars Local and Bebiano branco, according to the results of the experiment, may show a higher root colonisation and, consequently, an increased P uptake.

4.2. RGR and leaf growth

A significant and positive correlation between RGR, net assimilation rate (NAR) and leaf weight ratio (LWR) was found at 42 days after planting (data not shown). The relationship between NAR and RGR was found to be inconsistent in dicots (Meerts and Garnier 1996), conferring to the leaf area ratio (LAR), via SLA, the major explanation for the high RGR in small-seeded seedlings as reported by Mooney *et al.* (1978) in *Eucalyptus*, Poorter *et al.* (1990) in 24 wild species, Maranon and Grubb (1993) in Mediterranean annuals and Poorter and Van der Werf (1998) in herbaceous species. In contrast to the previous results, Roumet *et al.* (1996) found that NAR explained the increase in RGR under elevated CO_2. The results of the present experiment are not consistent enough to ascribe the increase of RGR either to NAR (data not shown) or LAR.

4.3. RGR and root growth

RGR showed a significant and negative correlation with RWR (Figure 2.4b), a result similar to that reported by Lambers and Poorter (1992), who stated that at optimum nutrient supply, inherent fast-growing species have a somewhat lower RWR than slow-growing species. This relationship shows, additionally, that large root systems from large seeds may provide an advantage by allowing better access to limited soil moisture and nutrient resources. It can be speculated that the cultivars Local and Bebiano Branco may show a better performance under water and nutrient limitation.

Several studies suggest that a low RGR is associated with increased cell wall material (Niemann *et al.*, 1992) and stress tolerance (Chapin 1988; Grime *et al.*, 1988).

The cultivars Natal Common and Falcon, with the highest RGR, are the results of breeding programs under high-input agriculture in South Africa and Zimbabwe, while the cultivars Local and Bebiano Branco are landraces. The high RGR shown by the cultivars Natal Common and Falcon, may not result only from the experimental conditions, but be inherent, since it has been found that high RGR is linked to species, originating from fertile sites (Van der Werf *et al.*, 1993).

Several authors found that even when grown under optimum conditions, species

which naturally occur in nutrient-poor or dry habitats (Rozijn and Van der Werf 1986), still have a low RGR, compared to plants characteristic of fertile soils (e. g. Poorter *et al.*, 1990). It may, therefore, be suggested that the low RGR of the cultivars Local and Bebiano Branco, also may have resulted from their inherent RGR and not only from the seed size, as they originated from low-input agricultural systems.
The allocation pattern of biomass varied between the cultivars and did not show a very clear trend, which does not allow to assign a specific relation to RGR.

5. CONCLUSIONS

Under the conditions of the present growth analysis experiment, it is concluded that: (i) the initial seedling weight (ISW) tended to influence the relative growth rate (RGR), though not significantly, showing that the small-seeded peanut cultivars tended to have a higher RGR; (ii) both types of cultivars (small-seeded and large seeded) showed traits which enable tolerance to stress, such as high MRLAR for the small-seeded and high RWR for the large-seeded ones and (iii) the small-seeded cultivars, particularly Falcon, allocated more dry matter to the reproductive organs, whereas the large-seeded cultivar Local allocated more dry matter to the leaves and roots. The differences in allocation pattern will be exploited in the next Chapters in relation to drought and nutrient stress tolerance.

3 Growth response of two peanut cultivars to nitrogen limitation

SUMMARY

Two peanut cultivars Falcon (F) and Local (L) were grown in a glasshouse in plastic pots for 13 weeks, under well-supplied and low N conditions. At the vegetative stage relative growth rate (RGR) was reduced in cultivar Falcon, while relative leaf expansion rate (RLAER) was substantially decreased in both cultivars at low N. At the reproductive stage RGR and RLAER were reduced in control plants, resulting in higher growth and yield of the low N peanut plants.
No significant differences were found in yield and yield components.

1. INTRODUCTION

Peanuts have been grown in Mozambique for many years, mainly in light soils along the coastal zone. These soils give a poor yield because mineral nutrients are low and symptoms of deficiency in nitrogen (N), phosphorus (P), calcium (Ca), and zinc (Zn) have been observed (Malithano *et al.*, 1984a). In the peanut growing areas of southern Mozambique, plants showed deficiency symptoms of N and P, whereas Ca deficiency was mainly observed in the North, where long duration and prostrate types of peanut are grown (Malithano *et al.*, 1984a). Nitrogen deficiency is characterised by a poor vegetative growth and pale yellow foliage, particularly in coarse, sandy soil (Ramanaiah *et al.*, 1984).

Plants, subjected to low external concentrations of nitrate and phosphate in the soil, in general, exhibit multiple stress symptoms. Particularly common is an increase in root shoot ratio (R/S) Gutschik and Kay (1995), as a result of a shift in carbon allocation to roots, presumably to minimise the effect of N and/or P deficiency-induced multiple stress.

The peanut plant usually is resource limited, frequently because of low soil fertility, in particular N. This situation will affect assimilation and partitioning of photosynthates (Pell *et al.*, 1990).

Chapin *et al.* (1987) suggested that plants grown in an environment, which is deficient in a given element, will respond by allocation of carbon to the organ which acquires the nutrient in question.

For proper growth of peanut, small quantities of N in the form of NO_3^- or NH_4^+ are needed by young plants, until N becomes available through the N_2 fixation process. N deficiency in peanut plants is characterised by varying degrees of foliar chlorosis. Young plants, not yet adequately colonised by *Bradyrhizobium*, usually are paler green than normal. In severe cases the entire leaf becomes a uniform pale yellow and stems may be slender and elongated (Smith *et al.*, 1994).

N deficiency-induced chlorosis can result from: (i) lack of nodulation, associated with an inadequate amount of soil bacteria, (ii) insufficient N reduction, attributable to molybdenum deficiency, a condition associated with extreme soil acidity, (iii) translocation of N to developing fruits late in the season and (iv) water-logged conditions that limit root respiration and inhibit N_2 fixation (Smith *et al.*, 1994).

N deficiency can alter the physiological responses of crop plants to water deficits (Bennet *et al.*, 1986; Jones *et al.*, 1986), and may result in changes in plant characteristics, often associated with drought resistance, such as increased root weight ratio (RWR) and reduced vegetative growth (Radin and Parker 1979).

Lambers and Poorter (1992) compared fast and slow growing species under conditions of nutrient limitation. Fast growing species still had at least a relative

growth rate (RGR) comparable to that of slow growing species. McDonald *et al.* (1992) have shown that a growth-limiting N availability primarily affects leaf area development, while the rate of photosynthates per unit leaf area is almost unaffected. The exact mechanism behind the N limitation-induced reduction in leaf growth is not fully known, but cell wall loosening and incorporation of new cell material are processes which are negatively affected by N limiting growth conditions (Taylor *et al.*, 1993). The rapid decrease in leaf growth is in line with the N-C balance concept (e.g. Thornley 1972): when N is limiting, the concentration of N in the shoot will be lower than in the root, because the shoot system is further away from the N supply.

Wright *et al.* (1994) found that the specific leaf area (SLA) was negatively correlated with the water use efficiency (WUE), suggesting that genotypes with a lower SLA (greater leaf thickness) had a greater WUE. WUE is an important physiological characteristic which is directly related to the ability to cope with water stress. Plant and soil N play a role in WUE: soil N level was positively related to WUE in wheat (Roy and Singh 1983).

When photosynthates, water or nutrients are growth limiting, a larger fraction of water and nutrients taken up is retained in the roots, resulting in a decrease in the shoot/root ratio (S/R). The S/R drastically decreased with a reduction in N supply, as a result of root growth stimulation (Klepper 1991).

Peanut in southern Mozambique is grown in sandy soils with a low water holding capacity and low retention of nutrients as N and K. Generally, crops subjected to a low soil N have a low growth rate and low S/R (Russell 1977), which probably affects the delicate balance between crop transpiration and nutrient and water absorption.

The objective of the present study was to investigate the response of two peanut cultivars, differing in their carbon allocation pattern, to a limiting nitrogen supply in a glasshouse experiment, using a slow release fertiliser. It is expected to identify physiological and morphological traits associated with N deficiency. The results obtained may contribute to the development of good screening procedures.

2. MATERIAL AND METHODS

2.1. Plant material

Two peanut cultivars (*Arachis hypogaea* L.), Local (L) and Falcon (F), were grown in 1.5 l plastic pots (for the harvests in week 1 to week 3), 5 l pots (for the harvests in week 4 to week 10) and 12 l (for the harvests in week 10 to week 13).

The characteristics of the cultivars were given in Chapter 2 (Table 2.1).

2.2. Growth conditions

The plants were grown from July to October 1998 in a glasshouse, in Haren, The Netherlands. Temperature, relative humidity and light regimes were as described in Chapter 2.

The pots were filled with a 1:1 (v/v) washed sand/vermiculite mixture supplied with a slow release fertiliser osmocote 13:13:13 NPK (Scotts Europe, NL) at a ratio of 3.6 $g.l^{-1}$ soil substrate. The mineral composition of the osmocote is described in Chapter 2. The plants grown under these conditions were considered control plants. The following manipulations allowed plants to receive low levels of nitrogen, while maintaining a constant rate of phosphorus and potassium fertilisation.

Nitrogen deficient plants were grown in soil mix, containing 0.72 $g.l^{-1}$ osmocote NPK 13:13:13, 0.982 $g.l^{-1}$ osmocote NPK 0:41:0 and 0.908 $g.l^{-1}$ osmocote NPK 0:0:45. This procedure was adapted from Pell *et al.* (1990). The moisture of the mixture was kept near the field capacity by weighing representative pots of each size and cultivar, twice a week and adding water, when needed.

2.3. Growth analysis

Eight plants per cultivar and treatment were harvested every week, from week 1 through week 13. Growth analysis was performed as described in Chapter 2.

The relative leaf expansion rate (RLAER) was calculated as the slope of the natural logarithm of the leaf area versus time, according to Lynch *et al.* (1991).

The N deficiency tolerance index (NDTI) was calculated on dry weight matter of different plant parts for each variable according to the formula:

NDTI = Component dry weight (g) under N deficiency/Component dry weight (g) under well-supplied N conditions (Maiti *et al.*, 1996).

2.4. Data analysis

Data were analysed with the SPSS statistical package, version 4.0.1. Differences in growth parameters between cultivars and treatments were analysed by one way ANOVA. Trends in LWR, RWR, SLA, LAR, NAR and RGR were analysed by a linear regression, using a GraphPad Prism package, version 2.01.

3. RESULTS

3.1. Phenological development

Low nitrogen supply did not influence the phenological development at the vegetative stage and the time of 50% flowering in both cultivars, Local (L) and Falcon (F). At the reproductive stage, N deficient plants in larger pots showed a pale yellow foliage, elongated stems and pegs, while the control plants were dark green and

stunted (results not shown). These deficiency symptoms of the N limited plants at the later development stages, indicated that new N was required for pod fill: translocation of N from vegetative parts to the pods was intensive, as found in other legumes (da Silva *et al.*, 1993).

3.2. Leaf and root growth

At the vegetative stage, a significant reduction was observed in the RGR in the cultivar Falcon under low N, expressed on a basis of total dry weight, leaf dry weight, dry weight of the roots, as well as of the RLAER in cultivar Falcon and Local (Table 3.1).

RLAER of the cultivar Local, seems to be highly sensitive to low N, it showed a reduction of 34 %, compared to only 16 % in the cultivar Falcon. The reduction in RGR of plant, leaf and root dry weight, shows that cultivar Falcon was more sensitive to low nitrogen supply, than cultivar Local.

At the reproductive stage RGR and RLAER were much reduced in control plants, while N deficient plants showed a slightly higher RGR for the whole plant, leaves, roots and RLAER. RGR values of the roots in cultivar Local, were lower, while in the cultivar Falcon, it was the same (Table 3.1).

A similar pattern was also observed in the last harvests, when plants were grown in 12 l plastic pots (large soil volume), particularly for the whole plant, leaves and leaf area. Thus, the pot size seems not to have influenced the observed results (not shown).

Table 3.1 Effect of nitrogen limitation on RGR (plant, leaf, root dry weight) and RLAER at the vegetative stage and reproductive stages. Values are means of 8 plants (± SD). Dap, are days after planting. Within columns, per growth stage, values of each cultivar followed by the same letter, are not significantly different at the $P<0.05$ level, using the Student t-test.

Treatment	RGR ($g.g^{-1}.day^{-1}$)			RLAER ($cm^2.g^{-1}.day^{-1}$)
	Whole plant	Leaves	Roots	
	Vegetative stage (0-35 dap)			
Local control	0.19±0.02a	0.21±0.01a	0.15±0.06a	0.21±0.01a
Local low N	0.17±0.01a	0.19±0.01a	0.15±0.02a	0.14±0.00b
Falcon control	0.16±0.01a	0.15±0.01a	0.14±0.01a	0.13±0.01b
Falcon low N	0.11±0.01b	0.13±0.01b	0.10±0.01b	0.11±0.01b
	Reproductive stage (42-91 dap)			
Local control	0.03±0.00a	0.02±0.00a	0.07±0.00b	0.02±0.00b
Local low N	0.05±0.00b	0.04±0.00b	0.01±0.00a	0.03±0.00b
Falcon control	0.02±0.00a	0.02±0.00a	0.01±0.00a	0.01±0.00a
Falcon low N	0.05±0.00b	0.03±0.00b	0.01±0.00a	0.02±0.00b

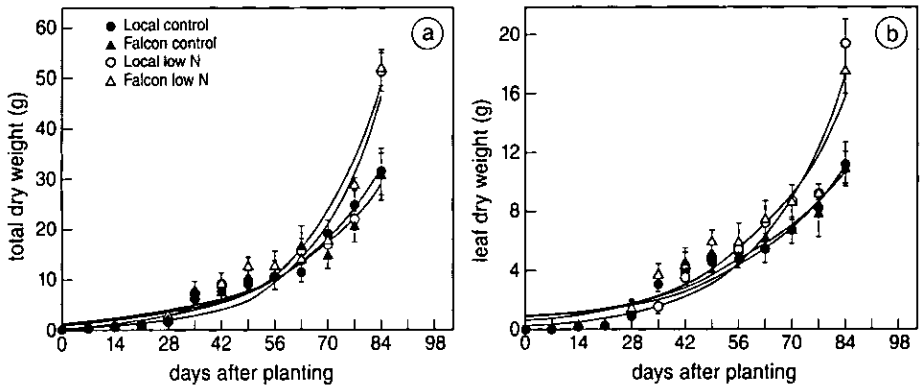

Figure 3.1 Growth of the cultivars L and F under optimal and low N supply, on a dry weight basis of the whole plant (a) and leaf (b). Data represent mean of 8 plants (± SD).

With the exception of RGR of roots, the cultivar Falcon showed significantly higher values of RGR under N deficiency in relation to the control, namely 118 % for the whole plant, 115 % for the leaf dry weight, and 53.7 % for the RLAER. Cultivar Local, showed a reduction of 76 % of the root RGR, in relation to control.

Despite some significant differences in RGR, particularly in the cultivar Falcon, the total dry weight of the whole plant and leaves did not show significant differences at the vegetative stage (Figure 3.1).

No statistically significant changes were observed in parameters, known to be sensitive to N deficiency, such as specific leaf area (SLA), leaf area ratio (LAR) and root weight ratio (RWR). Low N plants showed even slightly higher values of total dry weight, and leaf dry weight, while other parameters did not show any consistent change.

In a comparison of the data, low N plants showed a significantly higher root weight, when expressed on fresh weight basis and a high root volume. The culti-

Table 3.2 Effect of nitrogen limitation in the reproductive stage (day 42 to 91), on root fresh weight (RFW) and root volume (Rvolume) in two peanut cultivars. Within column, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	RFW	RFW (% of control)	Rvolume	Rvolume (% of control)
Local control	12.2a	100	14.3a	100
Local low N	15.6b	128	19.1b	133
Falcon control	12.6a	100	14.8a	100
Falcon low N	17.9b	143	21.9b	148

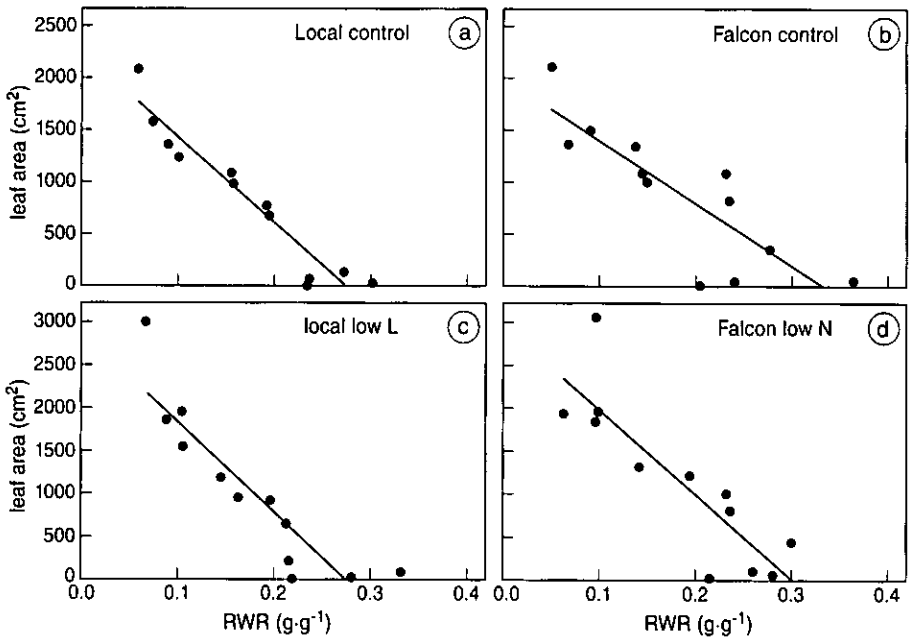

Figure 3.2 Correlation between RWR and leaf area of peanut cultivars L and F, under good and low N supply (35-91 dap).

var Falcon had the strongest response to N supply. This observation indicates again that cultivar Falcon seems to be more sensitive to low nitrogen than cultivar Local (Table 3.2).

On a dry weight basis, although not statistically different, N deficient plants always had higher root and stem weight (data not shown).

Leaf area was less sensitive to low N supply, while root and stem growth were stimulated by low N supply. However, a strong and negative relationship was found between RWR and leaf area ($r = 0.95$ at $P<0.0001$ for Local control, $r = 0.83$ at $P<0.005$ for Falcon control, $r = 0.89$ at $P<0.0001$ for Local low N and $r = 0.85$ at $P<0.0005$ for Falcon low N, Figure 3.2).

The relationship between RWR and SLA showed a different pattern from leaf area (data not shown). On a fresh weight basis, a significant and positive correlation between RWR and SLA was found in the cultivar Local under good N supply ($r = 0.65$ at $P<0.05$) and Falcon under low N supply ($r = 0.59$ at $P<0.05$). Using the data on a dry weight basis, the cultivar Falcon under low N supply did not show a significant positive relationship between RWR and SLA, but all treatments did (data not shown). In fact, the cultivar Falcon showed a slightly higher value of SLA, under N deficiency (data not shown).

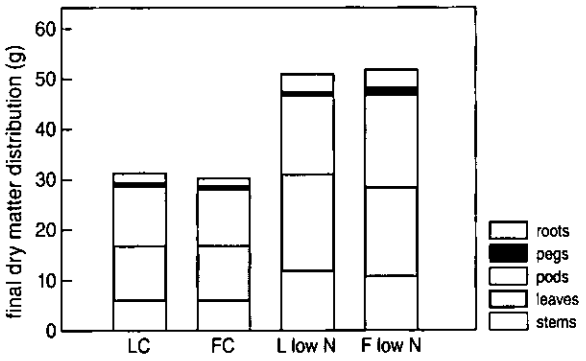

Figure 3.3 Dry matter accumulation in peanut cultivars Local and Falcon, under good and low N supply conditions (35-91 dap). LC and FC are the control plants. Data represent the mean of 8 plants.

3.3. Dry matter accumulation

Both cultivars produced more dry matter in the maturity stage under low N, compared to the control plants (Figure 3.3).

Cultivar Falcon always allocated more dry matter to pegs and pods than cultivar Local, even under adverse conditions, as described in Chapter 2.

3.4. Yield and yield components

N limitation did not affect the yield and its components (Table 3.3).

Although not statistically significant, pod number and pod dry weight were increased by low N in cultivar Falcon, while TSN was increased, but not significantly, in both cultivars. Harvest index (HI), however, was reduced significantly in cultivar Local, under low N.

Table 3.3 Effect of nitrogen limitation on pod dry weight, pod number, total sink number (TSN) and harvest index (HI). Values are means of 8 plants. Within column, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Pod dry weight (g)	Pod number	TSN	HI ($g.g^{-1}$)
Local control	5.80a	15.6a	25.7a	0.68a
Local low N	5.90a	15.1a	28.8a	0.50b
Falcon control	5.62a	14.3a	33.4a	0.66a
Falcon low N	7.31a	17.4a	40.0a	0.64a

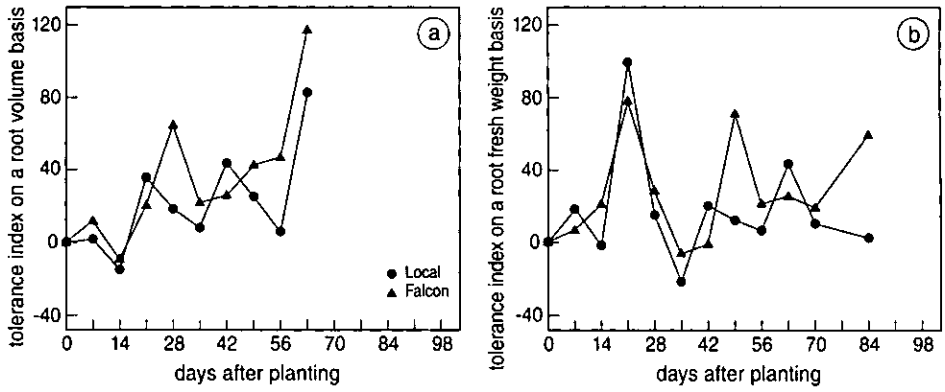

Figure 3.4 Nitrogen tolerance indices (TI) of the cultivars Local and Falcon.

4. EFFECT OF NITROGEN LIMITATION ON TOLERANCE INDICES

Low N supply did not show a significant reduction in biomass production on a dry weight basis. However, in the peanut cultivars used in the present study, some traits sensitive to environmental stress showed the usual reaction pattern to stress: e.g. the root growth on a fresh weight basis and the root volume increased in the low N supply treatment. No significant changes were observed in root weight ratio (RWR) or root shoot ratio (R/S). The tolerance indices (the component plant part under low N/component plant part under well-supplied N) for root fresh weight and root volume show that both cultivars were relatively little sensitive to low N supply in early stages of growth and that the sensitivity increased in the later stages of growth (Figure 3.4).

5. DISCUSSION

5.1. Performance of the peanut in the vegetative stage

The results of this study on the effects of low N, were obtained under conditions in which the peanut plants did not nodulate. The only N sources were nitrate (6.2 %) and ammonium (5.8 %), supplied by a slow release fertiliser (osmocote). Since results on the effect of low N on non-nodulating peanut plants are still very few, comparisons with similar results are difficult.

At the vegetative stage, under low N, a significant reduction in relative growth rate (RGR) of the whole plant, root and leaf dry weight was observed in cultivar

Falcon, in accordance with Lambers *et al.* (1995), while a reduction in leaf expansion rate (RLAER) was observed in both cultivars (Table 3.1).
Parameters, generally sensitive to low N, such as root shoot ratio (R/S) (Klepper 1991) or root weight ratio (RWR) (Radin and Parker 1979) did not show a significant response. A reduction in RGR has been partly accounted for by a smaller investment of resources in leaves and a greater investment in roots, mediated via a reduction in cytokinin export from the roots (Kuiper and Staal 1987). Abscisic acid has also been implicated in the process by which root growth increases in response to nitrogen deficiency in soil (Munns and Cramer 1996). At this growth stage however, no great investments in the roots were observed, as RGR of the roots was reduced in cultivar Falcon, and no response was observed in cultivar Local. It appears from the results that a smaller investment in the leaves does not linearly follow a greater investment in the roots. The sensitivity of the RLAER, which was significantly reduced in both cultivars (Table 3.1), confirms the results of McDonald *et al.* (1992) in *Betula pendula*, who found that photosynthesis was less sensitive than leaf area development under low N. In fact, at this growth stage cultivar Local did not show significant changes, either in the RGR of the whole plant, or in the dry matter content (Figure 3.1), but the RLAER, was reduced significantly under low N. In *Festuca arundinacea* a reduction in investment in the leaves lead to a decrease in the leaf production and consequently to a lower leaf area per plant dry matter and leaf area ratio (LAR) (Gastal and Belanger 1993; Belanger *et al.*, 1994). In the peanut cultivars used, however, no significant changes were found regarding a decrease in LAR, SLA or an increase in RWR.
The lack of significant differences between optimal N and low N plants in growth parameters regarding SLA, LAR or RWR, contradicts recent reports, which attribute the decrease in RGR to a reduced SLA or LAR (e.g. Van den Boogaard *et al.*, 1996). The changes in leaf growth, found in other studies have been ascribed to lower cytokinin export into the leaves, reducing protein synthesis and growth of the leaves (Simpson *et al.*, 1982; Kuiper and Staal 1987), as well as to low cytokinin export from the roots, due to insufficient N supply (Fetane and Beck 1993). Results with N fertilisation in peanut under field conditions differ substantially and there are many other reports showing inconsistent peanut response to fertiliser N (Nambiar *et al.*, 1986). The lack of response, as found in the present study, has been attributed to the fact that peanut is a poor utiliser of fertiliser N (Nambiar 1998), so that consistent responses can not be obtained unless the site is very poor in N or the soil conditions are not suitable for nodulation (Reid and Cox 1973). It seems therefore that in the absence of nodulation a 20-fold reduction (from 3.36 gl^{-1} to 0.72 gl^{-1}) of N in the substrate did not represent a significant lowering of N for the peanut plants to induce general symptoms of N deficiency.

The high sensitivity of the cultivar Falcon to low N supply, expressed as RGR reduction at the vegetative stage, compared to cultivar Local, may be ascribed to its high SLA under well-watered conditions (Chapter 5), since high SLA has been associated with a high fertility demand of the species (Lusk *et al.*, 1997).

5.2. Performance of peanut under low N supply in the reproductive stage

In large soil volumes the low N plants have elongated stems and yellow foliage (results not shown). Despite these deficiency symptoms, biomass production was not reduced but even stimulated. Nambiar *et al.* (1986) reported that a peanut cv Robut without fertiliser N showed normal coloured foliage and application of fertiliser N did not significantly influence N concentration in the plant. Clearly, the visible low N symptoms, observed in this study, were not necessarily expressed as reduced growth and yield of the peanut crop. RGR was stimulated by low N in all cultivars (Table 3.1) as a result of increased leaf growth in both cultivars. Smith *et al.* (1994) indicated that green leaves, abundant foliage and a poorly developed root system may be symptoms of excessive supply of N and that for proper growth only small quantities of N are needed by young seedlings. On the other hand, Salisbury and Ross (1991) indicated that plants, grown with excessive N, usually have dark green leaves and abundant foliage, usually with a root system of minimal size and therefore a high S/R; the reverse ratio occurs when N is limiting. In the present results the SLA, LAR and LWR, as well as the RWR, did not show any significant change on a dry weight basis. Using fresh weights, however, low N plants showed a significant increase in root growth, which may be induced by a higher cytokinin level (Kuiper *et al.*, 1989). An increase in RGR in low N plants (Table 3.1) might support the findings of Cox *et al.* (1982), indicating that high rates of N may not be beneficial and they may even have a detrimental effect, according to the amount applied. The tolerance indices (component plant part under low N/component plant part under well supplied N, Figure 3.4) showed that although not many changes occurred in N deficient plants, the root growth was affected by a stress factor, which may not have been sufficient to change the other parameters (Figure 3.3). Nambiar (1988) observed that, with the exception of a few circumstances, application of N fertiliser at early growth stages (basal application) or during all growth stages as split application or only during the pod filling stage may not influence peanut pod yield. This could be explained because peanut is a poor utiliser of fertiliser N compared with cereal crops, like sorghum. Therefore, they suggested that a response to low N is more likely if P is simultaneously low, apparently due to a mutual influence in plant metabolism of N and P.

Synergistic effects between N and P were shown in non-legumes, where the effect

of N on P resulted in increased top growth, increased root growth, altered metabolism and increased solubility of soil P (Adams 1976). No attempt was made during the research period to explore the synergistic effects of P and N supply.
In the generative phase the RGR is 6 and 8 times less for the cultivars Local and Falcon than in the vegetative stage, under optimal conditions respectively and 3 and 2 times less under low nitrogen conditions.
This may indicate that under conditions of "high N" senescence is postponed (cytokinin effect) and the leaves are longer green and producing dry weight, while under "low N" the RGR is lower and the leaves are yellowing earlier.
The peanut cultivars used can be classified as N efficient non-responders (i.e. they can yield high at low levels of N, but do not respond to nutrient addition (Gerloff 1977).

6. CONCLUSIONS

The effects of low N varied with the cultivars at the vegetative stage, showing a high sensitivity of the RLAER. At the reproductive stage a 20-fold reduction in N supply had no negative effects on the growth and yield of the peanut cultivars, on the contrary, it stimulated their growth. It is concluded that under the conditions of the present experiment (no inoculuation with *Bradyrhizobium* and no natural nodulation) N fertilisation did not have any beneficial effects on the growth and yield of the peanut cultivars.

4 Growth response of two peanut cultivars to phosphorus limitation

SUMMARY

Two peanut cultivars local (L) and Falcon (F) were grown in plastic pots for 13 weeks, under good and low P supply conditions. At the vegetative stage relative growth rate (RGR) of whole plant, leaf and root dry weight as well as of the leaf number, was significantly reduced in both cultivars at low P. At the reproductive stage the RGR of the root dry weight and the relative leaf expansion rate (RLAER), were reduced in cultivar Local, as well as leaf number in cultivar Falcon.
Yield and yield components were not significantly affected by low P.

1. INTRODUCTION

In low input agriculture in semi-arid regions, highly weathered soil conditions limit phosphorus (P) supply to plants (Ascencio 1996). Thus, P unavailability or deficiency as well as lack of moisture play an important role as primary determinants of crop production.

Soil P levels required for peanut are often lower than those required for other crops (Cope *et al.*, 1984) and data from different regions and with a large number of extractants suggest very low critical levels of approximately 10 $mg.kg^{-1}$ (Gascho and Davies 1994), while Otani and Ae (1995) estimated 44 $mg.kg^{-1}$ as the optimum value for crop production. However, on a global scale, where peanut is grown, P may still be the most deficient element (Gascho and Davies 1994). P deficiency is probably the major limitation to the growth of legumes in many soils, and insufficient P levels in soil can limit N_2-fixation (Salih *et al.*, 1986).

In southern Mozambique, peanut is grown in light soils, where P deficiency symptoms have been noticed (Malithano *et al.*, 1984a). In other regions of Africa, N and P deficiencies are also considered as major factors underlying low plant productivity (Elsheik and Mohamedzein 1998).

Plants species and even genotypes within species differ widely in P uptake efficiency under low P conditions. Peanut together with pigeonpea and rice have the capacity to absorb a large amount of inorganic P from normally unavailable forms, such as Al and Fe bound P. Peanut in particular shows a high absorption capacity in P limiting soils but not in vermiculite, apparently due to arbuscular-mycorrhizal (AM) associations (Ae and Otani 1997).

The superior ability of absorption of P by peanut was not related to extensive root development, root exudates, which solubilize sparingly soluble P fixed by iron (III) and/or aluminium containing clay minerals (Ae *et al.*, 1997), but to "active sites" in the cell wall, whose chemical nature has to be identified (Ae and Otani 1997).

Several authors have referred to (i) a decrease in relative growth rate (RGR) and root length under P deficiency in bean, cowpea and pigeonpea, but without a decrease of the specific leaf weight (SLW) (Ascencio 1994), (ii) a decrease in plant dry weight, RGR, relative leaf expansion rate (RLAER) and unit leaf rate (ULR) as a result of reduction in leaf area ratio (LAR) but not in SLW in common bean (Lynch *et al.*, 1991), (iii) growth inhibition and induction of acid phosphatase in *Ruellia tuberosa*, *Euphorbia heterophylla* and *Cajanus cajan* (Ascencio 1997), (iv) reduction in stem and leaf dry matter accumulation, particularly in early stages of growth in wheat (Rodriguez and Goudriaan 1995). The sensitivity of leaf area expansion to P availability has been ascribed to reduced C-availability to the leaves and greater biomass partitioning to the heterotrophic tissues (Lynch

et al., 1991) and to hormonal changes, as P deficiency is known to reduce cytokinins supply to the shoot (Horgan and Wareing 1980). In some legume species P deficiency has been demonstrated to increase/induce rhizosphere acidification (Elliot *et al.*, 1997) and increase mycorrhizal colonisation (Lynch *et al.*, 1991).

It is well established that P and N exert a pronounced influence on photosynthate and dry matter partitioning between shoots and roots. The reduction in biomass at the end of the low P treatment with leguminous plants has been primarily associated with a reduced development of leaves instead of with large differences in net assimilation rate (NAR) (Ascencio 1996).

Other authors observed that root growth is less sensitive to P deficiency than shoot growth (Lynch *et al.*, 1991) and this could reflect reduced transport of P from root to shoot (Lindgreen *et al.*, 1977), higher export rates of photosynthates to the roots (Ericsson *et al.*, 1992) and reduced shoot water availability (Radin and Eidenbock 1984). Root length and final number of leaves could be used as morphological indicators of P stress (Ascencio 1996).

Marschner (1995) stated that the decrease in shoot root ratio (S/R) in P deficient plants was correlated with an increase in partitioning of carbohydrates towards roots, as indicated by a steep increase in the sucrose content of roots (Khamis *et al.*, 1990). However, despite the adaptive response to increase P-acquisition by roots, flower initiation was delayed in subterranean clover (Rossiter 1978), the number of leaves was decreased in apple (Bould and Parfitt 1973) and seed formation was restricted in maize (Barry and Miller 1989), mainly due to premature senescence of leaves. While mild P deficiency stimulated root growth relative to shoot growth, acute P deficiency in wheat lead to visible symptoms such as stunted and spindly growth (Elliot *et al.*, 1997), necrosis and dead tips of the oldest blades (Grundon 1987) and reddening of veins of mature leaves (Atkinson 1973).

Since phosphorus is the most limiting nutrient for growth of crops in tropical regions (Casanova 1991) and soluble fertilisers in these regions are often scarce, identification of morphological and physiological traits associated with P deficiency is an important goal for plant breeding programs and non conventional agricultural practices such as intercropping, multiple cropping systems and mixed weed-crop agriculture (Ascencio and Lazo 1997).

Therefore, the objective of this study was to investigate the response of two peanut cultivars differing in their carbon allocation, to limiting phosphorus in a slow release fertiliser in order to identify traits associated with P deficiency. The results obtained may contribute to the development of appropriate screening procedures.

2. MATERIALS AND METHODS

2.1. Plant material

Two peanut (*Arachis hypogaea* L.) cultivars Local (L) and Falcon (F) were grown in plastic pots of 1.5 l (for the harvests in week 1 to week 3), 5 l (for the harvests in week 4 to week 10) or 12 l (for the harvests in week 11 to week 13).
The characteristics of the cultivars are described in Chapter 2 (Table 2.1).

2.2. Growth conditions

The plants were grown from June to September 1999 in a glasshouse, in Haren, The Netherlands. The temperature, relative humidity and light regime were as described in Chapter 2.
The pots were filled with a washed 1:1 (v/v) sand/vermiculite mixture with a slow release fertiliser osmocote (Scotts Europe, the Netherlands) 14:14:14 NPK at a ratio of 3.36 $g.l^{-1}$ and 0.3 $g.l^{-1}$ "Micromax" (Scotts Europe, the Netherlands) as supplier of macro- and micronutrients, respectively. The mineral composition of the osmocote was as follows: 6.8 % nitrogen as ammonium and 7.2 % as nitrate; phosphorus as phosphoric acid and potassium as potassium oxide. The plants grown under these conditions were considered as control plants.
P deficient plants were grown in a soil mix containing 0.335 $g.l^{-1}$ NPK 14:14:14, 1.84 $g.l^{-1}$ NPK 23:0:0, 0.90 $g.l^{-1}$ NPK 0:0:47 as macronutrients and 0.3 $g.l^{-1}$ Micromax as supplier of micronutrients. This procedure was adapted from Pell *et al.* (1990). Results of a preliminary experiment indicated that this treatment would allow plants to receive low levels of P, while maintaining a constant rate of N and K fertilisation.
The moisture of the mixture was kept near the field capacity by weighing representative pots of each size and cultivar twice a week and adding water when needed.

2.3. Growth analysis

The growth analysis was performed as described in Chapter 2.
Relative leaf expansion rate (RLAER) was calculated by analogy with RGR, as the slope of (ln transformed) leaf area against time (Lynch *et al.*, 1991).
P deficiency tolerance index (PDTI) was calculated on the dry weight matter of different plant parts for each cultivar variable according to the formula:
PDTI = Component dry weight under P deficiency/Component dry weight under well-supplied P conditions (Maiti *et al.*, 1996).

2.4 Data analysis

Data were analysed using the SPSS/PCX statistical package, version 4.0.1. Differences between cultivars and treatments were analysed by one-way ANOVA.

Trends in different growth parameters were analysed by a linear regression, using the GraphPad Prism package, version 2.01. Student t-test was used to analyse trends in RGR.

3. RESULTS

3.1. Phenological development

No differences were found in phenological development between the cultivars or between the treatments at the vegetative stage. The time of 50 % flowering did also not show any difference between cultivars and treatments (data not shown). Similarly at the reproductive stage, no clear differences were observed between the cultivars and treatments. However, cultivar Falcon showed slightly elongated plants under low P (results not shown).

3.2. Leaf growth

At the vegetative stage, no significant differences were found in leaf area, leaf number, leaf dry weight and leaf weight ratio (LWR) between both cultivars, but a slight reduction of these parameters was observed under low P conditions (Table 4.1). While leaf dry weight was reduced under low P by 22 % and 20 %, leaf area was reduced by 12 % and 18 % in cultivars Local and Falcon, respectively (not significant). Contrary to the above indicated parameters, the specific leaf area (SLA) was significantly increased by 8 % and 6 % for the cultivars Local and Falcon, respectively (Table 4.1). A statistically significant and positive correlation was found between leaf area and leaf number: Local control ($r = 0.99$ at $P<0.0005$), Falcon control ($r = 0.98$ at $P<0.005$), Local low P ($r = 0.99$ at $P<0.0001$) and Falcon low P ($r = 0.98$ at $P<0.05$).

Although not statistically significant, the ratio leaf area to root dry weight as well as LWR was reduced by 16 % and 17 % for cultivars Local and Falcon, respectively. At the reproductive stage no significant differences were found in leaf dry weight, leaf area, leaf number, and LWR in both cultivars and treatments (Table 4.1).

However, the LAR was significantly reduced by 19 % in both cultivars under low P. Similarly SLA was significantly reduced by 6 % in cultivar Local and by 16 % in cultivar Falcon. Although the leaf area was not significantly reduced in both cultivars, the ratio leaf area to root dry weight was reduced by 35.5 % and 47 % in cultivars Local and Falcon, respectively (Table 4.1).

This result indicates that under low P, less leaf area was available to supply a large portion of root dry mass. Indeed, though not significant, the reduction in leaf area and leaf number was more pronounced for the cultivar Falcon (both 23

Table 4.1 Effect of P limitation on leaf growth and leaf parameters at the vegetative and reproductive stages. LDW, LA, LN and LARDW is leaf dry weight, leaf area, leaf number and leaf area to root dry weight ratio, respectively. Values are means of 8 plants. Dap, are days after planting. Within column, per growth stage, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	LDW (g)	LA (cm^2)	LN	LAR ($cm^2.g^{-1}$)	LWR ($g.g^{-1}$)	SLA ($cm^2.g^{-1}$)	LARDWR ($g.g^{-1}$)
			Vegetative stage (35 dap)				
Local control	0.60a	118a	7.53a	77.1a	0.36a	217b	251a
Local low P	0.47a	104a	6.83a	73.8a	0.33a	234ab	213a
Falcon control	0.51a	125a	8.22a	91.4a	0.37a	252ab	332a
Falcon low P	0.41a	102a	7.20a	87.4a	0.33a	267a	275a
			Reproductive stage (91 dap)				
Local control	7.05a	1303a	43.0a	58.2ab	0.33a	185b	517ab
Local low P	6.61a	1077a	40.0a	47.2b	0.28a	175c	334b
Falcon control	6.30a	1230a	43.1a	63.4a	0.34a	197a	715a
Falcon low P	5.50a	9478a	33.6a	52.0ab	0.30a	185b	380ab

%), than for cultivar Local, (17 % and 7 % for leaf area and leaf number, respectively, Figure 4.1)

LWR, as in the vegetative stage, decreased under low P in both cultivars. Similarly, the ratio leaf area to root dry weight decreased in both cultivars with a more substantial decrease in cultivar Falcon (47 %) than in cultivar Local (35 %).

3.3. Root growth

At the vegetative stage, root dry weight was increased under low P, by 8 % and 22 %, in cultivars Local and Falcon, respectively (Table 4.2), but these increases were not statistically significant. Although not significant, root volume was increased by 12 % in cultivar Local, while a reduction of 3 % was observed in cultivar Falcon (Table 4.2).

A statistically significant increase in root weight ratio (RWR, 11 %) was found in cultivar Falcon, while in cultivar Local no significant change was found for RWR. Root shoot ratio (R/S) showed an increase under low P in both cultivars, but this was not statistically significant. At the reproductive stage root dry weight, was significantly increased by 31 % and 26 % in cultivar Local and Falcon, respectively. Similarly, the increase in root volume, was more pronounced in cultivar Local (25 %) than in cultivar Falcon, (7 %, Table 4.2).

Despite these changes the partitioning parameters, RWR and R/S, were not significantly affected although they showed a general tendency to increase under low P.

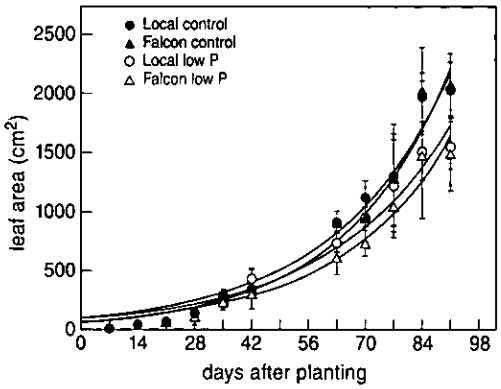

Figure 4.1 Effect of P limitation on leaf area of two peanut cultivars. Data represent the mean of 8 plants (± SD).

Table 4.2 Effect of P limitation on root growth and root growth parameters at the vegetative and reproductive stages. Values are means of 8 plants. Dap, are days after planting. Within column, per growth stage, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Root dry weight (g)	Root volume (cm^3)	RWR ($g.g^{-1}$)	R/S ($g.g^{-1}$)
		Vegetative stage (0-35 dap)		
Local control	0.45a	6.05a	0.31b	0.63a
Local low P	0.48a	6.80a	0.35b	0.66a
Falcon control	0.32a	5.20a	0.29a	0.53a
Falcon low P	0.40a	5.05a	0.32b	0.67a
		Reproductive stage (42-91 dap)		
Local control	2.74ab	23.3b	0.14a	0.26a
Local low P	3.61b	29.3a	0.15a	0.37a
Falcon control	2.30a	22.8b	0.20a	0.26a
Falcon low P	2.91ab	24.5b	0.20a	0.41a

A significant and negative correlation was found between RWR and leaf area, being ($r = 0.95$ at $P<0.0001$) for Local control, ($r = 0.91$ at $P<0.0001$) for Local low P, ($r = 0.95$ at $P<0.0001$) for Falcon control and ($r= 0.88$ at $P<0.005$) for Falcon low P.

3.4. Total dry matter accumulation

P limitation did not reduce significantly the total dry weight of the cultivars, in both growth stages (Figure 4.2).

However, the effect of P limitation was more evident when expressed as RGR on

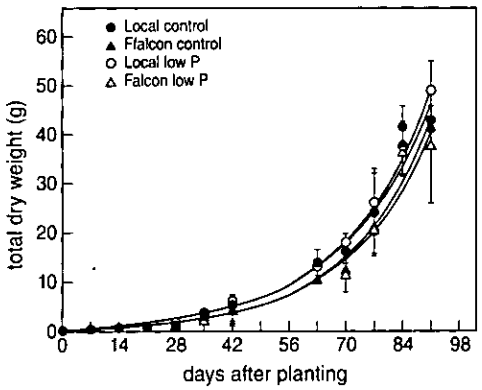

Figure 4.2 Effect of P limitation on dry matter accumulation of two peanut cultivars. Data represent mean of 8 plants (± SD).

Table 4.3 Effect of P limitation on RGR (plant, leaf, root dry weight, leaf number) and RLAER at the vegetative and reproductive stages. TDW, LDW, RDW, LA and LN, is total dry weight, leaf dry weight, root dry weight and leaf number, respectively. Dap, are days after planting. Values are means of 8 plants (± SD). Within columns, per growth stage, values of each cultivar followed by the same letter, are not significantly different at $P<0.05$ level, using the Student t-test.

	RGR ($g.g^{-1}.day^{-1}$)		RLAER ($cm^2.day^{-1}$)		
Treatment	TDW	LDW	RDW	LA	LN
	Vegetative stage (0-35 dap)				
Local control	0.11±0.02a	0.10±0.00a	0.08±0.01a	0.10±0.00a	0.07±0.00a
Local low P	0.07±0.01b	0.07±0.00b	0.07±0.00b	0.07±0.00b	0.06±0.00b
Falcon control	0.07±0.00a	0.07±0.00a	0.06±0.01a	0.10±0.00a	0.06±0.00a
Falcon low P	0.09±0.01b	0.11±0.00b	0.09±0.01b	0.11±0.00b	0.07±0.00a
	Reproductive stage (42-91 dap)				
Local control	0.04±0.00a	0.02±0.00a	0.01±0.00a	0.03±0.00a	0.02±0.00a
Local low P	0.04±0.00a	0.02±0.00a	0.01±0.00b	0.02±0.00b	0.01±0.00a
Falcon control	0.04±0.00a	0.03±0.00a	0.01±0.00a	0.03±0.00a	0.03±0.00a
Falcon low P	0.04±0.01a	0.03±0.00a	0.01±0.00a	0.03±0.00a	0.02±0.00b

a dry weight basis of the whole plant, root, and leaf and leaf number and as relative leaf expansion rate (RLAER).

While cultivar Local showed a reduction of plant growth under P limitation, the cultivar Falcon showed a slight increase in RGR, leaf number and RLAER (Table 4.3). Thus, in cultivar Local, the RGR of the whole plant on a dry weight basis was reduced by 18 %, as RLAER by 28.5 % and as leaf number by 17 %.

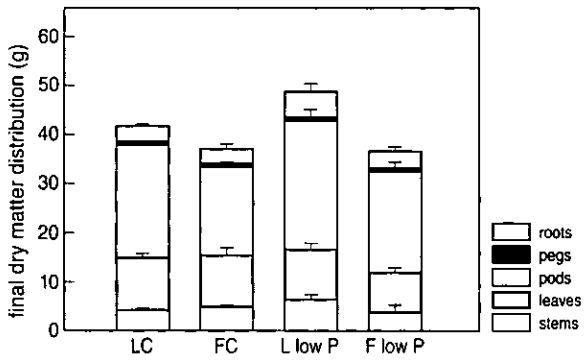

Figure 4.3 Effect of P limitation on final dry matter distribution (91 dap) of two peanut cultivars Local (L) and Falcon (F). LC and FC, are the control treatments. Data represent the mean of 8 plants (± SD).

In cultivar Falcon, P limitation had no reductive effects on RGR. On the contrary, RGR expressed as total dry weight was increased by 23 %, as leaf dry weight by 42 %, as root dry weight by 44.5 %, as RLAER by 53.4 % and as leaf number by 5 %. The final distribution of dry matter, however, showed different responses of the cultivars. The cultivar Local under low P showed the highest total dry matter. The high dry matter accumulation under low P, can mainly be ascribed to increased root dry weight (51 %), since pods were increased by 13 % and leaf dry weight was reduced by 17.6 % (Figure 4.3).

In cultivar Falcon under low P, although an increase in root dry weight by 21% was observed, leaf dry weight was decreased by 20%, and shoot dry weight by 22%. As with cultivar Local, pod dry weight was increased under low P in cultivar Falcon by 22.3%.

The peg dry weight was increased by 16%, whereas in cultivar Local no differences were observed.

3.5. Yield and yield components

Despite some changes in leaf and root growth, P deficiency did not significantly affect the yield and yield components in the peanut cultivars (Table 4.4).

While a general, but not significant pattern of decrease in yield and yield components was observed in cultivar Falcon, cultivar Local under low P showed slightly higher values of TSN and pod number (also not significant). Both cultivars showed a slight but not significant reduction, in pod dry weight and harvest index (HI), under low P conditions.

Table 4.4 Effect of P limitation on pod dry weight, pod number, total sink number (TSN, number of pegs and pods) and harvest index (HI) of two peanut cultivars. Values are means of 8 plants. Within columns means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Pod dry weight (g)	Pod number	Total Sink Number (TSN)	HI ($g.g^{-1}$)
Local control	11.9a	21.6a	34.3a	0.45a
Local low P	11.2a	22.3a	34.7a	0.40a
Falcon control	11.1a	19.4a	32.2a	0.47a
Falcon low P	10.5a	14.6a	26.3a	0.41a

4. EFFECT OF P LIMITATION ON TOLERANCE INDEXES

The tolerance indexes did not show significant differences, between cultivars and growth stages (Figure 4.4).
Cultivar Local showed a slightly higher TI, particularly at the beginning and the end of the growth period.

5. DISCUSSION

5.1. Performance of peanut in the vegetative stage

5.1.2. Leaf growth

A ten-fold reduction of P (from 3.36 $g.l^{-1}$ to 0.335 $g.l^{-1}$) in the substrate had some negative effects on leaf and root growth, partitioning pattern and RGR of the cultivars Local and Falcon (though not always statistically significant). Leaf area showed a reduction under low P in both cultivars (Table 4.1 and Figure 4.1), but the reduction was not significant. A decrease in photosynthetic leaf surface area is generally observed before the rate of photosynthesis per area is affected (Natr 1975), when plants are deprived of P. At this growth stage, dry matter production did not show differences between the treatments, suggesting no great influence of low P on photosynthesis. In common bean, Lynch *et al.* (1991) found that reduced leaf area development was associated more with reduced leaf appearance than with reduced expansion, elongation and final leaf size. In this study the sensitivity of leaf growth to low P differed between the two cultivars. While in cultivar Local the slight reduction in leaf area and leaf number resulted in a significant reduction in relative leaf expansion rate (RLAER), no reduction of RLAER in cultivar

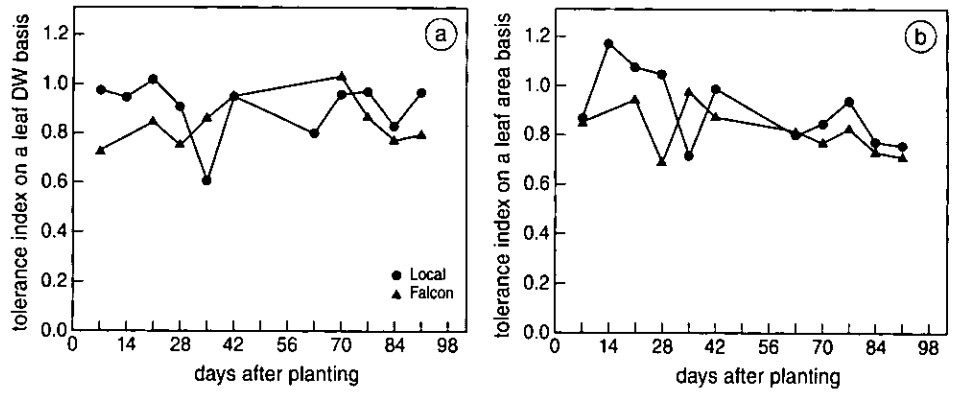

Figure 4.4 Effect of P limitation on tolerance indexes (TI) of the peanut cultivars: (a) tolerance for leaf dry weight and (b) for leaf area (LA).

Falcon was observed. On the contrary, cultivar Falcon increased the values of RGR of leaves under P limitation, which possibly could be ascribed to a positive response of the RGR of the roots on the RGR of the leaves (Table 4.3). Despite contrasting results regarding the RGR, leaf weight ratio (LWR) was slightly and equally reduced in both cultivars under low P. This is an indication that less carbon is being allocated to the leaves, under low P conditions. The specific leaf area (SLA) was increased, as reported too by Rodriguez *et al.* (1998), particularly in the early stages of growth (Table 4.1). An increase in SLA in cereals has been associated with a lack of assimilates required for growth (Kemp 1981). The SLA seems therefore to be very sensitive to low P at the vegetative stage, since dry matter content resulting from photosynthesis, did not show any significant response (Figure 4.2).

5.1.3. Root growth

Root weight ratio (RWR) showed a significant increase in cultivar Falcon, while root shoot ratio (R/S) increased in both cultivars, but never significantly (Table 4.2). The observed sensitivity of root growth to low P in comparison to leaf growth, is consistent with results reported by Gascho and Davies (1994) and Cassman *et al.* (1980) and has been interpreted as reflecting a reduced transport of P from root to shoot (Lindgreen *et al.*, 1977), in addition to reduced water availability (Radin and Eidenbock 1984) and other shoot specific responses. Water content of the plants did not show any significant decrease under low P in this study, making water availability as a less probable explanation for increased R/S. Many authors have reported on increased R/S under P deficiency: Lynch *et al.* (1991) in *Phaseolus vulgaris*, Thomson *et al.* (1992) in *Lupinus angustifolius* and Ascencio (1996) in *Desmodium tortuosum*, despite reduced root dry weight.

The results of this study showed that the slight increase in R/S, was a result of a slight increase in root dry weight. Therefore, the enhanced root growth enables the shoot to be supplied with enough P and water, resulting in maintaining its growth. These results are in agreement with those of Munns and Cramer (1996), when they stated that carbon allocation often alters in response to adverse soil conditions before the shoot is acutely limited by the resource.
The effect of low P on increasing root growth is also reflected in high root weight ratio (RWR) values of low P plants, particularly in cultivar Falcon (Table 4.2). Ascencio (1996), who found an increase in RWR 28 days and 40 days after emergence in *Phaseolus vulgaris*, described this as a common feature for most plants grown under nutrient deficiency. The superior response of the RWR in cultivar Falcon, compared to cultivar Local, support our previous findings (Chapter 2), that small-seeded cultivars always invest more heavily in the roots.

5.2. Performance of peanut in the reproductive stage

5.2.1. Leaf growth

Leaf growth showed a certain degree of sensitivity to low P in both cultivars. Contrary to the vegetative stage, the cultivars showed a similar pattern, characterised by a significant decrease in specific leaf area (SLA), relative leaf expansion rate (RLAER) in cultivar Local and leaf number in cultivar Falcon (Tables 4.1 and 4.3).
As discussed in the vegetative stage, a reduced leaf growth is attributed to a reduced C availability, which in turn affects the subsequent morphological development. A reduction in leaf expansion in particular, as observed in cultivar Local, may result alternatively from hormonal changes, since P deficiency is known to reduce cytokinin supply to the shoot (Horgan and Wareing 1980) and alter leaf response to ABA (Radin 1984). It has also been suggested that P nutrition itself might regulate plant growth (Trewavas 1981) via reduced phosphate for the expansion of the epidermal cells.
The sensitivity of the peanut cultivars to low P at the reproductive stage contradicts the results reported by Tewolde and Fernandez (1997) in cotton, which indicated that P deficiency suppressed vegetative growth more severely than growth in the reproductive stage. Additionally, they found that P deficiency reduced plant leaf area by producing less and smaller leaves, caused by direct effects, such as lack of turgor in leaves (Radin and Eidenbock 1984). In the peanut plants, used in this experiment, water content expressed as dry/fresh weight ratio was reduced by 8 and 13 % in cultivars Local and Falcon respectively, under low P (data not shown), making the turgor effect less valid. In fact, recent evidence indicates that the expansion properties of the cell wall rather than a lack of turgor for cell expansion, are more likely to limit leaf expansion (Pritchard *et al.*, 1990), particu-

8. Is drought stress not a simple human concern? Which higher plant would exist without drought stress?
   Every higher plant experiences drying, at least once during its life cycle, when the seed matures.

9. The arbuscular mycorrhizal fungi are an example of modesty. They are more active when there is a shortage of water and nutrients.

10. If politics is the art of making successful compromises, then the peanut plants are extraordinary politicians. Under drought and nutrient stress, they compromise light interception and assimilate production for a successful exploration of water from deeper soil zones and therefore guarantee the success in their career.

11. The effects of the debt on the Mozambican economy are compared to the effects of a severe stress in plants. Unless it is relieved no economic growth will be possible, as a plant resistant to severe drought stress is never profitable.

Stellingen behorend bij het proefschrift

Functioning of peanut (*Arachis hypoagea* L.) under nutrient deficiency and drought stress in relation to symbiotic associations

Orlando Quilambo

1. Symptoms of N deficiency reported in the field are not an adequate indication of the N requirements of peanut and do not correspond to a low yield at harvest.
(*This thesis*)

2. A reduced relative growth rate, relative leaf expansion rate and an increased root weight ratio, are trais related to P limitation.
(*This thesis*)

3. Nodulation of peanut is insensitive to a drought stress occurring at the vegetative stage, independently of its severity.
(*This thesis* and Sinclair *et al.* (1995). Peanut Sci. **22**:162-166)

4. Under drought stress conditions, inoculation of peanut with arbuscular mycorrhizal fungal inoculant, leads to allocation of more dry matter to the pods, followed by leaves, whereas non- inoculation leads to allocation of more dry matter to the roots and to a delay in pod formation.
(*This thesis*)

5. Indigenous arbuscular mycorrhizal inoculant, increases drought tolerance in non-sterile soil, regardless the seed size and drought stress tolerance strategy of the peanut cultivar.
(*This thesis*)

6. An ecophysiologist in Mozambique, where nature still drives life, must be as plastic as a razor blade, because while specialising in water deficit, water excess may suddenly become the major constraint, as it happened in the year 2000.

7. Drought tolerance is a nebulous term, which becomes more nebulous, the more closely we look at it.
(Passioura 1996. Plant Growth Regulation **20**:79-83)

larly since nutrients have been observed to have direct effects on these properties (Palmer *et al.*, 1996). In the present study, a positive and significant correlation was found between leaf area and leaf number supporting the hypothesis that leaf area reduction under low P mainly resulted from a reduced number of leaves. At this growth stage cultivar Falcon showed a more substantial reduction in RGR for leaf number (Table 4.3) than in cultivar Local.

5.2.2. Root growth

Contrary to the vegetative stage, P limitation in the generative stage significantly increased root dry weight in both cultivars and root volume in cultivar Falcon (Table 4.2). These results agree with others, indicating that P deficiency shifted biomass partitioning in favour of non-photosynthetic tissues, by limiting shoot growth more than root growth (Lynch *et al.*, 1991; Radin and Eidenbock 1986). It is suggested that increased root weight is a result of several changes occurring in response to P stress such as increased P uptake or higher export rates of photosynthates to the roots (Fredeen *et al.*, 1989; Aloni *et al.*, 1991 and Ericsson *et al*, 1992). In addition to root dry weight, root volume, a parameter not analysed in most other studies, showed a significant increase in both cultivars upon P deficiency. It is therefore clear that roots become more competitive for photosynthates than shoots, which leads to a higher export of carbohydrates to roots with correspondingly lower S/R (Rufty *et al.*, 1993). Under these conditions, leaves of P deficient plants may only remain as storage of sugars or cell wall materials, which have a very low maintenance requirement as suggested by Rodriguez *et al.* (1998). This may be the explanation for the significant reduction of SLA, as found in the present experiment (Table 4.1). However, Qiu and Israel (1992) have argued that increased R/S in P deficient plants is not caused by higher export of photosynthates to roots, but by a more efficient utilisation of carbohydrates in the roots of P deficient plants.

The continuous root growth, found in this experiment, contradicts the results of Mollier and Pillerin (1999) in maize, Rychter and Randall (1994) in bean and Fredeen *et al.* (1989) in soybean, who found a slight initial enhancement of root growth, but a strong reduction thereafter. On the basis of their results they concluded that P deficiency mainly affects the root morphology through its effect on the carbon budget of the plant with no additional specific effect of P deficiency on root morphogenesis. Data available from this experiment do not allow to draw a conclusion on the direct relation between low P and inference in root morphogenesis.

Despite limiting leaf and root growth, P limitation did not have a significant effect on the final yield. The present results contradicts part of those reported by Elliot *et al.* (1997) in spring wheat, where P deficiency markedly reduced grain yield and grain weight, but some components of yield such as number of grains

per ear and harvest index remained unaffected or only slightly reduced. Chauhan *et al.* (1988) found an increase in dry-pod yield, pods/plant and 100 kernel weight in peanut under low P, but kernels/pod did not show any remarkable response. Thus, low P does not necessarily reduce yield in the peanut cultivars. The two cultivars appeared to develop different strategies to cope with P limitation particularly at the vegetative stage, as long as they are grown without inoculation with arbuscular mycorrhiza (AM). Cultivar Local decreased its RGR, while cultivar Falcon increased it. Decreased growth rates are considered an adaptive mechanism that lowers the requirements for P to match more closely the availability of P from deficient soil (Elliot *et al.*, 1997), while increased RGR enables plants to acquire more nutrients and water under drought conditions, those species with high RGR showing a superior resistance (Hendrix and Trapp 1992). The strategy observed in cultivar Local, appeared to be most appropriate, since it had a higher dry matter content at harvest compared to the other cultivar and treatments (Figure 4.3).

The present results suggest that, under glasshouse conditions, if N, K and micronutrients are not at a low level, yield and yield components in peanut may not be substantially affected by low P. The explanation may be partly found in our previous findings, that RWR correlated positively with final mean pods/plant. As P deficiency increased RWR, it may have led to normal yield, compensating the effects of low P on leaf growth.

6. CONCLUSIONS

P deficiency affected peanut growth more severely in the reproductive than in the vegetative stage. When plants were exposed to low P at the vegetative stage, RLAER as well as SLA were reduced, as result of partitioning greater biomass to the roots. Yield and yield components may not be significantly affected by low P, if the levels of N and K are not limiting.

It is suggested that in low-input agriculture the enhancement of P via management of arbuscular mycorrhizal fungi (AMF) may be of importance in improving peanut growth and yield, since (i) P deficiency imposed by a ten-fold reduction of the control level, showed some negative effects on plant growth as compared to no significant differences found in twenty-fold reduction on N in the substrate (Chapter 3), (ii) insufficient P levels in soil can limit N_2-fixation and (iii) adequate P levels have been found to alleviate water stress symptoms.

5 Response of two peanut cultivars to imposed drought stress

SUMMARY

Two peanut cultivars Local (L) and Falcon (F) were grown in ceramic pots, lined with plastic bags, under water-controlled conditions. Drought-stress was created by withholding water from week 2 onwards until a moisture water content of the soil of 3 % was reached, while the control plants were irrigated to a soil moisture above 20 %.
At the vegetative stage RGR was significantly reduced in both cultivars. Leaf area, leaf dry weight and leaf number were only significantly reduced in cultivar L, while nodulation was not affected. Similarly, root weight ratio (RWR), root shoot ratio (R/S) and maximum root length leaf area ratio (MRLAR), were only increased in cultivar Local.
At the reproductive stage, drought stress reduced in a similar manner RGR, leaf dry weight, area and number, as well as yield and yield components.
The cultivars showed different mechanisms of coping with drought-stress: drought avoidance and drought-tolerance.

1 INTRODUCTION

Peanut is a major cash crop in the semi-arid tropics, where it is mainly grown under rainfed conditions. Inadequate soil fertility (especially N and P) and disease are important factors causing low yields (Gibbons 1980; Patanothai and Ong 1987), but drought is often the major cause of a low yield. Besides a direct effect in reducing peanut yield, drought stress discourages farmers to alleviate other yield reducing constraints such as pests, diseases and nutrient stress (Busolo-Bulafu 1992). N_2-fixation, a very important physiological process in legumes, is negatively affected by soil water deficit and water stress limits N accumulation, dry matter and yield (Venkateswarlu *et al.*, 1989).

Drought is a complex phenomenon with 3 widely varying components: (i) timing of occurrence of the drought during the season, (ii) duration of the drought and (iii) intensity of the drought (Nageswara Rao *et al.*, 1989).

Sensitivity of a genotype to drought stress generally increases with the yield potential and it is increasing the closer the drought occurs to final harvest (Williams *et al.*, 1986). Yield appears to be little affected by drought stress during the peanut vegetative phase (Boote 1982) and may even promote yield since it prevents excessive vegetative growth. Yield is drastically reduced by drought during flowering and pod fill. A high rate of irrigation gave maximum yield in peanut (Reddy *et al.*, 1982), which increase is attributed to an increase in available soil moisture and nutrients, especially nitrogen and phosphorus.

Under optimal growth conditions plants with a higher inherent relative growth rate (RGR) potentially produce more biomass and exploit nutrient and water resources to a larger extent, than those with a lower RGR. Under unfavourable conditions such as limited water availability, a low RGR might be advantageous since traits linked with low RGR such as small leaf area, may reduce the demand for water on a per plant basis (Van den Boogaard *et al.*, 1996). Muchow (1985) conducted detailed studies on six grain legume species and demonstrated differences in crop development, physiology, root growth and yield under both well-watered and water-stressed conditions.

Genetic variability of root and shoot characters (Ketring 1984) possibly associated with drought resistance (Ketring *et al.*, 1982) has been reported among several breeding lines and peanut introductions. Nour and Weibel (1978) found that more drought tolerant lines had a higher root/shoot ratio (R/S). An increase in the ratio of root growth to leaf growth occurs as well as when the soil is N deficient (Van der Werf and Nagel 1996), and in many other adverse soil conditions such as dryness and salinity (Munns and Sharp 1993).

A selection of cultivars with a more extensive root system should extract more water from a greater soil volume than a selection with a limited root system.

Hence, the former should be able to develop and maintain a greater leaf area during a period of drought (Bolanos *et al.*, 1993).

Water deficit reduces dry matter production of vegetative components, crop growth rate, leaf expansion and stem elongation through a reduction of turgidity (e.g. Munns and Cramer 1996). Excessive rainfall or irrigation may promote vegetative growth at the expense of reproductive growth (Vivekanandan and Gunasena 1976). These authors also observed, that a high soil water availability resulted in a greater leaf area index (LAI) and excessive vegetative growth, but not to an increase in pod yield.

Various types of responses to drought can be divided into: (ia) avoidance by escape from drought, (ib) avoidance by access to deep groundwater and (ii) tolerance of water deficit (Ludlow 1989). Escape from drought occurs e.g. in plants which loose all their leaves and enter into a dorment state during a dry period, or plants which survive drought as dorment seeds in the soil, which germinate after sufficient rainfall has occurred (e.g. Atwell *et al.*, 1999).

Avoidance can be realised by minimising water loss by reduction of stomatal conductance and transpiring leaf surface and by maintenance of an adequate water uptake by increased root density and depth of rooting. A tolerance mechanism enables the plant to function well despite a low water potential of the plant and comprise mechanisms such as maintenance of turgor by osmotic adjustment of cell sap (Jones 1993).

Improved acclimation to drought may well occur at cost of productivity. For example, turgor maintenance confers metabolic costs, and a reduced stomatal conductance and leaf area may lead not only to reduced water loss but also to decreased photosynthesis. Also, an increased biomass allocation to roots implies that less carbohydrates are available for investment on above-ground CO_2 assimilating plant parts (Jones 1993).

To stabilise and raise yields of peanut in the semi-arid tropics, including Mozambique, there is a need for genotypes that are tolerant to different patterns of encountered drought (Mathews *et al.*, 1988), in particular to match cultivars to specific agroecological zones (Busolo-Bulafu 1992). However, selection for drought resistance proved to be difficult because of the large variation in: (i) environments and (ii) between years and sites. The use of large numbers of genotypes in multiple seasons and locations has been costly in terms of time and space (Mathews *et al.*, 1988). Thus, the search for morphological and physiological traits, contributing to a higher performance of peanut under drought stress, combined with an analysis of RGR and its components, can help to understand the variation in growth and development in peanut under limiting resources as low water availability (see Lambers *et al.*, 1989).

The desirable traits include those, which allow plants to gain access to and

Table 5.1 Physical and chemical characteristics of the soil, used in the experiment.

Parameters (Units) Texture		Value
Sand (%)		85.60
Silt (%)		13.40
Clay (%)		1.00
PH		6.80
Bulk density ($g.cm^{-3}$)		2.37
Electrical conductivity ($ms.cm^{-1}$)*		0.06
Cation exchange capacity ($meq.kg^{-1}$)	Ca^{2+}	26.00
	Mg^{2+}	9.10
	Na^{+}	0.60
	K^{+}	0.90
Carbon (%)		0.07
Organic matter (%)		0.12
Total N (%)		0.08
Total P ($mg.kg^{-1}$)		188.00
P-Bray (II) ($mg.100g^{-1}$)		30.70

* Electrical conductivity (EC) was determined by diluting soil in distilled water at a rate of (1:2.5 v/v) and measuring the EC of the solution.

absorb a greater volume of water, to reduce water loss and to maintain a high physiological activity at low water potential (Turner 1986; Ludlow and Muchow 1990).

The objective of this study was to investigate the response of two peanut cultivars, differing in their carbon or dry weight partitioning pattern to imposed drought stress, taking into account an analysis of desirable traits, as discussed above.

2. MATERIAL AND METHODS

2.1. Plant material and soil

Two peanut cultivars (*Arachis hypogaea* L.), Local (L) and Falcon (F) were grown for 13 weeks in 12 l ceramic pots, lined with plastic bags, filled with soil, collected from the experimental farm of the Faculty of Agronomy and Forestry Engineering of the Eduardo Mondlane University in Maputo, Mozambique.

The soil is classified as arenosol and the characteristics are given in the Table 5.1.

The characteristics of the cultivars were presented in Chapter 2 (Table 2.1).

The plants were grown from pre-germinated seeds and planted one plant per pot.

2.2. Growth conditions

The plants were grown in a plant nursery in Maputo-Mozambique (25° 28' S and 32° 36' E), from February to May 1998, under water-controlled conditions.

The minimum and maximum air temperatures during the growth period were: 22.9 °C to 37.9 °C in the morning, 24.5 °C to 38.8 °C in midday and 24 °C to 41.2 °C in the afternoon.

The minimum and maximum relative humidity of the air ranged from 50.2 % to 90.9 %, in the morning, 31.5 % to 90.6 %, in midday and 29.8 % to 79.1 % in the afternoon. The illumination was by screened natural light, resulting in an average photon flux density at the canopy level of 366 ± 55 $\mu mol.m^{-2}.s^{-1}$ in the morning, 539 ± 14 $\mu mol.m^{-2}.s^{-1}$ in midday and 213 ± 160 $\mu mol.m^{-2}.s^{-1}$ in the afternoon, measured with a quantum sensor (SK P215, Skye Llandrindod Wells, UK).

During the first week the plants were irrigated to field capacity with normal tap water. The plants were water-stressed by soil drying (in preference to other methods), since this procedure would more accurately reflect the field characteristics. Water deficit was created by withholding irrigation water from week 2 onwards until the moisture content of the soil reached 3 % (near the wilting point, according to preliminary experiments), measured with the use of a Thermal Domain Reflectometer (TDR, Eijkelkamp, Giesbeek, The Netherlands).

The control plants were regularly watered, according to the evaporative condition and transpiration demand to a soil moisture content of above 20 %, using TDR, a moisture level near to field capacity. Whenever the soil had dried out beyond this limit, water was added in the morning to restore the soil moisture content back to the pre-determined level.

2.3. Growth analysis

The growth analysis was performed as described in previous Chapters. The plants were harvested every week and separated into roots, cotyledons, stems, leaves, pegs and pods, and their fresh and dry weights were determined.

Leaf area was measured using the gravimetric method for the first 4 weeks and a leaf area meter (Delta-T Area System, ΔT-devices Ltd, Burwell, Cambridge, England) for the rest of the experiment. Relative leaf expansion rate (RLAER) was calculated as described in Chapters 3 and 4. Drought stress tolerance index (DSTI) was calculated on the basis of dry matter of different plant parts for each variable according to the formula:

DTSI = Component dry weight under drought stress/Component dry weight under watered conditions (Maiti *et al.*, 1996).

2.4. Data analysis

Data were analysed with the SPSS/PC statistical package, version 4.0.1.
Differences in growth parameters between cultivars and between drought stress treatments were analysed by one-way ANOVA.
Trends of LWR, RWR, SLA, LAR, NAR and RGR were analysed by a linear regression, using the package GraphPad Prism version 2.01. RGR was determined as the slope of the natural logarithm of dry weight versus time (Poorter 1989).

3. RESULTS

3.1. Phenological development

Drought-stress reduced leaf number and size, as explained below, and did not influence the time of (50 %) flowering (data not shown). From 77 days after planting (dap) the control plants showed a yellowish colour, a sign of senescence, while the stressed plants still showed a dark green colour.
Similarly, the roots of the control plants showed signs of rotting and some nodules became darker. The stressed plants were more susceptible to infestation of leaves by mites in the later stages of growth, but no treatment was applied, to avoid collateral effects on plant growth.

Table 5.2 Effect of drought stress on RGR of the total dry weight, leaf dry weight, root dry weight basis and RLAER at the vegetative and reproductive stages. LC and FC are the control plants of cultivar Local and Falcon and LS and FS, the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Values are means ± standard deviations (±SD). Within column, per growth stage, means of each cultivar followed by the same letter, are not significantly different at $P<0.05$ level, using the Student t-test.

	RGR ($g.g^{-1}.day^{-1}$)			RLAER ($cm^2.g^{-1}.day^{-1}$)
	Whole plant	Leaves	Roots	
	Vegetative stage (0-35 dap)			
Local control	0.089±0.009a	0.082±0.010a	0.100±0.010a	0.070±0.009a
Local stress	0.063±0.007b	0.062±0.008b	0.047±0.009b	0.063±0.008a
Falcon control	0.111±0.006a	0.104±0.009a	0.111±0.004a	0.095±0.001a
Falcon stress	0.086±0.008b	0.089±0.006b	0.105±0.005a	0.088±0.007a
	Reproductive stage (42-91 dap)			
Local control	0.033±0.003a	0.010±0.001a	0.022±0.004a	0.025±0.0049a
Local stress	0.029±0.004a	0.014±0.001a	0.020±0.002a	0.025±0.0035a
Falcon control	0.026±0.004a	0.013±0.003a	0.020±0.005a	0.021±0.0054a
Falcon stress	0.025±0.004a	0.012±0.003a	0.020±0.005a	0.020±0.0055a

3.2. Relative growth rate

RGR of the whole plant, leaves and roots, and RLAER are presented in Table 5.2. At the vegetative stage both cultivars showed a high RGR in well-watered conditions. Under drought stress, the RGR on a total dry weight basis was reduced by 29 % and 22.5 % in the cultivars Local and Falcon, respectively.

RGR of the leaves was reduced significantly by 24 % and 15 % for the cultivars Local and Falcon, RGR of the roots by 54 % and 5 % (not significantly) and the relative leaf area expansion rate (RLAER) by 10 % and 7 %, respectively (not significant). At the reproductive stage, no differences were found either on RGR or RLAER (Table 5.2).

3.3. Leaf and root growth

3.3.1. Leaf growth

At the vegetative stage, drought-stress significantly reduced the leaf number in both cultivars to 55 % and 42 % for Local and Falcon, respectively. Leaf dry weight and leaf area in the cultivar Local was also significantly decreased to 72 % and 78 %, respectively (Figures 5.1a, b and c).

As in the vegetative stage, drought-stressed plants showed a significant reduction in leaf number, leaf area and leaf dry weight (Figures 5.1a, b and c), in the reproductive stage.

In the vegetative stage drought stress significantly reduced specific leaf area (SLA) by 15 % and 12 % in cultivars Local and Falcon, respectively and leaf area ratio (LAR) by 22 % in cultivar Local. A significant reduction in LWR in cultivar Local by 8 % in cultivar Falcon by 7 % was observed (Table 5.3).

SLW was significantly 25 % higher in stressed plants than in control plants of cultivar Local, whereas the cultivar Falcon did not show any response (data not shown). At the reproductive stage, LAR in both cultivars did not respond significantly to drought, while LWR, which is considered to be less sensitive to environmental factors (see Cramer *et al.*, 1994) significantly increased to 128 % and 112 %, for the cultivars Local and Falcon, respectively.

SLA, which is very sensitive to environmental stresses, was reduced significantly in both cultivars, as shown in Table 5.3.

SLW was higher in both cultivars under drought stress conditions, with an increase of 16% for both cultivars (data not shown).

3.3.2. Nodulation and root growth

The root dry weight did not respond to drought stress, but the root weight ratio (RWR) responded to drought and was significantly higher (82 %) in the cultivar Local under drought, while the cultivar Falcon did not show any significant

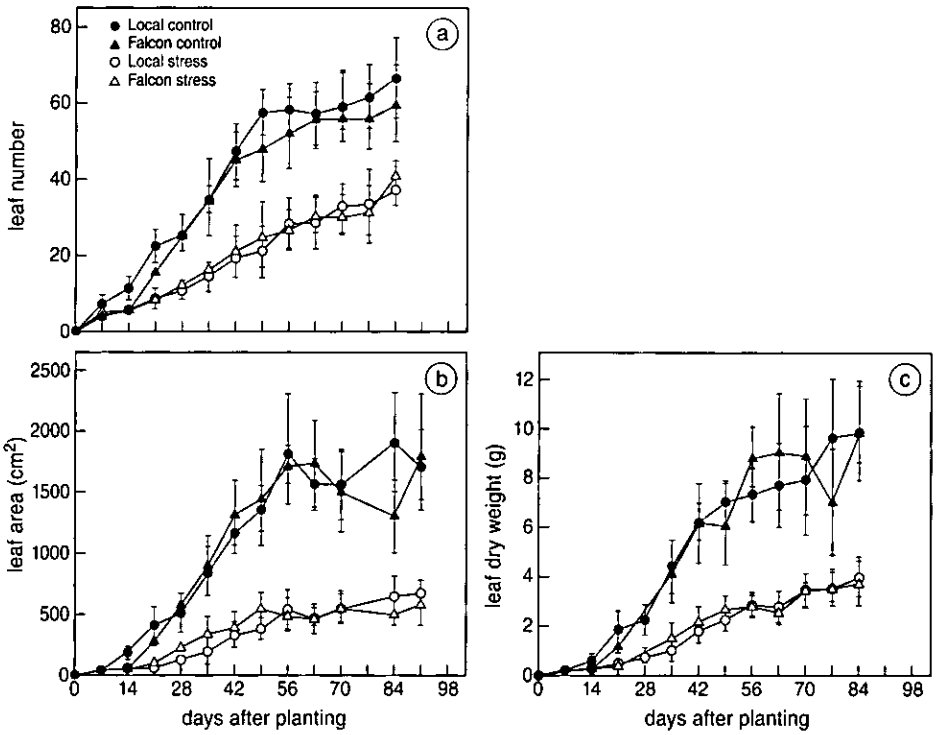

Figure 5.1 Effect of drought stress on leaf number (a), leaf area (b) and leaf dry weight (c) of the peanut cultivars at the vegetative and reproductive stages. Data represent mean of 8 plants (± SD).

response of RWR to drought stress, at the vegetative stage.

On the other hand, the maximum root length leaf area ratio (MRLAR), a characteristic potentially important for screening seedlings to withstand drought, was significantly higher under drought conditions in the cultivar Local (314 %), while the cultivar Falcon again did not show any significant response (Table 5.4).

At the reproductive stage, the root dry weight again did not show a significant response to drought in both cultivars, but the RWR did. RWR increased under drought stress to 175 and 144 % for the cultivars Local and Falcon, respectively, although these differences were not statistically significant.

Contrary to the vegetative stage, MRLAR was now significantly increased by drought: 305 % in cultivar Local and 331 % in cultivar Falcon (Table 5.4).

At fourteen days after planting nodules were formed in both cultivars but the number was not significantly affected by drought stress, suggesting that the nodulation might not have been affected by drought stress at this early developmental stage (Figure 5.2).

During the rest of the growth period of the plants, the stressed plants always had

Table 5.3 Effect of drought stress on LAR, SLA and LWR in two peanut cultivars. LC and FC are the control plants of cultivar Local and Falcon and LS and FS, the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Within column, per growth stage, means followed by the same letter, are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	LAR ($g.cm^2$)	LAR (% of control)	SLA ($cm^2.g^{-1}$)	SLA (% of control)	LWR	LWR (% of control)
			Vegetative stage (0-35 dap)			
LC	118a	100	229b	100	0.51a	100
LS	92b	78	195c	85	0.47a	92
FC	121a	100	236a	100	0.50a	100
FS	102a	84	208b	88	0.46b	94
			Reproductive stage (42 91 dap)			
LC	73a	100	200a	100	0.36c	100
LS	80a	110	172b	86	0.46a	128
FC	83a	100	195a	100	0.42b	100
FS	79a	95	169b	87	0.47a	112

Table 5.4 Effect of drought stress on, RWR, MRLAR and root shoot ratio (R/S) in two peanut cultivars at the vegetative and reproductive stages. LC and FC are the control plants of cultivar Local and Falcon and LS and FS, the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Within column, per growth stage, means followed by the same letter, are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	RWR	RWR (% of control)	MRLAR	MRLAR (% of control)	R/S	R/S (% of control)
			Vegetative stage (0-35 dap)			
LC	0.071b	100	0.042b	100	0.084b	100
LS	0.129a	182	0.132a	314	0.169a	201
FC	0.072b	100	0.034b	100	0.049b	100
FS	0.091b	126	0.034b	100	0.073b	149
			Reproductive stage (42-91 dap)			
LC	0.036a	100	0.020c	100	0.059a	100
LS	0.063a	175	0.061a	305	0.092a	156
FC	0.035a	100	0.016c	100	0.049a	100
FS	0.051a	146	0.053b	331	0.073a	149

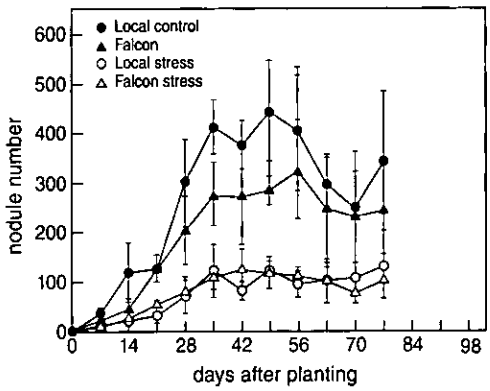

Figure 5.2 Effect of drought stress on nodule number/plant of two peanut cultivars, at vegetative and reproductive stages. Data represent mean of 8 plants (± SD).

a lower number of nodules than the control plants. There were no significant differences between the two stressed varieties in number of nodules. Under control conditions the cultivar Local showed a significantly higher number of nodules compared to the cultivar Falcon.
After about 50 days after planting the number of nodules decreased to about 60-70 % of the maximum number of nodules observed. The cultivar Local showed a significantly higher number of nodules compared to the cultivar Falcon (Figure 5.2).

3.4. Root shoot ratio (R/S) and root weight ratio (RWR)

At the vegetative stage, R/S, in cultivar Falcon, did not respond significantly to drought stress, but cultivar Local showed a 100 % increase. Similarly, RWR increased in the cultivar Local by 82 %, indicating that RWR is a usable indicator in drought stress experiments (Table 5.4).
At the reproductive stage, the R/S and RWR did not respond to drought stress, although the cultivar Local showed slightly higher values of R/S and RWR in relation to Falcon.

3.5. % Dry matter

High dry weight/fresh weight ratios (i.e. low water content) were found in the cultivar Local as a response to drought, in leaves, roots and on the whole plant level in the vegetative stage. The observed values were not only higher in relation to well-watered plants, but also in relation to drought-stressed plants of the cultivar Falcon.
Drought stress accounted for a significant increase in DW/FW ratios of 39 % for the leaves, 57 % for the roots and 34 % for the whole plant (Table 5.5).
At the reproductive stage, increased leaf percentage dry matter was found in both

cultivars under drought stress, representing 23 % and 18 % increase for Local and Falcon, respectively in relation to control, while the root and whole plant ratios did not show any significant response (Table 5.5).

3.6. Pod development

Pod number and dry weight were significantly reduced by drought, 68 % for pod number of both cultivars, pod dry weight was reduced to 73 % and 63 % for the cultivars Local and Falcon, respectively (Figure 3a and 3b). Cultivar Local showed a higher total sink number (TSN), but drought stress reduced TSN in a similar manner in both cultivars, 69 % and 58 % for cultivars Local and Falcon, respectively (data not shown).

Cultivar Falcon had fewer pods (29 %), partitioned more biomass to leaf (under drought) and a positive relationship between leaf number and pod number ($r = 0.83$ at $P<0.05$), was observed in this cultivar.

3.7. Total dry matter accumulation and distribution

At the vegetative stage a reduction in dry matter under drought stress was not statistically significant, suggesting that a short-term drought period may not affect the final yield in these cultivars to a large extent (Table 5.6).

In contrast to the vegetative stage, drought stressed plants showed a significant

Table 5.5 Effects of drought stress on water content in leaves, roots and whole plant, expressed as % dry matter, at the vegetative and reproductive stages. LC and FC are the control plants of cultivar Local and Falcon and LS and FS, the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Within column, per growth stage, means followed by the same letter, are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Leaf	Leaf (% of control)	Root	Root (% of control)	Whole plant	Whole plant (% of control)
			Vegetative stage (0-35 dap)			
LC	0.156b	100	0.125b	100	0.150b	100
LS	0.217a	139	0.196a	157	0.201a	134
FC	0.142b	100	0.111b	100	0.122b	100
FS	0.157b	111	0.132b	119	0.153b	129
			Reproductive stage (42-91 dap)			
LC	0.182c	100	0.144a	100	0.219a	100
LS	0.224a	123	0.156a	108	0.223a	102
FC	0.201b	100	0.158a	100	0.207a	100
FC	0.238a	118	0.172a	109	0.227a	105

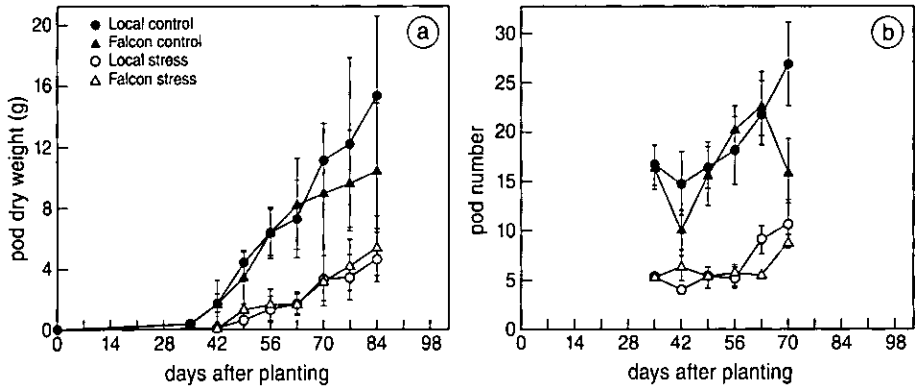

Figure 5.3 Effects of drought stress on pod dry weight and number of two peanut cultivars. Data represent mean of 8 plants (±SD).

Table 5.6 Effect of drought stress on total dry weight (TDW) in two peanut cultivars, at the vegetative and reproductive stages. LC and FC are the control plants of cultivar Local and Falcon and LS and FS, the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Within column, per growth stage, means followed by the same letter, are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	TDW (g)	TDW (% of control
Vegetative stage (0-35 dap)		
Local control	3.90a	100
Local stress	1.10a	28
Falcon control	3.03a	100
Falcon stress	1.26a	42
Reproductive stage (42-91 dap)		
Local control	23.2a	100
Local stress	7.0b	30
Falcon control	20.0a	100
Falcon stress	8.0b	40

reduction in total dry weight (Table 5) at the reproductive stage.
Both cultivars responded to drought stress by reducing dry-matter by 70 % and 60 % for the cultivar Local and Falcon respectively, as Table 5.6 shows.
The final distribution of dry matter is shown in Figure 5.4. Clearly, a long term drought affected dry matter accumulation in both cultivars.

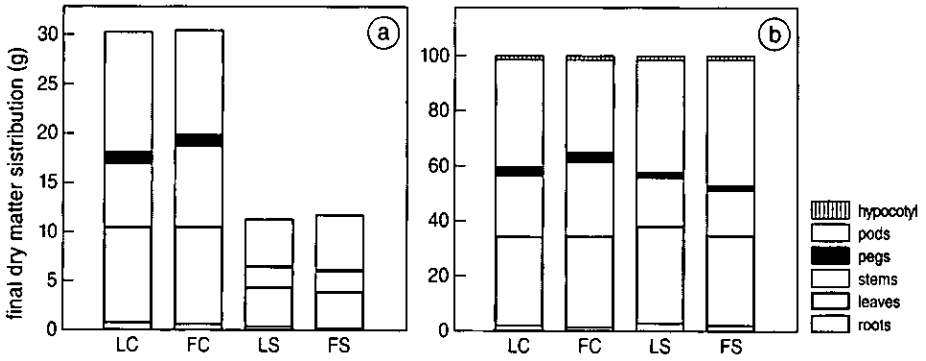

Figure 5.4 Effect of drought stress on absolute (a) and relative (b) final dry matter distribution (91 dap) of two peanut cultivars. Local (L) and Falcon (F). LC and FC are the well-watered controls and LS and FS the drought-stressed treatments, respectively.

4. EFFECTS OF DROUGHT STRESS ON TOLERANCE INDEXES

In general both cultivars showed a two-phase tolerance pattern, corresponding to responses in the vegetative and reproductive stages (Figures 5.5a, b, c, d and e for TDW, Ln, LDW, LA and RDW, respectively).

The tolerance index decreased sharply after drought imposition and it showed a slight recovery pattern in the reproductive stage. The cultivar Falcon showed a higher tolerance index (low drought susceptibility) in the vegetative stage (except for the roots), while in the reproductive stage the pattern of change was similar for both cultivars.

In cultivar Falcon a weak positive correlation was found between drought stress tolerance index (DSTI) and leaf water content under well watered ($r = 0.73$ at $P<0.05$) and drought conditions ($r = 0.63$ at $P<0.05$). This relation was not found in the cultivar Local in either treatments.

5. DISCUSSION

5.1. Drought stress: performance of peanut in the vegetative stage

5.1.1. Relative growth rate (RGR) and seed size

A previous experiment (Chapter 2) showed a strong and negative correlation between RGR and seed size ($r= 0.95$ at $P<0.05$). In the present experiment the small-seeded cultivar Falcon (F) again showed a high RGR under well watered and drought-stress conditions relative to the large-seeded cultivar Local (L)

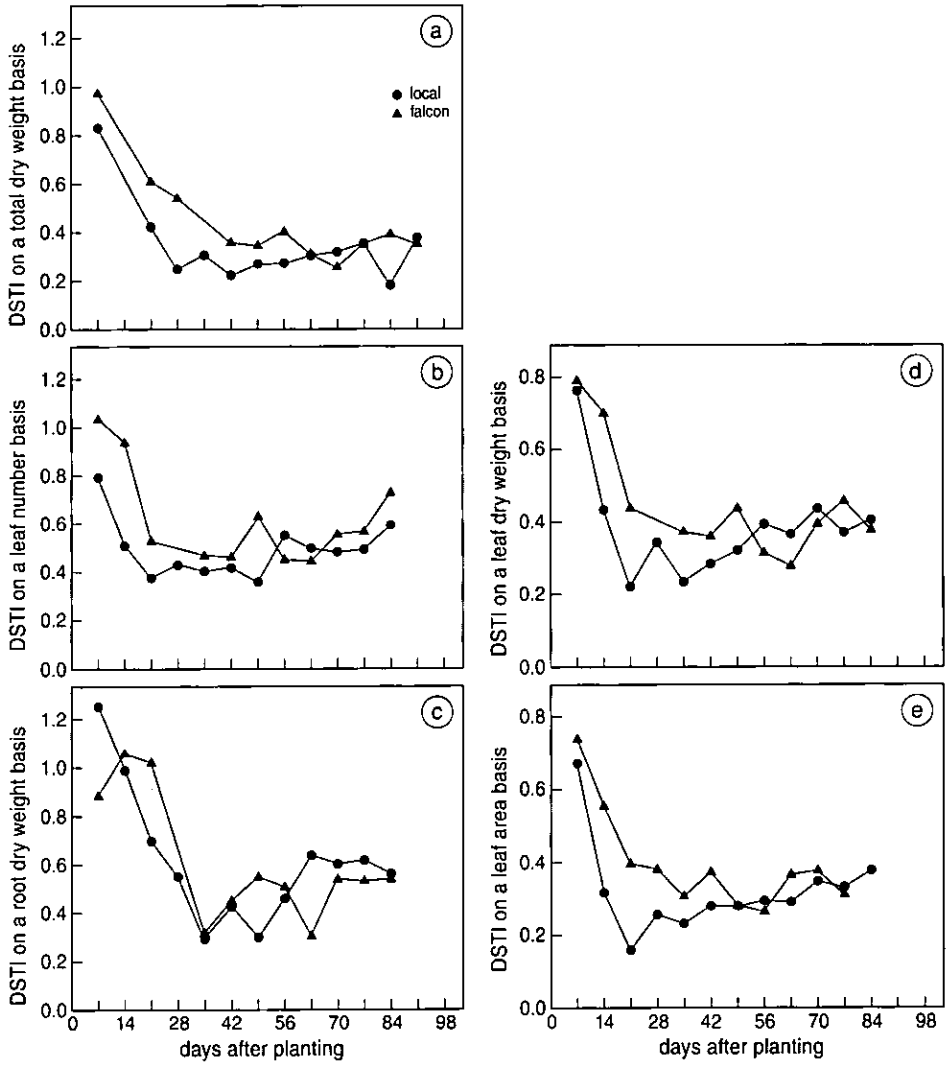

Figure 5.5 Tolerance indexes of the peanut cultivars for total dry weight (a), leaf number (b), leaf dry weight (c), leaf area (d) and root dry weight (e).

(Table 5.1). These results agree with those of Hendrix and Trapp (1992) in *Pastinaca sativa*, who found a superior drought resistance of seedlings from small seeds during a short-term drought. The better performance of the small-seeded cultivar is related to its high root length as found in Chapter 2, allowing a better access of water and nutrients from deeper sources. Wullf (1986) also suggested that in non competitive situations a small seed size might be advantageous under conditions of limited water availability.

The small-seeded cultivar Falcon also showed a high drought tolerance index at this growth stage (Figure 5.5). The low susceptibility of this cultivar in the vegetative stage is in agreement with other results on peanut, indicating that moderate water deficit (within 50 days after planting, Vivekanandan and Gunasena 1976) or water deficit during vegetative stage (Pallas *et al.*, 1979), is expected to have only minor effects on peanut growth and final yield. In fact, as drought-stress developed, the tolerance decreased (Figure 5.5).
The large-seeded cultivar Local showed a relatively low RGR during this stage. Although low RGR has been associated with increased cell wall material (Nieman *et al.*, 1992) and stress tolerance (Chapin 1988; Grime *et al.*, 1988), the cultivar showed an increase in drought-avoidance parameters like root weight ratio (RWR), maximum root length leaf area ratio (MRLAR) and root shoot ratio (R/S) (Table 5.4), suggesting higher susceptibility than cultivar Falcon.

5.1.2. Leaf growth

Under drought stress the cultivar Local, reduced its leaf area and leaf weight, traits linked to a low inherent RGR, which enables the plant to reduce the demand for water on a per plant basis (Van den Boogaard *et al.*, 1996). On the contrary, the cultivar Falcon maintained its leaf area under drought stress, a capacity expected to maintain light interception, radiation-use efficiency and to result in unaffected productivity. As several authors have reported, the expansion of leaves is very sensitive to moisture deficit and it responds more rapidly to changes in leaf water status than photosynthesis and transpiration (Michelena and Boyer 1982; Muchow 1985; Hoogenboom *et al.*, 1987). The lack of response of leaf area in cultivar Falcon, lead to the speculation that in the present experiment the cultivar Falcon possessed a mechanism to maintain leaf expansion for a longer period of drought stress.
Another possible explanation for this lack of response could be that the cultivar has a lower rate of transpiration, maintaining the same rate of photosynthesis, i.e. high water use efficiency and high leaf area ratio (LAR). Thus, the high RGR did not lead to a higher water demand as suggested by Van den Boogaard *et al.* (1996). Maintenance of leaf water content in the leaves could be another explanation, since Shippley (1995) indicated that plants with a higher water content in their leaves, can maintain the same surface area per leaf and, therefore, the same light interception per leaf, with less investment in biomass. The cultivar Falcon did indeed not show a significant reduction in leaf dry weight (Figure 5.1) as well as in the leaf water content (Table 5.5).
On the other hand, the specific leaf weight (SLW) did not change under drought stress, only in the cultivar Local, where it decreased by 24.4 %.
A change in SLW under drought stress would indicate the effects of drought in

restricting the movement of photosynthates to sinks (Munns and Weir 1981). As no measurements on transpiration were performed, one can only speculate that this may be the explanation.
The reduction in leaf area found in the present experiment with cultivar Local is a widely adopted alternative and is commonly associated with thicker leaves, resulting in a higher ratio of photosynthesis to transpiring area and low specific leaf area (SLA). Wright *et al.* (1993) found that SLA is closely and negatively correlated with WUE. Both cultivars reduced the SLA under drought stress conditions, indicating a tendency of reducing the transpiring area in both cultivars. LAR decreased significantly in cultivar Local, mainly as a result of low SLA, while in the cultivar Falcon it was a result of both SLA and LWR (Table 5.3). Since no decrease in root dry weight occurred, the present results indicate that drought stress inhibits more the growth of the leaves, than that of the roots.

5.1.3. Root growth

The root dry weight did not show any significant response to drought, although Reid and Renquist (1997) reported that increased root growth of tomato appeared to be an even clearer example than reduced leaf area of a feed-forward mechanism of acclimation to drought. Despite a lack of response of the root dry weight, an increase in drought-avoiding parameters, such as root weight ratio (RWR), maximum root length to leaf area ratio (MRLAR) and root shoot ratio (R/S), was observed (Table 5.4). Clearly R/S, seems to be suitable to be used as a drought indicator, in contrast to results reported by Simane *et al.* (1993) for wheat.
The observed high RWR values demonstrate a change in biomass partitioning between roots and leaves, increasing the proportion of dry matter allocation to the roots and decreasing the proportion of dry matter allocated to the leaves (Kalapos *et al.*, 1996).
Passioura (1983) argued, that a small root system may be more efficient than a large one, as roots are sinks of assimilates and root density normally exceeds the minimum, needed for water uptake. However, White *et al.* (1990) showed that in common bean a greater rooting density can delay dehydration and allow a prolonged maintenance of relatively large stomatal conductance and hence results in increased growth and yield. The present results indicate that it is not the root *per se* which confers advantageous characters to drought stress, but mainly its relation to shoot characteristics, like leaf area and leaf and stem weight. In fact root/shoot ratio related parameters showed significant changes in the cultivar Local after exposure to drought (Table 5.4).
RWR increased by 82 %, which corresponds to several similar findings (Munns and Sharp 1993; Cramer *et al.*, 1994) and as a result of a simultaneous reduction observed in LAR, a unit of dry weight of root supplied a smaller leaf area with

water in drought-stressed conditions. An increase in RWR as found in cultivar Local has been interpreted as a means of alleviating water stress in crops (Husain *et al.*, 1990; Reid 1990; Rengasamy and Reid 1993). Farrar (1996) indicated that RWR is important for resource capture, the higher the RWR the higher photosynthesis is, relative to nutrient uptake, suggesting sucrose as playing a role in its regulation.

In a previous experiment (Chapter 2) a significant and negative correlation was found between RGR and RWR ($r = 0.95$ at $P<0.01$), indicating that slow growth rate was linked to a large root system, which is advantageous for allowing better access to soil moisture and other resources, under limited conditions. Cultivar Local showed a similar pattern, resulting in a reduced transpiring area and an increased absorptive surface area.

5.1.4. Nodulation

N_2-fixation was measured, using the number of nodules, although Wang *et al.* (1993) indicated that nodule mass was a more reliable predictor of N_2-fixation potential, than nodule number. However, a strong correlation was found ($r = 0.90$ at $P<0.0\ 5$) between nodule number and nodule mass, so we could use the nodule number as an indirect predictor of N_2-fixation potential.

Nodulation measured as the number of nodules, was not affected by drought stress, which contradicts the results of Kulkarni *et al.* (1988a,b), who found a significant reduction in nodule number by drought stress in two peanut cultivars with uninoculated and inoculated seeds. Several studies have reported a reduction in N_2-fixation by drought stress by a direct effect on the nodules and its effects can be aggravated by the inability of the drought-stressed leaves to supply photosynthates to the nodules (Finn and Brun 1982), which are sensitive to water stress (Antolin *et al.*, 1995).

Since total dry matter was not reduced by drought stress in both cultivars and neither was leaf area in cultivar Falcon, it may be argued that no limitation of supply in carbon for growth and development of nodules occurred. In fact, a strong positive correlation between nodule number and leaf area and leaf weights was observed. Although a significant correlation was found between nodule number and leaf area, which were both reduced in cultivar Local, it seems that this reduction was not sufficient to reduce the supply of photosynthates to the nodules. Thus the correlation between nodule number and leaf area did not represent an exact measure of the relation between N_2-fixation and photosynthesis.

The insensitivity of the symbiotic N_2-fixation to water stress, when compared to plant growth, has been reported in *Vicia faba* (Plies-Balzer *et al.*, 1995) and peanut (Sinclair and Serraj 1995) and has been linked to the transport of the nitrogen products from the nodules. A possible explanation could be, that peanut

does not transport nitrogen products from the nodule in the form of ureides (Peoples *et al.*, 1991), in contrast to other grain legumes. This difference could have an important feedback role in regulating the overall metabolism of nodules (Silsbury *et al.*, 1986; Parsons *et al.*, 1993). Since Sinclair *et al.* (1995) assumed that soil drying results in early inhibition of nitrogen fixation via ureide activity, peanut would be exempted from this hypothetical feedback and could sustain nitrogen fixation activity at a low water content. HPLC analysis had failed to detect the presence of ureides in the xylem sap of peanut (Sinclair and Serraj 1995).

5.2. Drought stress: performance of peanut in the reproductive stage

5.2.1. Leaf and root growth

Continuous drought negatively affected growth and development of both cultivars in the reproductive stage in a similar manner. The results are in agreement with those found under salinity stress showing that a short-term salinity stress may limit growth by inhibition of leaf expansion, whereas long-term salinity stress may limit growth by inhibiting the carbon supply (Munns and Termaat 1986). Indeed, in contrast to observations during the vegetative phase, both cultivars showed a reduction in leaf weight ratio (LWR); a clear sign of reduced carbon allocation to the leaves. The reproductive stage has been reported as the most sensitive to drought (Pallas *et al.*, 1979; Boote and Hammonds 1981).

Leaf water content was significantly reduced by 23 % in cultivar Local. This decrease may contribute to a decrease in surface area per leaf as suggested by Shippley (1995). However, the whole plant dry weight to fresh weight ratios, did not show any response, which can be ascribed to a contribution of the water content of the roots, which was not significantly affected by drought stress. Neither was the water content of the pegs and pods, structures, which evidently are in direct contact with the water supply by the roots

The root dry weight did not show any response to drought stress, a result which can be ascribed to root death, which was found to occur within 30 days after withholding water in *Lycopersicum esculentum* (Reid *et al.*, 1996).

5.2.2. Nodulation

The reduction in nodulation in the reproductive generative stage of 70 and 60 % for the cultivars Local and Falcon respectively, may result, at least partly, from accelerated senescence of the nodules, as clearly found in control plants and also observed by Pate *et al.* (1969). Since the development of leaf area and dry matter production depends on the availability of N-compounds from the roots, the restricted production and transport of these compounds to root and shoot, under drought stress conditions, may reduce N_2-fixation *per se* (Pate *et al.*, 1969). The

results clearly show that regardless of the cultivar a prolonged drought stress is, in fact, a serious problem in peanut, in contradiction with the results of Sinclair *et al.* (1995). The mechanisms by which drought stress affects nitrogen fixation are still poorly understood, but they may be linked to increased nodule permeability to oxygen (Pankhurst and Sprent 1975) and to an effect of drought stress on a permeability barrier to gas diffusion located in the nodule cortex (Serraj *et al.*, 1995).

5.2.3. Yield and yield components

The reduction in light absorbing and assimilatory surface area drastically reduced the yield expressed as pod number and pod weight (Figure 5.3).

Several authors have referred to the reduction of yield as a result of drought stress (Gibbons 1980; Williams *et al.*, 1986). Contrary to the glasshouse experiment (Chapter 2), the cultivar Local showed a higher TSN and the cultivar Falcon partitioned more biomass to leaf and initiated new sinks. Chapman *et al.* (1993) found the same results in the Virginia Bunch as in the cultivar Falcon. Harris *et al.* (1988) reported a linear relationship between leaf number and pod number, a result that we only observed in the cultivar F under drought stress.

Differences between cultivars in this respect may be due to differences in a high priority for leaf production, possibly through a requirement for a critical number of leaves to be initiated, before filling of existing pods continues. Chapman *et al.* (1993) interpreted this as a mechanism which may be useful when water stress affects assimilate production potential, but it may waste assimilate, when the time available for pod filling is limited.

5.3. Drought stress and traits, which contribute to adaptation.

Cultivars differed in response to drought and the sensitivity of a growth parameter or plant part seemed to depend on the inherent strategy of the cultivar in question. RWR, R/S, reduction in leaf area, reduction in leaf water content of the leaves and MRLAR, were all associated with drought avoidance.

Dry matter accumulation was relatively less sensitive to drought stress. R/S was inconsistent as a drought stress indicator, since it did not show a regular pattern in both cultivars.

SLA was sensitive to drought, while LWR, showed sensitivity only in cultivar Falcon in the vegetative stage and in both cultivars in the reproductive stage.

Comparing the water content in leaves and roots, the water content of the leaves was the factor most likely to determine a high tolerance index of cultivar Falcon.

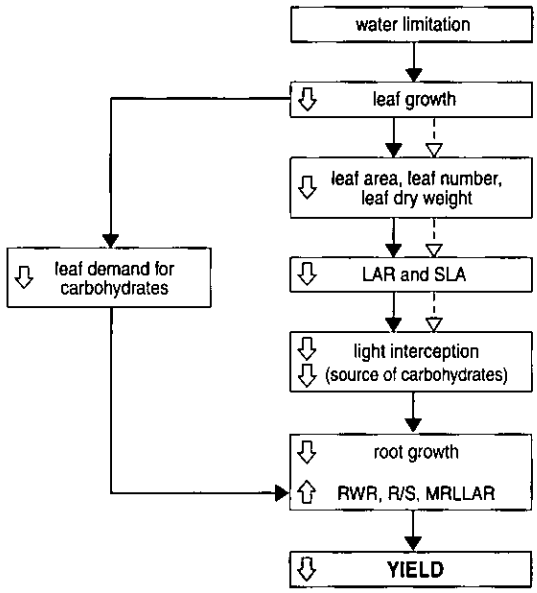

Figure 5.6 Synoptic diagram of the water-limitation effects on leaf and root growth and yield in the two peanut cultivars. Arrows indicate a statistically significance of the effect for cultivar Local at both growth stages and for cultivar Falcon only at the reproductive stage. Dashed lines indicate no statistically significant effect for the cultivar Falcon at the vegetative stage. ⇧ or ⇩ indicate increase or decrease, respectively

6. CONCLUSIONS

The effects of drought on growth and yield of peanut are summarised in Figure 5.6. Continuous drought stress drastically affected growth, development and yield of the two peanut cultivars, but the reproductive stage was the most sensitive.

From the present results it can be concluded that small seed size together with high RGR, can be associated with a relatively low sensitivity to drought, while large seed and low RGR is associated with a high sensitivity to drought.

The differential responses of the two peanut cultivars at the vegetative stage, suggested that cultivar Local is a drought-avoider, since it exhibits characteristics which increase water acquisition and conservation, such as reduced leaf area and leaf number, low SLA and RWR and MRLAR.

Cultivar Falcon seemed to have an osmotic adjustment mechanism, since leaf area, a very sensitive parameter to drought stress, was not significantly affected by drought.

RWR, MRLAR and RSR were consistent drought stress indicators, particularly in the vegetative stage, loosing their significance as drought stress developed.

6 Leaf water relations of two peanut cultivars in response to imposed drought stress: proline content, water retention capability and cell membrane integrity as parameters for drought tolerance

SUMMARY

Two peanut cultivars were grown for 13 weeks under water controlled conditions in ceramic pots, lined with plastic bags. The cultivar Falcon (F) showed characteristics of drought tolerance, while cultivar Local (L) showed those of drought-susceptibility.
The relative water content (RWC), relative saturation deficit (RSD), cell membrane integrity (CMI) and proline content, were effective criteria for detecting drought-tolerance strategies taking into account the growth stage and duration of the stress period, while the water retention capacity (WRC) did not show any significant relation with drought-tolerance.

1. INTRODUCTION

Plant survival and production under environmental stress is conditioned by a complex of mechanisms. Many studies point to the cell membrane as an initial site of stress injury, i.e. the function and structure of plant cell membranes is drastically damaged by environmental stress (Agarie *et al.*, 1995). Thus, evaluation of cellular membrane integrity as a measure of environmental stress tolerance appears to be a relevant criterion (Sullivan 1972). In recent years, the polyethylene glycol (PEG) test for measuring cell membrane stability (CMS) has been claimed as an efficient method to determine drought sensitivity (Premachandra *et al.*, 1990).

Most commonly changes in the electrical impedance and leakage of intact plant cells or tissue have been measured to detect stress injury of plasma membrane. Leakage will vary in relation to the membranes' ability to take up and retain solutes and, therefore, will reflect drought stress-induced changes in both membrane potentials and membrane permeability (Agarie *et al.*, 1995). Sullivan and Ross (1979) found for sorghum that membrane integrity and stability to stress, as evaluated by electrical leakage, correlated well with drought tolerance of other plant processes to stress.

Among the symptoms of membrane damage are: (i) lessening of the Hill reaction of chloroplasts (Boyer 1971), (ii) a lowering of the quantum efficiency of photosynthesis (Boyer 1976), (iii) a lowering of the respiratory rate of mitochondria (Koepe *et al.*, 1973), (iv) an increase in leakage of solutes from leaf tissue (Bramlage *et al.*, 1978) and (v) hastening of senescence (Boyer 1976).

Some authors referred to genetic variability and heritability of CMS and then concluded that the technique could be used as an efficient means for selection of drought-tolerant genotypes in wheat (Premachandra and Shimada 1987). The same authors (1988) measured the CMS in naturally dehydrated excised leaves and found that drought tolerance was highly correlated with CMS, as measured by the PEG-test.

Natural dehydration of plants exposed to drought can be measured as excised leaf water retention capability, which is mainly affected by cuticular and stomatal resistances. A comparison of these characteristics and other physiological measurements with the CMS, measured by the PEG test, may increase our understanding of the physiological processes involved in the differential ion leakage (Premachandra *et al.*, 1989).

Premachandra and Shimada (1988) indicated that CMS, measured by the PEG test, was significantly and positively correlated with leaf water potential, osmotic potential of leaf tissues, excised-leaf water retention, degree of leaf rolling, total plant weight and total root length under varied soil moisture levels. Worku

(1995) reported a close relationship between high water retention capability, drought hardiness and high yield in wheat.

In peanut Venkateswarlu and Ramesh (1993) reported that cell membranes of cultured cells, originating from a drought tolerant cultivar, had suffered much less injury than those from a drought sensitive one. Levels of organic osmotic solutes as sugars and proline in the cell sap of sensitive peanut accesions with a low CMS were much lower than those of tolerant ones with high CMS (Deb *et al.*, 1996).

Relative water content (RWC) has been successfully used to monitor water content and drought status in peanut (Bennett *et al.*, 1984). Sinclair and Ludlow (1985) argued that RWC is a more useful parameter of a plant's water balance than the leaf water potential and it should provide a universal relationship between physiological traits and level of drought stress. RWC values in well-watered plants were typically in the range of 85-98 % (Prabowo *et al.*, 1990). Under drought conditions RWC as low as 29 % has been measured (Bhagsari *et al.*, 1976), indicating that peanut has a very low lethal water status, which should contribute to a high level of dehydration tolerance and leaf survival in peanut during intermittent drought periods.

Leaf dehydration is related to tissue hydration and resistance to dehydration should be related to the manner, in which tissue water replacement and maintenance occurs. Turner (1986) reported, that a number of researchers have observed a relatively small decrease in RWC per unit water potential decrease in drought resistant species (Sanchez-Diaz and Kramer 1973).

The ability to maintain a high stomatal conductance at low leaf water potential, known as stomatal adjustment, is a common adaptive response to drought in peanut (Ludlow 1980). Such adjustment has been observed in peanut (Black *et al.*, 1985) and within peanut cultivars (Joshi *et al.*, 1988), however it should be realized that the physiological mechanism responsible for this acclimation is unknown.

Osmotic adjustment (OA) has been suggested as a mechanism that leads to smaller changes in RWC per unit decrease of water potential (Steudle *et al.*, 1977) and consequently it should help to maintain a positive and high turgor potential during water stress.

OA has received increasing attention in the last decades and refers to active accumulation of solutes in cells beyond the increase in concentration caused by loss of water. OA provides certain advantages: lowering of the leaf osmotic potential permits turgor to remain more positive under stress conditions. As a result, cell growth can continue, root cells can penetrate a greater soil volume, stomata will remain longer open and therefore photosynthesis can continue at greater drought levels (Parsons and Howe 1984). OA reduces sensitivity of turgor-dependent processes, such as leaf expansion, stomatal conductance and leaf rolling, to declining

leaf water potentials (Jones *et al.*, 1980; Morgan 1984). However, Munns (1988) argued that OA is not the only factor for maintaining leaf turgor, since reduction in stomatal aperture can also accomplish maintenance of leaf turgor.

A decrease in osmotic potential, leading to osmotic adjustment is partly a consequence of reduction in leaf expansion rate rather than an active acclimation process: concentration of osmotic solutes in elongating cells is related not only to deposition rate of the osmotic solutes in these cells, but also to the dilution by the water influx allowing increase in cell volume. Voetberg and Sharp (1991) have shown that only some osmotic solutes, in particular proline, have an increase in accumulation rate, when roots are stressed, while most leaves accumulate it as a consequence of decreased expansion rate.

Proline is believed to act as an (i) osmotic solute in plant cells (Hu *et al.*, 1992; Delauney and Verma 1993); (ii) as a stabilising agent for membranes, through an effect on the hydration layer surrounding phospholipids (Rudolph *et al.*, 1986); and (iii) as a source of nitrogen and carbon during recovery from stress. Proline content correlated positively with membrane integrity, measured as ion leakage, in tobacco leaves (Van Rensburg *et al.*, 1993), suggesting its use as a selection criterion for drought tolerance in *Nicotiana tabacum*. While some authors associate proline with drought tolerance, Hanson (1979), Andrade *et al.* (1995) found accumulation of proline in drought sensitive cultivars of barley and beans, associating this change with a more rapid decline in water potential or as a symptom of severe stress. Andrade *et al.* (1995) confirmed the suggestion that proline was synthesised in the leaves and translocated to the roots and other organs and that it may act as a mechanism for drought tolerance .

The peanut is a legume that under many conditions fixes N_2 through symbiotic relations, to avoid N deficiency. However, factors such as peanut cultivar, variety, presence of inoculum, crop rotation, soil type, moisture and temperature, all can affect N_2-fixation (Gascho and Davis 1994).

Peanut is grown on P deficient soils in Mozambique. Phosphorus is the most deficient element, although this deficiency is limited to areas which have never been fertilised with P, where fertilisers are not available or where their cost is prohibitive.

The objective of the present study is to determine and compare the leaf water relations' responses of two peanut cultivars to water stress. Differences that might be observed may partially explain the observed differences in growth of the cultivars response to imposed drought stress in Chapter 5. A further objective is to evaluate how proline levels differs among the cultivars (drought avoider and tolerator) and how its contribution changes with increasing drought stress. For this reason cultivar Falcon, a drought-tolerator, and cultivar Local, a drought avoider, were selected for this study.

2. MATERIAL AND METHODS

2.1. Plant material

Two peanut (*Arachis hypoagaea* L.) cultivars Falcon (F) and Local (L) were grown in 12 l ceramic pots, lined with plastic bags, as described in Chapter 5.

2.2. Growth conditions

The plants were grown in a plant nursery in Maputo-Mozambique (25° 28' S and 32° 36' E), from November 1998 to February 1999, under water-controlled conditions.

The mean air temperature during the growth period was 28.3 ± 3.0 °C in the morning, 31.5 ± 2.5 °C in midday and 29.1 ± 3 °C in the afternoon. The mean relative humidity ranged from 57 ± 12 % in the morning, 47.8 ± 10 % in midday and 55 ± 9 % in the afternoon. The illumination was by screened natural light, resulting in an average photon flux density at canopy level of 285 ± 18 μmol.m^{-2}.s^{-1} in the morning, 436 ± 24 μmol.m^{-2}.s^{-1} in midday and 577 ± 26 μmol.m^{-2}.s^{-1} in the afternoon, measured with a quantum sensor (SK P215, Skye Llandrindod Wells, UK).

During the first week the plants were irrigated to field capacity with normal tap water. Drought stress imposition was as described in Chapter 5.

The plants were harvested at week four after initiation of drought stress (vegetative stage) and at ten and thirteen weeks after drought stress initiation (pod-setting) and maturity stages.

At each harvest the following determinations were made:

a) Water retention capacity of the leaves.
b) Leaf water relations.
c) Cell membrane integrity of the leaves.
d) Proline content of leaves and roots.
e) Total N and P in the leaves.

2.3. Water retention capacity of the leaves

The water retention capacity (WRC) of the leaves was determined according to Worku (1995). Pots were covered in the night, preceding the measurement with a black plastic sheet to avoid water loss due to pot evaporation.

One leaf per branch (8 replicates) was detached and weighed immediately. The leaves were kept at room temperature (20-25 °C) for free transpiration. The weight of these excised leaves was recorded every h for a period of 8 h and once again after 24 h.

The WRC was calculated as the relative decrease of weight in percent per h, using the formula: (fresh weight of the excised leaf x 100)/fresh weight of the leaf after n hs of free transpiration.

2.4. Leaf water relations

The leaf water relations were determined as relative water content (RWC), water saturation deficit (WSD) and relative saturation deficit (RSD) as described by Turner (1986) and Ashraf *et al.* (1996). The second fully expanded tetrafoliate leaf of the main stem, was used for this determination (8 replicates). The leaves were excised in the morning and immediately weighed (fresh weight = FW).
Leaves were then kept in a humid chamber in test tubes, containing 10 ml of distilled water, for at least 12 h at room temperature.
The leaves were then taken out, the water was removed from the surface and weighed again [turgid (or saturated) weight = TW]. The dry weight (DW) was obtained by weighing after placing the leaves in an oven at 70 °C for 48 h.
The RWC was determined as follows: [(FW-DW)/(TW-DW)]100. The WSD was computed as follows: WSD = 100-RWC, and the RSD as [(TW-FW)/TW]100

2.5. Cell membrane integrity of the leaves

For measurements of the cell membrane integrity the PEG test was used, as adapted from Agarie *et al.* (1995) and Ashraf *et al.* (1996). Thirty leaf discs, obtained from the uppermost fully expanded leaves, were washed three times with deionized water in a test tube. The leaf discs were then submerged in 30 ml of 40 % PEG 600-solution (T1) or deionized water as a control (C1) and both were left for 24 h at 10 °C. The leaf discs were then quickly washed with deionized water and allowed to remain in 30 ml deionized water for another 24 h at 10 °C. The electrical conductivity (EC) of the liquid was measured afterwards. The leaf discs, still in the same solution, were then killed by autoclaving for 20 minutes to release all ions from the tissue, cooled to 25 °C (T2 and C2) and the EC was again determined.
The cell membrane integrity was evaluated as percentage of injury (PI), using the formula:

$$PI = [(1-T_1/T_2)/(1-C_1/C_2)]100.$$

2.6. Proline content of the leaves

The free proline was determined according to Bates *et al.* (1973). The ninhydrine derivate was extracted with toluene and analysed spectrophotometrically at 520 nm. The proline concentration was determined from a standard curve and expressed as μmol proline g FW^{-1}.

2.7. Total nitrogen and phosphorus in leaf material

Total N and P, were determined on a dry weight bais according to Houba *et al.* (1989), at the National Institute for Agricultural Research, Maputo, Mozambique. Material was digested with a digestion mixture [sulphuric acid, salicylic acid and hydrogen proxide (v:v:v, 0.3:2.5:1)] and selenium added as a catalyst. For N, the ammonium was determined colorimetrically on a segment flow analyser, measuring the absorption at 660 nm (Houba *et al.*, 1989).

For P the phosphorus-molybdenum blue complex was determined spectrophotometrically at 882 nm (Houba *et al.*, 1989).

2.8. Data analysis

Differences in the parameters measured between the treatments were analysed using the Student t-test.

Trends in RWC, proline content and cell membrane integrity were analysed by a linear regression, using the GraphPad Prism package, version 2.01.

3. RESULTS

3.1. Water retention capability (WRC) of the leaves

No differences between the two cultivars were found under well-watered conditions. Under water-stressed conditions, the cultivar Local had a slightly, not significantly, lower WRC than cultivar Falcon (Figure 6.1).

Water-stressed plants showed a slightly, not significantly, higher WRC, compared to well-watered control plants. The cultivar Local showed under drought stress a

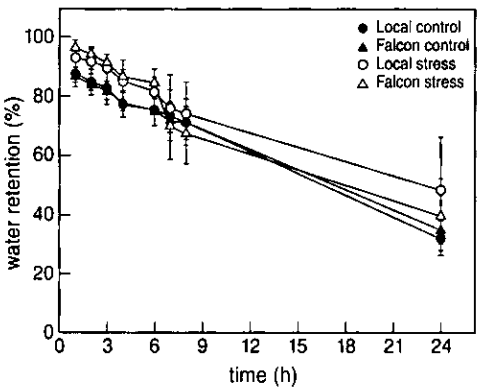

Figure 6.1 Water retention capability of two peanut cultivars under well-watered and water-stressed conditions. Data represent mean of 8 plants (± SD).

Table 6.1 Effect of drought stress on water retention capacity after 24 hs of free transpiration (%) at the pod-setting and maturity stages. LC and FC, are the control plants of the cultivars Local and Falcon and LS and FS, are the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap are days after planting. Each value is the mean of 8 replicates for each parameter (± SD). Per growth stage, values of the same cultivar followed by the same letter, are not significantly different at $P<0.05$ level, using the Student t-test.

	LC	FC	LS	FS
Pod-setting stage (77 dap)	56.2±1.7 a	43.4±9.0 b	56.3±3.3 a	55.4±4.9 a
Maturity stage (91dap)	61.6±6.9 a	57.1±8.4 a	69.3±4.4 b	65.5±8.9 a

lower WRC in the first 6 h of free dehydration, but it retained more water after 8 and 24 h. No significant differences were found in WRC in the later growth stages: pod setting and maturity. Both cultivars increased WRC during growth and during the drought stress, up to 97 %. Under well-watered conditions the cultivar Falcon showed the lowest water retention capability 24 h after free dehydration, both in the pod-setting and maturity stage as shown in the Table 6.1.
Cultivar Local seems to better retain water after 24 h dehydration, even if it retained less in the first 8 h of dehydration than cultivar Falcon.

3.2. Relative water content (RWC), water saturation deficit (WSD) and relative saturation deficit (RSD).

RWC of well-watered plants did not differ significantly between the two cultivars. However, under water-stressed conditions the cultivar Local showed the lowest RWC value (Table 6.2).
At the vegetative stage cultivar Local showed the highest values for WSD and RSD, characteristic of drought-susceptible plants (Ashraf *et al.*, 1996). At the pod-setting stage, no differences were found in RWC, WSD and RSD. At the maturity stage drought-stressed plants showed a lower RWC value and higher WSD and RSD values.
Cultivar Falcon showed a low RWC value under drought stress conditions (68 %), compared to the control treatment (91 %, Table 6.2). This result is in contrast to RWC in the initial growth stage, where RWC was hardly decreased (Table 6.2).
Apparently, a long duration of the drought stress affected the cultivar Falcon more negatively than cultivar Local.

3.3. Cell membrane integrity

At the vegetative stage, the cell membrane injury as measured with the PEG-test was relatively high in the drought susceptible cultivar Local under both well watered and water-stressed conditions (Table 6.3).

Table 6.2 Effects of drought stress on relative water content RWC (%), water saturation deficit WSD (%) and relative saturation deficit RSD (%) of two peanut cultivars, at the vegetative and maturity stages. LC and FC, are the control plants of the cultivars Local and Falcon and LS and FS, are the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Each value is the mean of 8 replicates for each parameter (± SD).
Per growth stage, values of the same cultivar followed by the same letter, are not significantly different at $P<0.05$ P level, using the Student t-test.

Treatment	RWC (%)	WSD (%)	RSD (%)
		Vegetative stage (0-35dap)	
Local control	99.0±0.6a	0.92±0.05a	1.1±0.37a
Local stress	86.0±7.0b	14.0±6.72b	11.7±5.31b
Falcon control	98.5±0.4a	1.53±0.31a	1.2±0.31a
Falcon stress	92.3±3.0b	7.71±2.9b	7.4±2.35b
		Reproductive stage (42-91dap)	
Local control	90.0±4.0a	10.5±3.5a	8.3±2.8a
Local stress	74.0±9.0b	26.3 ±8.5b	21.3±6.7b
Falcon control	92.0±4.1a	9.5±3.6a	8.5±3.5a
Falcon stress	68.0±6.0b	32.1±5.6b	26.0±5.4b

Table 6.3 Effects of drought stress on percentage cell membrane injury with polyethylene glycol (PEG) test in two peanut cultivars at the vegetative and pod-setting stages. LC and FC, are the control plants of the cultivars Local and Falcon and LS and FS, are the drought-stressed plants of the cultivars Local and Falcon, respectively. Dap, are days after planting. Each value is the mean of 8 replicates for each parameter (± SD). Per growth stage, values of each cultivar followed by the same letter, are not significantly different at $P<0.05$ level, using the Student t-test.

Treatment	Injury in PEG test (%)
	Vegetative stage (0-35dap)
Local control	23.7±10.0a
Local stress	21.2±7.6a
Falcon control	15.0±3.5a
Falcon stress	17.2±4.2a
	Reproductive stage (42-91dap)
Local control	48.4±9.4a
Local stress	33.7±10.8a
Falcon control	33.7±10.8a
Falcon stress	16.0±4.0b

The two cultivars showed different responses, when control and stress treatment plants were compared. Cultivar Falcon, showed a higher but not significant percentage of injury under drought stress conditions and the cultivar Local showed a slightly higher but not significant value, under well-watered conditions in the vegetative stage.
At the pod-setting stage, no significant differences were found in cultivar Local, whereas in cultivar Falcon under drought stress, a significant lower percentage injury was observed.
At the maturity stage, the cultivar Falcon showed slightly higher values in percentage injury (data not shown). Drought tolerance of cultivar Falcon apparently decreased with increasing duration of the drought stress period.

3.4. Proline content

Roots and leaves showed a higher proline content under drought stress conditions (Figures 6.2a and b).
Leaves showed an increased proline content under drought stress (Figure 6.2a) except for Falcon at week 10. The increase varied from 700 % at week 5, 100 % at week 10 and 166 % at week 13 for cultivar Local and 1700 % at week 5, to 270 % at week 13, for cultivar Falcon.
Roots also showed a high proline content under drought stress (Figure 6.2b) except for Local at week 10.
The increase varied from 400 % at week 5 to 125 % in week 13, while in week 10 no differences were found in cultivar Local. In cultivar Falcon an increase of 300 % at week 5, 300 % at week 10 and 250 % at week 13 was observed (Figure 6.2b).

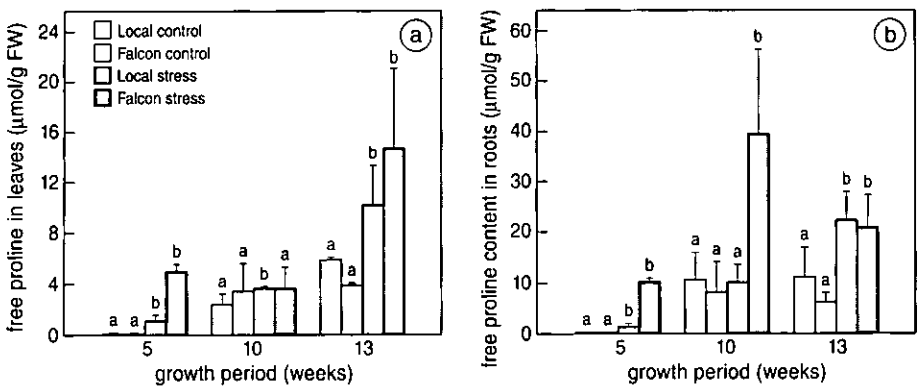

Figure 6.2 Proline content in two peanut cultivars, leaves (a) and roots (b) as affected by drought stress. Data represent mean of 8 plants (± SD). Different letters at a particular growth period denote values that differed significantly at $P<0.05$ after a Student t-test.

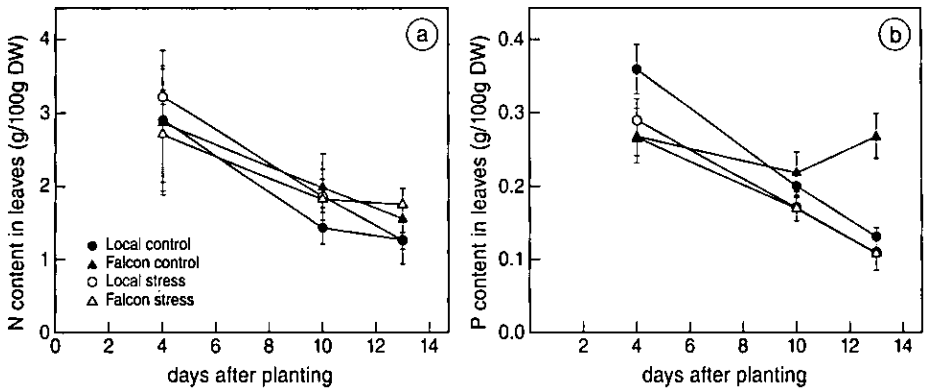

Figure 6.3 N (a) and P (b) content of the leaves of two peanut cultivars as affected by drought stress. Data represent mean of 8 plants (± SD).

3.5. N and P content

The N content of the leaves of cultivar Local was unaffected by drought stress, while in the cultivar Falcon a 5 % reduction in N content was observed in the initial phases of growth. At the pod-setting stage a 28% increase in the N content in the drought-stressed leaves of the cultivar Local was observed, while in the cultivar Falcon a reduction of 9 % was measured. Phosphorus content at the vegetative stage was reduced by 20 % in the cultivar Local, while the cultivar Falcon did not show any significant change (Figures 6.3a and b).

At the pod-setting stage P was reduced by 15 % and 30 % for the cultivars Local and Falcon, respectively. At the maturity stage drought stress lead to an increase in N of 38% in the cultivar Local, while in the cultivar Falcon, a 1.9 % increase was only observed. In contrast, P was reduced by 18% in the cultivar Local, while in the cultivar Falcon a reduction in the order of 45% was observed.

4. DISCUSSION

4.1. Drought stress and water relations

4.1.1. Water retention capability (WRC)

WRC, which could be strongly related to drought hardiness, did not show any significant trend in both growth stages, in contrast to the results of Worku (1995), working with wheat under well-watered conditions. However, drought-stressed plants, at least in the early stages of growth showed a higher WRC than well watered plants (Figure 6.1).

In the first 6 h under drought stress cultivar Local showed a slightly lower WRC

than cultivar Falcon, but Local retained relatively more water after 8 and 24 h of dehydration. Few experiments have reported results on WRC after 8 and 24 h, but assuming that a low WRC in the first 6 hs of natural dehydration is associated with drought-susceptibility, as in wheat (Worku 1995), it may be speculated that a high WRC after 8 and 24 h may also have a relation with drought-sensitivity, in this case drought-susceptibility. In fact, Rascio (1985) showed that water retention capability of durum wheat leaves 24 h after excision was higher in the cultivars susceptible to a shortage of water than in those resistant to drought. Thus, under stressed conditions the cultivar Local was slightly more susceptible to drought than the cultivar Falcon. The relation between WRC and drought resistance after 8 and 24 hs needs further testing.

The susceptibilty of the cultivar Local to drought stress confirms the results reported in Chapter 5 of this thesis, when the cultivar Local was tentatively classified as a drought-avoider and drought-susceptible, whereas the cultivar Falcon was indicated as a drought-tolerator on the basis of changes in leaf area, specific leaf area (SLA), root weight ratio (RWR) and maximum root length to leaf area ratio (MRLAR). In the pod-setting and maturity stage no changes in WRC were found under either well-watered or stressed conditions, which may indicate that in peanut the water retention capability as a selection criterion for drought resistance depends on the growth stage.

4.1.2. Relative water content (RWC) and relative saturation deficit (RSD)

From emergence until peg-initiation, the cultivar Falcon, the drought-tolerator, maintained a higher RWC (WSD) and a relatively low relative saturation deficit (RSD) as compared with cultivar Local, when both cultivars were subjected to drought. These results are consistent with several reports, indicating that drought tolerant species exhibit significantly higher RWC and lower RSD (Premachandra *et al.*, 1995; Ashraf *et al.*, 1996).

At the pod-setting stage drought did not significantly change RWC and RSD in either cultivar and treatment, although slightly higher values in RSD were observed in the cultivar Local. Drought did not affect the water content of the leaves at this growth stage, in contrast to the results of Nageswara Rao *et al.* (1985) in peanut cultivar. At the maturity stage significant differences among the cultivars and treatments were observed. The well-watered plants showed higher RWC, while under stressed conditions the cultivar Falcon showed the lowest RWC value (Table 6.2). The high sensitivity of this cultivar to drought is also supported by the percentage of injury of the cell membranes, as discussed below. Although showing the lowest RWC value, the percentage of reduction in RWC in relation to control was slightly higher in the cultivar Local (82 %), than in cultivar Falcon (74 %). RSD, however, increased by 255 % in the cultivar Local and 308 % in cultivar Falcon.

The high values of RSD indicate a high sensitivity of the peanut plants at this growth stage to drought, since water is required not only to maintain the regular growth but to maintain an adequate peg turgor in order allow the peg to penetrate the soil.

4.2. Drought stress and proline content

Osmotic adjustment has been suggested as a mechanism, that leads to smaller changes in RWC per unit decrease in water potential in drought resistant species (Steudle *et al.*, 1977) and consequently may help to maintain a positive turgor potential during water stress. This is a result of an increase in content of solutes such as proline. Both cultivars accumulated significantly more proline under drought stress, showing the highest values for the roots (Figure 2b) in early stages of growth.

The drought-tolerant cultivar Falcon had a much greater accumulation of proline in the leaves than the drought-avoider Local. Ali Dib *et al.* (1994) found that proline accumulation in wheat explained 59 % of the drought sensitivity index (DSI) in wheat suggesting that the capacity of a genotype to accumulate proline under stress with respect to the same genotype without stress, could give a good prediction of grain yield sensitivity to water stress, even if the physiological role of this aminoacid is not fully understood. No yield differences were observed in this experiment, which could be ascribed to the level of proline content, making it questionable as a selection criterion in these peanut cultivars.

In the pod-setting stage no significant changes in proline content were observed among the cultivars and treatments in accordance with the apparent insensitivity to drought stress of the cultivars at this stage, as was also shown by observations that no changes in RWC occurred.

At the maturity stage proline continued to accumulate under stress conditions (Figure 6.2). At this stage there was a strong and negative relationship between proline content and RWC ($r = 0.97$ at $P<0.05$), and a strong positive relationship between proline content and RSD ($r = 0.97$ at $P<0.05$), an indication that a low water content was associated with a high proline content. This relationship, which is contrary to the results at the vegetative stage, was used to question the use of proline content as a selection marker in durum wheat (Ali Dib *et al.*, 1994). On the other hand, the continuous increase in proline content even in the well-watered plants makes clear that it is a result of several growth factors.

Summarizing, the cultivar Falcon accumulated substantially more proline under stress conditions than the cultivar Local (24 times more in the initial stages, versus 9 times for the cultivar Local), in accordance with our previous supposition (Chapter 5) that the drought insensitivity of this cultivar, at least in the vegeta-

tive stage, may be linked to a mechanism of maintaining a positive turgor potential during prolonged water stress.

The results of the vegetative and pod-setting stages support the suggestion that a high level of proline in peanut leaves may indicate drought-resistance, as proposed by Singh and Paleg (1972) in barley, Karamanos (1983) in wheat and beans, and Ali Dib *et al.* (1994) in durum wheat; but it contradicts results of Andrade *et al.* (1995) who found that drought-susceptible *Phaseolus vulgaris* genotypes accumulated more proline than the drought-resistant ones. Premachandra *et al.* (1995) also found that in sorghum the contribution of osmotic adjustment and the rate of increase of proline with decreasing water potential were greater in the drought-susceptible line. The physiological significance of proline accumulation in stressed plants is not clearly understood. It has been described as a symptom of injury (Hanson *et al.*, 1979; Ibarra-Caballero *et al.*, 1988), playing a role in osmotic adjustment (Handa *et al.*, 1986; Hu *et al.*, 1992; Delauney and Verma 1993) or in the storage of C and N for stressed tissues (Singh and Paleg 1972; Purvis and Yelenosky 1982), which can be used again during recovery from stress and in involvement in cell osmoregulation and protection of proteins during dehydration (Sheyakova 1984).

4.3. Drought stress and cell membrane integrity

Cell membrane integrity (CMI), measured as electrolyte leakage in the initial stages of growth, showed a higher percentage of injury in the cultivar Local under both controlled and drought stressed conditions (Table 6.3) than in cultivar Falcon. Few authors have reported CMI in peanut crop.

Venkateswarlu and Ramesh (1993) showed, that the cell membranes of a drought-tolerant peanut cultivar suffered much less injury than those of a drought-susceptible one and that the differences were more marked in cultured cells.

Vasquez-Tello *et al.* (1990) indicated, that an important strategy for drought resistance is the maintenance of membrane integrity after water stress. Cultivar Falcon appeared to be drought-tolerant confirming our previous findings in Chapter 5. In contradiction to these results Deb *et al.* (1996), working with four *Arachis* accessions, found that sensitive accessions which retained a higher proline content suffered more injury to its membranes under stress. However, the molecular mechanism, underlying this effect of proline, is not completely understood. In the present study although no significant relationship between CMI and proline content was found (Premachandra *et al.*, 1992), the cultivar that accumulated more proline in the leaves, the cultivar Falcon, suffered relatively less membrane injury (Table 6.3).

Many other authors have reported a direct relation between CMI and osmotic adjustment (e.g. Rudolph *et al.*, 1986), but in the present study this relation was

not found particularly at the maturity stage. However, as the pods had developed, the cell membrane injury did not reduce the final yield.

4.4. Drought stress and nitrogen and phosphorus content

The pattern of N content in the two cultivars was not uniform. The general tendency was an increase of N content under stress conditions, as reported by Plies-Balzer *et al.* (1995) in stressed *Vicia faba* plants. This increase may be a result of an increased proline content under drought stress (Figure 6.2), since proline is believed to act as N source during recovering from stress (Fukutaku and Yamada 184). The reported negative effects of water shortage on nitrogenase activity did not result in a nitrogen deficiency of the host plant. This is in agreement with our previous results (Chapter 5) showing that growth was more sensitive to water stress than symbiotic N_2-fixation. Despite this tendency, which disappeared for cultivar Local at the end of the experiment, the levels of N in the two peanut cultivars were below the ones considered adequate for peanut, which is 3.5 % (Cox and Perry 1989). While drought did not negatively affect N content of the leaves, it reduced the P content with 15 % to 45 % in the pod-setting and maturity stage, respectively, in relation to the internal concentrations considered adequate (which is 0.2 %, see Cox and Perry 1989). It seemed therefore, that, as found in Chapters 3 and 4, low P may exert a more limiting influence on peanut growth and yield, making the assocation with arbuscular mycorrhizal fungi (AMF) of great importance.

5. CONCLUSION

From the results of this study it is concluded that: (i) cultivar Falcon exhibited characteristics of drought-tolerance as found in previous Chapters, and (ii) RWC, CMI and proline content were useful parameters to detect drought stress in peanut plants, but the pattern of change was much dependent on the growth stage.

7 Effects of arbuscular mycorrhizal (AM) inoculants on root colonisation and growth of two peanut cultivars in non-sterile Mozambican soil

SUMMARY

Inoculation of the peanut cultivars Local (L) and Falcon (F), grown for 13 weeks in 12 l ceramic pots lined with plastic bags, with the multiple-species arbuscular mycorrhizal (AM) inoculants "Soil Mozambique" and "Hannover", resulted in a significantly higher mycorrhizal colonisation for cultivar Local at the vegetative stage and in all growth stages for cultivar Falcon. At the reproductive stage, leaf number, leaf dry weight, and leaf area ratio (LAR) were significantly increased by the application of both inoculants, while other growth parameters showed a dependence on the inoculant. The genotypic variation in root colonisation indicated that the low and high yielding cultivars, Local and Falcon, respectively, preferred different AM fungal strains. The cultivar Falcon was more dependent on the symbiosis. These results indicate that inoculation with efficient AM inoculants can be an adequate strategy to improve peanut growth and yield, when the indigenous AM potential is low.

1. INTRODUCTION

Peanut can form two types of symbiotic associations with microorganisms, one with *Bradyrhizobium* involving N_2-fixation and with arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi (AMF) are one of the few plant fungus associations with a fossil record and AMF may even have facilitated the origin of land flora (Verma 1998). Mycorrhizas are a universal symbiosis, being 95 % of all terrestrial higher plant species characteristically mycorrhizal. Arbuscular mycorrhiza is the most common type appearing in all major crop species (Hooker and Black 1995).
Enhanced phosphorus nutrition, via an improved soil exploitation by the fungal mycelium is one of the benefits to host plants of colonisation by AMF (Harley and Smith 1983). Effects on phosphorus nutrition are particularly large because of (i) the poor mobility of the phosphate ion in soils, (ii) the rapid P fixation in many soils and (iii) utilisation of insoluble rock phosphate by fungi. There is also evidence of enhanced uptake of nitrogen (Barea *et al.*, 1989), particularly in conditions where nitrate is not readily available in sufficient quantities and where ammonium, which is much less mobile, is the dominant form.
Other nutritional benefits from AM include an enhanced uptake of potassium, sulphate, copper and zinc (Barea *et al.*, 1991). AMF also influence other growth promoting soil microorganisms in the rhizosphere. Nodule production and N_2-fixation by *Bradyrhizobium*, increase with mycorrhization, mainly due to increased phosphate uptake (Linderman 1992). Plants, which bear N_2-fixing nodules, are usually mycorrhizal, when grown in soil. This fact has great ecological relevance, because nodulation and N_2-fixation depend on a balanced nutrition of the host plant. They have a particularly high phosphate requirement, and the mycorrhiza can satisfy these demands. Due to this synergistic nutritional effect, nodulated and mycorrhizal plants are adapted to cope with nutrient deficiency and other stress situations (Smith and Read 1997).
AM fungi are also the soil organisms that most directly contribute to mineral uptake by plants, thus playing an important role in the soil-plant nutrient cycling (Hooker and Black 1995). Other mycorrhizal effects include formation and maintenance of soil structure (Miller and Jastrow 1992), protection against pathogens, among which are soil-borne root pathogens (Zambolim and Schenk 1983) and nematodes (Elliot *et al.*, 1984).
In the sustainable agriculture of the future, chemicals tend to be less important than biological land management practices and arbuscular mycorrhizas will play a crucial role, since (i) they are extremely widespread, existing in most of the common crop plants, (ii) they play multiple roles in the soil-plant system (Hoffman and Carroll 1995), and (iii) increase resistance of plants (Ruiz-Lozano and Ascón 1995).

Caution should however be exercised in interpreting the results of short-duration (less than the maturity period) or single-harvest experiments on host-fungal interrelationships, as the effects of different AM fungal isolates (even belonging to the same species) on growth and nutrition of legumes differ through the season (Ahiabor and Hirata 1994).
AM fungi can adapt to both low and high levels of soil nutrients. Lambert *et al.* (1980) compared the performance of several AM fungi in soils with low levels of extractable P and found that plant yield was always greatest when the inoculum used was indigenous to the soil in which the plants were grown. Peanut is a plant without root hairs, which suggests that its dependence on mycorrhiza for nutrient uptake will be high (Baylis 1970).
The objective of this study was to elucidate the response of two peanut cultivars differing in their dry matter partitioning pattern, to inoculation with mycorrhizal fungi. The experiment was carried out as a pot experiment using non-sterile Mozambican soil, collected at the experimental farm, of the Faculty of Agronomy and Forestry of the Eduardo Mondlane University in Maputo and one obtained from the Institute for Plant Diseases and Plant Protection in Hannover, Germany. In this way effects of indigenous and commercial mycorrhizal fungi on a traditional Mozambican cultivar Local, a cultivar with a relatively low yield and a modern high yielding cultivar Falcon can be compared.

2. MATERIAL AND METHODS

2.1. Plant material

The cultivars of peanut (*Arachis hypoagaea* L.) used (Local and Falcon) and the soil characteristics are described in Chapters 2 and 5, respectively.

2.2. Growth conditions

The plants were grown in a plant nursery in Maputo-Mozambique (25° 28' S and 32° 36' E), from February to May 1999, under conditions similar to those described in Chapter 5. A soil collected from the field was used, without any additional mineral fertilisation. The plants were irrigated with tap water to field capacity, which was controlled with a Thermal Domain Reflectometer (TDR).

2.3. Inoculum production

Mozambican soil (Chapters 5 and 6) was collected from the topsoil to a depth of 20 cm, including roots. The characteristics of this soil are described in Chapter 5. The same soil was used as the substrate for the experiments, performed and described in Chapters 5 and 6 of the present thesis. This soil was used as a source

of AM fungal propagules (start inoculum) for mass production of inoculum (Feldmann and Idczak 1992).
In a first step corn (*Zea mays* L.) plants were grown in small pots with a mixture of sand/vermiculite and Mozambican soil. These so-called "trap cultures" were checked after 5 weeks for AM root colonisation. Colonised plants were transferred to the mass production system.
Broken expanded clay (Lecadan) with a particle size of 4-8 mm was used as growth substrate and inoculum carrier material (Dehne and Backhaus 1986). It was washed and sterilised for 2 hours at 130 °C and filled in 5 l plastic pots, which were soaked overnight in 10 % sodium hypochlorite solution and then washed under running water for 10-20 min. Four mycorrhizal corn plants were planted in each pot and thoroughly watered. The pots were kept in a growth chamber with an air temperature of 25/20 °C day/night, relative humidity of 70 % and a light intensity to a maximum of 50000 lux for 12 h. The cultivation conditions included daily watering with distilled water and twice a week with a commercial liquid fertilizer "Wuxal NPK 12:4:6." When visible symptoms of deficiency appeared, the plants were fertilized more often. Once a month the plants were watered with an added solution of fungicide "Previcur N" (0.1 %), which is selective for oomycetes such as *Pythium*, an important plant pathogen which can occur in the substrate during inoculum production (Feldman and Idczak 1992) and does not affect mycorrhizal fungi.
In all following inoculum production cycles the host plant corn (*Zea mays* L.) was directly grown in 5 l pots, containing sterilized expanded clay with a layer of expanded clay inoculum (from a preceding production cycle) of about 10 % of the total pot volume. Untreated corn seeds were soaked in 1 % sodium hypochlorite solution for 10 min and subsequently rinsed under running water for another 10 min. They were pre-germinated in a germination chamber for 48 hours at 23 °C. Five seedlings with a 1-2 cm radicle were planted in each pot and covered with a 1 cm layer of expanded clay. Evaporation from the surface of the substrate during the first week was reduced by covering the pots with aluminium foil with small holes. After about 4 weeks root colonisation was checked.
After 3 months, irrigation was stopped and one week later plants were cut at the substrate level. The pots were allowed to dry for another 2 to 3 weeks. This procedure gives the formed spores time to mature. The expanded clay was sieved and coarse roots were removed. The inoculum was collected in paper bags and kept at room temperature under dry conditions until use. The inoculum will be further referred to as "Soil Mozambique" (SM)-inoculum.
The other inoculum used, in this study, was purchased at the company Mycotec Biotechnik in Hannover, Germany, which is associated to the Institute of Plant Diseases and Plant Protection of the University of Hannover. The commercial

name of this inoculum is "Mycoplant". It is produced using a similar procedure as described above with expanded clay 2-4 mm particle size and *Tagetes erecta* as a host plant, to diminish nematode attacks.
Mycoplant 510 *Glomus intraradices* (Schenk & Smith), Mycoplant 139 *Glomus etunicatum* (Becker & Gerdemann) and Mycoplant 49 (*Glomus intraradices*) were thoroughly mixed at a ratio of 1:1:1 prior to use. This inoculum will be further referred to as Hannover (H)-inoculum.

2.4. Inoculation

The mycorrhizal inoculant was mixed into the soil, at a ratio of 5 % of the pot volume, 2 cm below the surface before sowing. Non-inoculated plants served as controls.

2.5. Mycorrhizal parameters

The number of spores of mycorrhizal fungi in the soil was estimated after wet sieving and decanting of 50 g soil in 3 repetitions (Daniels and Skipper 1982).
Mycorrhizal root colonisation was determined after clearing the roots with 5 % KOH and staining with Trypan blue (Koske and Gemma 1989). Fourty root pieces of about 1 cm were randomly subsampled from the pooled roots of each treatment. They were mounted on slides and analysed under a compound microscope, at a magnification of 100 x.
The percentage of the root cortex colonised (M %) was estimated using the five class system, and calculated using the formula of Trouvelot *et al.* (1986):

0: no infection
1: trace
2: <10% of cortex colonised
3: 11-50% of cortex colonised
4: 51-90% of cortex colonised
5: >90% of cortex colonised

$$M\% = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / n,$$

Where n = number of observed root fragments
n_5, n_4, n_3, n_2, n_1 = number of fragments rated 5,4,3,2,1, respectively.

2.6. Growth analysis

Plants were harvested at week 4, 10 and 13, corresponding to the vegetative stage, flowering and pod-setting stage and maturity stage, respectively using 8 plants for each harvest. For the growth analysis the same procedure was used as in Chapters 2, 3, 4 and 5.

2.7. Data analysis

Differences in growth parameters, between the treatments, were analysed with the Student t-test. Differences in mycorrhizal colonisation were analysed with the Mann-Whitney U non-parametric test (Daniel 1978).

3. RESULTS

3.1. Phenological development

No significant differences were found in relation to time needed for full emergence and to 50 % of flowering. The inoculated plants showed greener leaves than the control plants, indicating that N nutrition of the plants was improved (results not shown).

3.2. Mycorrhizal colonisation

Cultivar Local (Figure 1a) showed a high indigenous mycorrhizal colonisation of up to 47 % (10 weeks after planting) growing without inoculation. In the pod setting stage (10 weeks after planting) and maturity stage (13 weeks after planting) there was no significant difference in percentage root colonisation between the non-inoculated control (LC) and both inoculated treatments (LSM and LH). Only at the vegetative stage (4 weeks after planting) there was a significant difference between LC and LSM (133 %) ($p = 0.003$) but not between LC and LH (49 %) ($p = 0.58$).

Cultivar Falcon (Figure 1b) showed a lower indigenous mycorrhizal colonisation of the non-inoculated control (20 %) than cultivar Local (47 %), particularly at week 10 ($p = 0.0001$). In all growth stages the percentage root colonisation in non-inoculated plants did not rise above 19 %. There was a significant effect of both inoculants (SM and H) on the percentage root colonisation, which was apparent already after 4 weeks growing (vegetative stage, 51% and 166 % for Soil Mozambique and Hannover inoculant, respectively) and more pronounced at the pod-setting and maturity stages, 148 % and 326 %; 119 % and 190 %, for the Soil Mozambique and Hannover inoculants, respectively.

The Hannover inoculant resulted at all growth stages in the highest root colonisation, up to 63% in the pod setting stage, compared with 20 % and 48 % for the non-inoculated and inoculated with Soil Mozambique plants. The difference with Soil Mozambique inoculant, however, was not statistically significant at any of the growth stages.

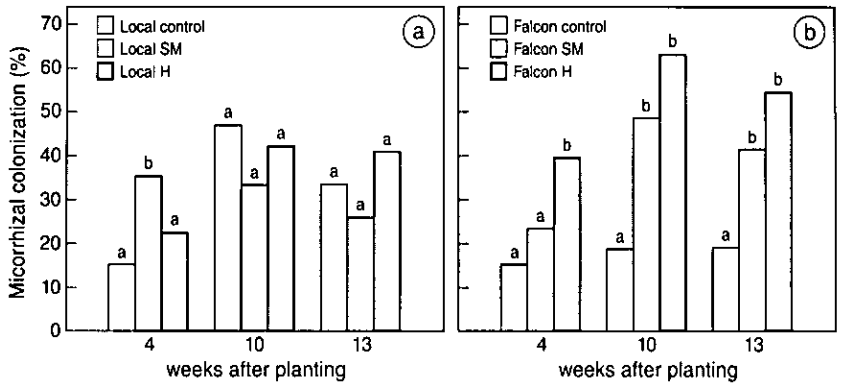

Figure 7.1 Effect of AM inoculation on mycorrhizal colonisation of the peanut cultivars Local (a) and Falcon (b). LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Values represent determinations in 40 root pieces randomly taken from the pool of 8 plants per treatment. Different letters at a particular growth stage denote values that differed significantly at $P<0.05$ after a Mann Whitney U non-parametric test.

3.3. Number of AM fungal spores

At the vegetative stage and at the pod-setting stage, inoculated pots had a lower number of spores than the non-inoculated controls (Table 7.1).

At the maturity stage inoculated pots had a higher number of spores. The number of spores increased in control pots from the vegetative to the pod-setting stage and then decreased towards the maturity stage. In inoculated pots the number of spores increased continuously towards the maturity stage. With cultivar Local the spore numbers at the maturity stage were significantly higher in the inoculated pots than in the control pots. With the cultivar Falcon this difference was less pronounced and not statistically significant (Table 7.1).

3.4. Leaf and root growth

Inoculation with AM fungi resulted in an increased leaf area, leaf number and leaf dry weight in both cultivars (Table 7.2).

Cultivar Local showed a slight increase of leaf area at the pod-setting stage (20 %) and a significant increase at the maturity stage (50 %) only with the Hannover inoculant. Leaf number responded significantly only at the vegetative stage when inoculated with the Soil Mozambique inoculant (39 %). Leaf dry weight did not significantly benefit from inoculation at all growth stages. In contrast to leaf dry weight, leaf area ratio responded at the pod-setting stage by a 22 % and 15 % increase, when inoculated with Soil Mozambique and Hannover

Table 7.1 Effect of AM inoculation on the number of AM fungal spores in the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Dap, are days after planting. Values are means of 3 repetitions. Within column, per cultivar, values followed by the same letter are not significantly different at $P<0.05$, using the Student t-test.

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Number of spores	
LC	63.0b	113b	62b
LSM	26.6a	77a	138a
LH	24.3a	58a	128a
FC	87.0b	127b	83a
FSM	29.0a	58a	114a
FH	37.3a	97b	117a

inoculants, respectively, while at the maturity stage an increase was only observed when plants were inoculated with Hannover inoculant (76 %). Leaf weight ratio (LWR) was significantly increased at maturity stage only, when inoculation with Hannover inoculant was applied.

Inoculation with Soil Mozambique inoculant lead to a significant increase of specific leaf area (SLA) by 10 % at the vegetative stage and to a decrease at the maturity stage (11 %); specific leaf weight (SLW) showed a significant decrease (11 %).

Although root dry weight did not show a significant response, root/shoot ratio (R/S) and root weight ratio (RWR) showed a significant decrease with Soil Mozambique inoculant at the vegetative stage (35 % and 38 %, respectively) and with Hannover inoculant at the maturity stage (Table 7.2).

In cultivar Falcon (Table 7.2), in contrast to cultivar Local, the leaf area already benefited significantly from inoculation at the pod-setting stage, when inoculated with both inoculants: 38 % for Soil Mozambique inoculant and 56 % for the Hannover inoculant. At the maturity stage inoculation increased leaf area by 44 % and 56 % for Soil Mozambique and Hannover inoculant, respectively. Similarly, the leaf number did not show a substantial response at the vegetative stage, but it responded significantly in the subsequent stages with maximum values at the maturity stage. Inoculation with AM fungi increased leaf number by about 60% in both treatments, at this stage.

As with cultivar Local, leaf dry weight of cultivar Falcon (Table 7.2) did not show a substantial response at the vegetative stage. At the pod-setting and maturity

Table 7.2 Effect of AM inoculation on the leaf and root growth parameters of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. LA, LN, LDW, LAR, LWR, SLA, SLW, RDW, R/S and RWR, is leaf area, leaf number, leaf dry weight, leaf area ratio, leaf weight ratio, specific leaf area, specific leaf weight, root dry weight, root shoot ratio and root weight ratio, respectively. Dap, are days after planting. Values are means of 8 plants. Within columns, per growth stage, values followed by the same letter are not significantly different at $P<0.05$, using the Student t-test.

Treatment	LA (cm^2)	LN	LDW (g)	LAR ($cm^2.g^{-1}$)	LWR ($g.g^{-1}$)	SLA ($cm^2.g^{-1}$)	SLW ($g.g^{-1}$)	RDW (g)	R/S ($g.g^{-1}$)	RWR ($g.g^{-1}$)
	Vegetative stage (28 dap)									
LC	203a	11.0b	0.78a	143a	0.551a	261a	0.038a	0.09a	0.079a	0.071a
LSM	267a	15.3a	0.93a	154a	0.534a	288b	0.034b	0.08a	0.049b	0.050b
LH	256a	13.1b	0.99a	149a	0.570a	263a	0.038a	0.09a	0.059a	0.054a
	Pod setting stage (77 dap)									
LC	680a	35.8a	3.80a	65a	0.382a	174a	0.059a	0.21a	0.034a	0.021a
LSM	735a	34.2a	3.91a	80b	0.422a	188a	0.052a	0.21a	0.036a	0.030a
LH	822a	41.0a	5.50a	75b	0.375a	200a	0.050b	0.24a	0.029a	0.017a
	Maturity stage (91 dap)									
LC	689a	34.2a	4.00a	53a	0.300a	177a	0.056a	0.22a	0.033a	0.020a
LSM	736a	37.4a	4.72a	54a	0.342a	158b	0.064b	0.25a	0.030a	0.020a
LH	1037b	39.6a	5.20a	94b	0.511b	182a	0.055a	0.25a	0.033a	0.030b
	Vegetative stage (28 dap)									
FC	218a	12.0a	0.88a	144a	0.584a	250a	0.040a	0.07a	0.065a	0.059a
FSM	268a	14.4a	1.03a	148a	0.566a	260a	0.038a	0.11b	0.060a	0.060a
FH	262a	12.2a	1.01a	151a	0.579a	261a	0.038a	0.12b	0.082a	0.073b
	Pod setting stage (77 dap)									
FC	530a	25.6a	2.90	77a	0.402a	192a	0.052a	0.12a	0.034a	0.022a
FSM	733b	34.0a	4.34b	63a	0.374a	168b	0.059a	0.22a	0.029a	0.020a
FH	828b	39.6b	5.10b	60b	0.387a	157b	0.065a	0.23b	0.036a	0.023a
	Maturity stage (91 dap)									
FC	588a	24.0a	3.54a	63a	0.384a	165a	0.060a	0.24a	0.038a	0.025a
FSM	848b	39.0b	5.10b	62a	0.377a	164a	0.061a	0.25a	0.025b	0.018b
FH	920b	38.5b	5.30b	58a	0.330a	175a	0.057a	0.25a	0.021b	0.013b

stages inoculation with both Hannover and Soil Mozambique inoculants lead to a significant increase of leaf dry weight (38 % and 56 % at the pod-setting and 44 % and 56 % at the maturity stage, for the Soil Mozambique and Hannover inoculants, respectively). As with cultivar Local, LWR and SLW, did not show significant differences (Table 7.2). However, SLA was decreased by 13 % and 18 %

when plants were inoculated with Soil Mozambique and with Hannover inoculants at the pod-setting stage, respectively (Table 7.2).
In contrast to cultivar Local, root dry weight was significantly increased by 45 % and 61 % in cultivar Falcon at the vegetative stage, when plants were inoculated with Soil Mozambique and Hannover inoculant, respectively. At the pod-setting stage the increase was 87 % and 115 % respectively, however statistically significant only for inoculation with Hannover. At the maturity stage no substantial differences between treatments were observed. The root partitioning parameters RWR and R/S of the cultivar Falcon did not follow this pattern (Table 7.2). RWR was significantly higher at the vegetative stage (24 %), when plants were inoculated with Soil Mozambique inoculant and both RWR and R/S were significantly lower at the maturity stage, when plants were inoculated.

3.5. Number of pegs and pods

The number of pegs and pods, also named total sink number (TSN), responded to inoculation in both cultivars, though not always significantly at the pod-setting stage and maturity stage (Table 7.3). The Hannover inoculant was more effective on TSN than the Soil Mozambique inoculant, but only significant with cultivar Falcon.

Table 7.3 Effect of AM inoculation on total sink number (TSN) of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Dap, are days after planting. Values are means of 8 plants. Within columns values of the same cultivar followed by the same letter are not significantly different at the $P<0.05$, using the Student t-test.

Treatment	Pod-setting stage (77 dap)	Maturity stage (91 dap)
	TSN	
LC	28.6a	40.0a
LSM	37.5a	39.5a
LH	38.3a	45.6a
FC	20.0a	27.6a
FSM	26.1a	32.0a
FH	35.6b	37.6a

3.6. Nodulation

In general, N_2-fixation measured as the number of nodules showed a direct relationship with mycorrhizal inoculation (Table 7.4 and Figure 7.1).

In the vegetative stage cultivar Local showed a significant increase in nodule number (392 and 113 %) with Soil Mozambique inoculant and with Hannover inoculant, respectively, and at the pod-setting stage only with Hannover inoculant (39 %). A significant reduction was observed at this stage, with Soil Mozambique inoculant. No substantial responses were found with both of the inoculants at the maturity stage.

Cultivar Falcon did not show any significant response at the vegetative stage. At the pod-setting stage, however Soil Mozambique and Hannover inoculant lead to an increase in nodule number of 79 % and 206 %, respectively. At the maturity stage this effect was less pronounced with only Hannover inoculant showing a significant increase (91 %).

3.7. Dry matter accumulation

No significant differences in dry matter accumulation between treatments were found in both cultivars at the vegetative stage (Table 7.5).

Cultivar Local showed at the vegetative stage a noticeable but not significant increase in dry matter with both types of mycorrhizal inoculation. Cultivar Falcon responded to inoculation at the pod-setting stage by a significant 55 % and 86 % increase in total dry matter, when inoculated with Soil Mozambique and

Table 7.4 Effect of AM inoculation on the nodulation of two peanut cultivars Local and Falcon. LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Dap, are days after planting. Nodule numbers are means of 8 plants. Within columns values of the same cultivar followed by the same letter are not significantly different at the $P<0.05$, using the Student t-test.

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Number of nodules	
LC	14.6a	82.8a	55.0a
LSM	72.0b	62.3b	54.5a
LH	31.2b	115.5b	66.6a
FC	27.8a	34.0a	47.5a
FSM	27.8a	60.8b	69.5a
FH	22.7a	104.2b	91.0b

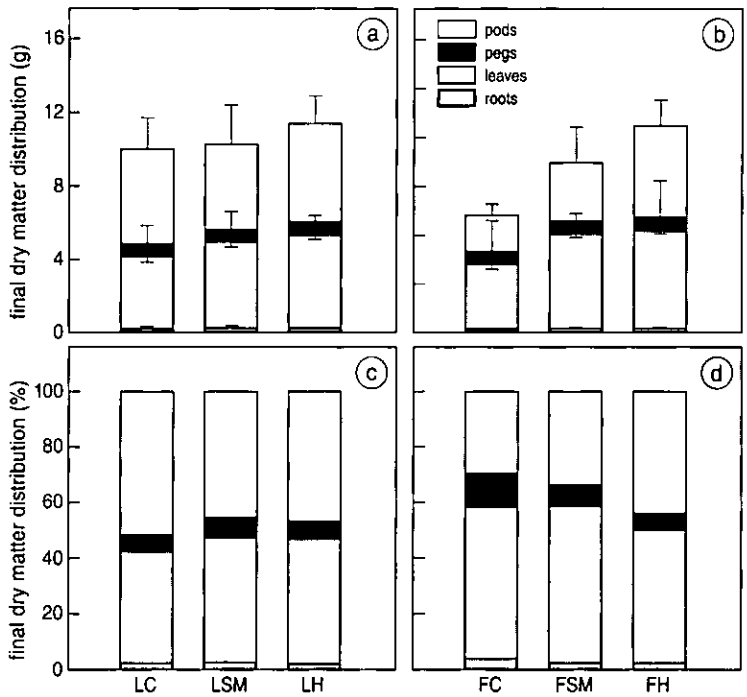

Figure 7.2 Effect of AM inoculation on final dry matter distribution of two peanut cultivars Local (a and c) and Falcon (b and d). LC and FC are the non-inoculated cultivars Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Values are means of 8 plants (± SD)..

Table 7.5 Effect of AM inoculation on dry matter accumulation of two peanut cultivars Local and Falcon. LC and FC are the non-inoculated controls Local and Falcon; LSM and FSM, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and LH and FH are the cultivars Local and Falcon, inoculated with Hannover inoculant, respectively. Dap, are days after planting. Values are means of 8 plants. Within columns values of the same cultivar followed by the same letter are not significantly different at the $P<0.05$, using the Student t-test.

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Dry matter (g)	
LC	1.4a	10.5a	13.0a
LSM	1.8a	9.4a	13.9a
LH	1.7a	8.7a	13.8a
FC	1.5a	7.4a	9.3a
FSM	1.8a	11.5b	13.8b
FH	1.7a	13.8b	15.1b

Hannover, respectively. A similar pattern was observed at the maturity stage, with Hannover inoculant accounting for the higher response (62 %), and Soil Mozambique for a lower response (47%).
The distribution of dry matter to the different plant organs was also influenced by mycorrhizal colonisation. In cultivar Local there was a tendency to increase peg and leaf dry weight by inoculation, but a decrease in pod dry matter occurred, particularly with Soil Mozambique inoculant (Figures 7.2a and c). In cultivar Falcon, the positive effect of both inoculants in final dry matter was substantial, particularly for pod and leaf dry weight (Figure 7.2b and d).

3.8. Summary of significant effects

The summary of all results indicates a more pronounced and positive response of both peanut cultivars to arbuscular mycorrhizal (AM) inoculants at the reproductive stage (Table 7.6).

Table 7.6 Summary of the significant responses of peanut growth and mycorrhizal parameters to AM fungal inoculation. – indicates no significant response, while SM or H, indicates a significant response of the parameter, when inoculated with Soil Mozambique or Hannover inoculant, respectively.

	Vegetative stage		Reproductive stage	
Parameter	Cultivar		Cultivar	
	Local	Falcon	Local	Falcon
Mycorrhizal colonisation	SM and H	SM and H	-	SM and H
Leaf area	-	-	H	SM and H
Leaf number	SM	-	-	SM and H
Leaf dry weight	-	-	SM and H	SM and H
Leaf area ratio	-	-	SM and H	SM and H
Specific leaf area	-	-	-	H
Root dry weight	-	SM and H	-	H
Root shoot ratio	-	-	-	H
Root weight ratio	-	-	H	SM and H
Nodulation	SM	-	H	SM and H
Total dry weight	-	-	-	SM and H

4. DISCUSSION

4.1. Mycorrhizal parameters

4.1.1. Root colonisation

An inoculant-cultivar dependent difference in mycorrhizal colonisation could be observed. The cultivar Local, a landrace frequently grown under the soil conditions in the experimental pots, seemed to be more compatible with the local (indigenous) AM fungal community. This was indicated by the high "background" root colonisation determined in the non-inoculated control, which at later growth stages reached the same levels as in the inoculated treatments (Figure 7.1a). Further, there was a better colonisation of this cultivar by the Soil Mozambique inoculant obtained from Mozambican soil (indigenous) than by the Hannover inoculant (foreign) at an early growth stage (4 weeks after planting). At later growth stages the differences between the treatments almost disappeared. The percentage root colonisation never rose above 50 %.

In contrast, the cultivar Falcon, a cultivar bred under a high input agriculture system and selected for a high yield, showed a substantial response to inoculation, particularly with the Hannover inoculant at all growth stages (Figure 7.1b). Percentage root colonisation of the non-inoculated plants was lower than with cultivar Local, whereas the level of root colonisation in inoculated treatments was higher, rising above 60 %. A genotypic variation in root colonisation, as observed with the cultivars Local and Falcon when inoculated with Soil Mozambique and Hannover inoculants, has been suggested to result from specific interactions between host plant genotype and AM fungal strain preferences (Mosse 1980; Ruiz-Lozano *et al.*, 1995) in such a way that in the present experiment the low and high yield cultivars apparently associate with different AMF strains.

4.1.2. Spore number

Four weeks after planting there were significantly more spores in the non-inoculated treatments of both cultivars than in the inoculated ones (Table 7.1). In all the treatments, the number of spores increased substantially towards later growth stages, with the difference, that non-inoculated pots showed a peak of maximum spore numbers at the pod-setting stage, whereas in both inoculated treatments the highest spore numbers were found at the maturity stage. Thus, with increasing mycorrhizal root colonisation more spores were formed. However, the "back-ground" AM fungal population as apparent in the non-inoculated treatments, seemed to produce spores earlier than the inoculated fungi, although the percentage root colonisation was not higher in non-inoculated plants (Figure 7.1). This indicates that root colonisation of the inoculated plants was of a different

nature than that of the non-inoculated plants. Different AMF species seemed to be involved, which sporulate at a different stage of plant development (Smith and Walker 1981), resulting in specific peanut cultivar-AM-strain interactions.
Inoculation with AM fungi did not increase the number of spores at the vegetative stage, in contrast to the results of Tarafdar and Rao (1997) in clusterbean, mung bean and moth bean and of Krishna and Bagyaraj (1984) in sterilised soils with peanut. However, spore production and root colonisation need not always to be correlated (Khalil *et al.*, 1992). At the maturity stage inoculation with AM fungi clearly stimulated the number of spores, in accordance with the observations of Tarafdar and Rao (1997). The numbers of spores found, even in the pod-setting stage, are above the level considered low by Sieverding (1991) in Colombian soils, which is 125 spores/100g soil.

4.2. Plant growth parameters

Inoculation with both inoculants did not influence the time to 50 % flowering, suggesting that either flowering of peanut was not dependent on nutrients and water, provided by the symbiosis, or the low level of mycorrhizal colonisation at the vegetative stage prevented differences in the flowering pattern. Another explanation could be that time of flowering is genetically strongly fixed.
A significant increase in leaf area was observed with inoculated plants of both cultivars at the pod-setting and maturity stages in both cultivars. This resulted from an increased leaf expansion *per se*, or was associated with increased leaf number, particularly in cultivar Falcon at the pod-setting and maturity stages (Table 7.2). The increase in leaf area allowed plants to intercept more light and to increase the radiation-use efficiency, resulting in an increased total sink number (TSN). An increase in leaf area has been ascribed to increased fungal nutrient uptake and transport from the soil to the plant (Kling and Jakobsen 1998; Van den Boogaard *et al.*, 1996) and increased photosynthesis (Wright *et al.*, 1998).
Leaf dry weight increased significantly with AM inoculation in cultivar Falcon at the pod-setting stage and maturity stage. This corresponds with the high mycorrhizal colonisation, observed at this growth stage (Figure 7.1b). Leaf dry weight in cultivar Local also tended to increase with inoculation, however not significantly. This could be explained with the higher "background" at later growth stages and the better "compatibility" of cultivar Local with the indigenous flora (Figure 7.1a). The benefit of mycorrhizal colonisation on leaf growth has been linked with an increased cytokinin activity, which has been found in arbuscular mycorrhizal plants or in their nodules (Baas and Kuiper 1989; Goicoechea *et al.*, 1996).
Root growth increased in inoculated plants of cultivar Falcon at the vegetative and pod-setting stage, while the partitioning components root/shoot ratio (R/S) and root weight ratio (RWR) showed a changing pattern in both cultivars. A

simultaneous increase in leaf and root growth shows that mycorrhizal colonisation helps to maintain the equilibrium between root and shoot growth, which is normally changed to the benefit of root growth under nutrient and water stress (Chapters 4 and 5).

However, the increase in root growth in cultivar Falcon agrees with earlier findings, that the cultivar Falcon may be able to withstand short-term drought stress (Chapter 5), since high values of root weight have been associated with greater drought tolerance (Jordan and Miller 1980; Al-Karaki *et al.*, 1995). At the maturity stage root dry weight was only slightly stimulated by inoculation. As water uptake is increased by AM inoculation and plants were under well-watered conditions, it might be argued that increased root growth was not necessarily important for these AM-plants. This is further supported by the low investment in the roots, as shown by the low RWR and R/S (Table 7.2).

Since RWR was found to correlate negatively with RGR (Chapter 2), the results indicate that inoculation contributed to an increased relative growth rate (RGR). Furthermore, a lower R/S in inoculated plants, as observed in the present experiment has been found by other authors and may also indicate partitioning of carbon to the fungus at the expense of root production while maintaining an overall neutral effect on carbon allocation within the plant, as proposed by Smith and Gianinazzi-Pearson (1990). Non-mycorrhizal or low mycorrhizal plants on the contrary often show high R/S values (Kothari *et al.*, 1990) as found in the non-inoculated Falcon treatment (Table 7.2). This has been associated with the need of avoiding stress (Wahbi and Gregory 1989).

As with root colonisation, leaf and root growth response to inoculation, tended to be more pronounced in cultivar Falcon, suggesting a higher dependence of this cultivar on the symbiosis and/or a lower compatibility with the indigenous AM flora. On the other hand, the cultivar Local with a low yield, showed a less pronounced growth pattern and yield, due to the high background colonisation.

4.3. Nodulation

A low number of nodules was found at the vegetative growth stage, a peak at the pod-setting stage and a slight decrease at the maturity stage. This corresponded to the pattern observed with the mycorrhizal root colonisation, although no significant correlation was found between nodulation and mycorrhizal colonisation (Figure 7.1 and Table 7.4). Similar results have been reported in other legumes (Abdel-Fattah 1997). An increase in nodule number has been related to the positive influence of arbuscular mycorrhizal fungi (AMF) on N_2-fixation through improved P-uptake (Subba Rao *et al.*, 1986; Linderman 1992), and also through direct beneficial effects to the bacteroid symbiont in the nodular tissue. This has been used to explain the close relationship between growth stage, mycorrhizal

colonisation, plant growth, increased leaf P, nodulation and N_2-fixation (Ahiabor and Hirata 1994).

4.4. Dry matter accumulation and distribution

The significant increase in dry matter accumulation with inoculated plants at pod-setting and maturity stages, particularly in cultivar Falcon, clearly supported the relationship between dry matter accumulation and root colonisation, although this relationship was not statistically significant (Figure 7.2). Similar results have been reported in sunflower at late growth stages (flowering and maturity) by Chandrashekara *et al.* (1995), who found that the percentage root colonisation, spore number, total dry mass and total P uptake were significantly higher in inoculated plants than in non-inoculated control plants. As stated previously, the dry matter content also showed a high mycorrhizal dependence on inoculation with AMF in the cultivar Falcon, independent of the type of inoculant applied (Table 7.5).

Dry matter distribution to the different plant organs was also influenced by mycorrhizal colonisation, particularly in cultivar Local in such a way that pods were significantly increased by inoculation. Beneficial effects were observed on leaves, pods and pegs, particularly in cultivar Falcon, the cultivar with the highest values of mycorrhizal root colonisation (Figure 7.2b and d). However, no direct relationship could yet be established between dry matter distribution and mycorrhizal colonisation.

4.5. Summary of significant responses

Growth of peanut, despite genotypic differences, can be improved by inoculation with a combination of AMF-species in a non-sterilised soil. This combination induced statistically significant changes in a number of growth parameters, particularly at the pod setting and maturity stages (Table 7.5), while in the vegetative stage only a few growth parameters showed a significant response: leaf number, root dry weight and nodulation. A response at the vegetative stage is more likely if seed P reserve is low and in small-seeded species (Zhao *et al.*, 1997). In fact, the small-seeded cultivar Falcon (Chapter 2) showed a larger growth response at the vegetative stage than the large-seeded cultivar Local (Table 7.2). The delay in growth response, has been attributed to (i) a lag phase before the onset of rapid root colonisation, (ii) a masking effect of the seed nutrient content, and (iii) a competition between various symbionts for photosynthate (Smith 1988). The increased number of parameters responding significantly to inoculation at the reproductive stage as compared to the vegetative stage, confirmed the results reported for chickpea, that plants start to benefit from inoculation at the flowering stage (Weber *et al.*, 1993).

The lack of responses of some parameters of the cultivar Local to inoculation, particularly with Soil Mozambique inoculant, may have resulted from adaptation of the cultivar to the AM fungal community existent in the soil, as the cultivar Local is a landrace, frequently grown under the soil conditions existing in the pots, and SM inoculant was isolated from this soil.
The results of this experiment additionally showed, that the SM inoculant isolated under the conditions, described earlier, was, mutualistic effective and can be used in the next experiment. Simpson and Daft (1990) reported that some inoculants reduced host plant weight, suggesting in these cases a parasitic effect due to limited carbohydrate availability in the plant (Bethlenfalvay *et al.*, 1982). It was found in the present study, that the indigenous or native AM fungi existing in the soil have a lower potential of colonisation of cultivar Falcon than the inoculant AM fungi. This is in contrast to the results of Weber *et al.* (1993) who failed to increase the level of mycorrhizal infection in non-sterilised soil, due to the high indigenous inoculant potential. The root colonisation in the non-inoculated plants and the lack of mycorrhizal response by some growth parameters, showed that in non-sterile soils, the presumed benefits of mycorrhizal colonisation might be difficult to demonstrate because of interactions between introduced and indigenous AM fungi (Arines and Vilarino 1989).

5. CONCLUSIONS

The results obtained by inoculation, lead to the following conclusions: (1) the effect of AM inoculation on root colonisation and growth of peanut plants depended on the cultivar and inoculant used (2) the indigenous endophytes in the non-sterile soil were relatively slow to colonise roots, so that inoculation was beneficial, particularly with the high-input bred cultivar Falcon, (3) with cultivar Local the introduction of new endophytes encountered considerable competition from the indigenous mycorrhizal flora resulting in a smaller benefit for plant growth (4) the Hannover inoculant was more efficient that the Soil Mozambique inoculant, which might partly explained, by the higher difference to the indigenous AM flora, and partly by the better compatibility with the high-input/high-yield cultivar Falcon and (5) inoculation with multiple-species inoculants may promote productivity, since an amount of only 5 % (v/v) inoculant increased growth, nodulation and total sink number significantly.

8 Effects of arbuscular mycorrhizal (AM) inoculants on drought tolerance of two peanut cultivars-Minimizing the effects of drought stress by AM inoculation

SUMMARY

The effect of arbuscular mycorrhizal (AM) inoculation with two different inoculants on root colonisation and root and leaf growth was studied in two peanut cultivars Local and Falcon, grown for 13 weeks, in a non-sterile Mozambican soil with and without drought stress. The indigenous Soil Mozambique inoculant significantly increased leaf and root growth in both cultivars under drought stress conditions, while preventing an increase in root weight ratio (RWR), root shoot ratio (R/S) and maximum root length to leaf area ratio (MRLAR). The commercial Hannover inoculant increased growth only under well watered conditions. In general, there was a tendency of allocating more dry matter to the pods with inoculation, followed by leaves, while drought stress and non-inoculation increased dry matter allocation to the roots and delayed the formation of pods. Drought stress effects on growth could be alleviated by inoculation with Soil Mozambique inoculant.

1. INTRODUCTION

Drought and P deficiency are major factors affecting growth and yield of peanut. N deficiency does not show significant and negative effects on growth and yield (Chapters 3, 4 and 5).

Arbuscular mycorrhizal fungi (AMF) form a most common symbiosis with higher plants. Their positive influence on plant growth and development make AMF a potentially very useful biological resource of assuring plant production with a minimum input of chemical fertilisers and pesticides (Ortas 1996).

The mycorrhizal effects on host plant water relations are more controversial; though there is little doubt that AM alters plant water relations. Various authors reported that mycorrhizal plants showed improved plant osmoregulation due to increased K^+ and Cl^- uptake (Buwalda *et al.*, 1983; Augé *et al.*, 1986a), altered hormonal balance (Levy and Krikun 1980), visible signs of wilting at low water potential (Allen 1982), increased drought resistance (Nelsen and Safir 1982; Busse and Ellis 1985), improved stomatal control (Allen and Allen 1986; Augé *et al.*, 1986a), modified hydraulic conductance (Levy *et al.*, 1983; Graham and Syvertsen 1984; Hardie 1985), and greater ability to extract water at low soil moisture content (Hardie and Leyton 1981). Other authors refer no effect in leaf water status when AM plants were exposed to an increasing level of drought stress (Bryla and Duniway 1997) or to the fact that drought resistance was unaffected or even decreased by mycorrhiza (Simpson and Daft 1990a).

Despite these contradictory results AM fungi allow drought hardiness (Davies *et al.*, 1992) and they clearly increase the absorptive surface area for water and nutrient uptake (Ruiz-Lozano and Azcón 1995). Thus, mycorrhizae as mediator of stress tolerance in plants may be more important to plant growth under dry conditions than when soil moisture is adequate (Sanchez-Diaz and Honrubia 1994). This beneficial effect may occur through improved host nutrition, in particular with phosphorus (Fitter 1988; Trimble and Knowles 1995; Al-Karaki and Al-Raddad 1997) or independent of plant P concentration (Augé *et al.*, 1986b; Bethlenfalvay *et al.*, 1988).

Huang *et al.* (1985) reported that in *Leucaena leucocephala* mycorrhiza could benefit the plant through direct drought avoidance, i.e. enhanced water absorption by the mycorrhizal root system and reduction of water stress in plant growth. Davies *et al.* (1992) indicated that drought resistance in AM plants was not attributable to leaf P concentration or confounded with leaf size, but to more developed extraradical hyphae, which could facilitate water uptake during high drought periods. Thus, osmotic adjustment combined with more extraradical hyphae could promote drought resistance (Davies *et al.*, 1993).

Plants, colonised with arbuscular mycorrhizal fungi (AMF) generally have more

growth and increased acquisition of mineral nutrients and often have a greater ability to withstand drought than non-mycorrhizal plants (Al-Karaki and Clark 1998). Several plant species, whose roots were colonised with AM fungi, had improved drought tolerance in greenhouse studies (Bethlenfalvay *et al.*, 1988; Wang 1996) and in field studies (Sylvia *et al.*, 1993). Under severe drought stress, however, beneficial mycorrhizal growth responses were eliminated in corn (Hetrick *et al.*, 1984). Other authors suggested that amelioration of drought stress by AM fungi can be ascribed to specific physiological (CO_2-fixation, transpiration and water use efficiency) and nutritional (P-and K-uptake) mechanisms, according to the fungus species involved in the symbiotic association (Ruiz-Lozano and Azcón 1995).

In a previous experiment with peanut (Chapter 7) it was found that inoculation with AMF of peanut plants resulted in high root colonisation and increased dry matter, in particular in cultivar Falcon, suggesting that inoculation could promote the productivity of the peanut cultivars.

Plant growth responses to symbiotic AM root fungi have been related to factors such as AM isolate, plant species/cultivar and growing conditions (Bryla and Koide 1990; Jakobsen *et al.*, 1992; Ruiz-Lozano *et al.*, 1995b). If tolerance of plants to drought differs with the AM isolate with which the plant is associated (Ruiz-Lozano and Azcón 1995) it would be important to determine effective host-plant-AM fungal combinations for practical use in the field. *Bradyrhizobium* and AMF are promising inoculants for peanut since they improve oil and protein content of the seeds. Therefore, the seed quality of peanut could be improved by selecting a good cultivar and competitive, infective and effective *Bradyrhizobium*/AMF (Elsheikh and Mohamedzein 1998). However, information is limited about effective host plant-AM fungal combinations under drought conditions (Al-Karaki *et al.*, 1998).

The objective of this study was to compare the effects of AM inoculants of different origin on growth of two peanut cultivars grown under drought stress and well-watered conditions (control). The cultivars differed in their dry matter partitioning pattern (Chapter 2), drought tolerance strategies (Chapter 5) and degree of colonisation with AM inoculants (Chapter 7).

2. MATERIAL AND METHODS

2.1. Plant material and soil

Two peanut (*Arachis hypogaea* L.) cultivars, Local (L) and Falcon (F) were grown for 13 weeks in 12 l ceramic pots, lined with plastic bags and filled with soil, collected from the experimental farm of the Faculty of Agronomy and Forestry Engineering of the Eduardo Mondlane University. The soil was collected at the

Table 8.1 Physical and chemical characteristics of the soil, used in the experiment.

Parameters (Units)		Value
Sand (%)		93.20
Clay (%)		5.30
Silt (%)		1.40
pH (H2O)		6.60
pH (KCl)		5.60
Electrical conductivity($ms.cm^{-1}$)*		0.03
Phosphorus-Bray ($mg.100g^{-1}$)		0.29
Total N (%)		0.02
Carbon (%)		0.20
Organic matter (%)		0.35
Cation exchange capacity (meq.100g-1):	Ca^{2+}	0.40
	Mg^{2+}	0.60
	K^{+}	0.08
	Na^{+}	0.02

* Electrical conductivity (EC) was determined by diluting soil in distilled water at a ratio of (1:2.5, v/v) and measuring the EC of the solution.

same site as for the experiments described in Chapters 5, 6 and 7. The soil is classified as arenosol and the characteristics are given in Table 8.1.
The characteristics of the cultivars are presented in Chapter 2 (Table 2.1).

2.2. Growth conditions

The plants were grown in a plant nursery in Maputo-Mozambique (25° 28' S and 32° 36' E), from December 1999 to March 2000, under irrigation-controlled conditions. The mean air temperature during the growth period was: 30.0 ± 2 °C in the morning, 32.3 ± 2.0 °C in midday and 30.3 ± 2.0 °C in the afternoon.
The mean relative humidity of the air ranged from 63.0 ± 11.0 % in the morning, 54.0 ± 7.0 % in midday and 53.0 ± 1.3 % in the afternoon.
The illumination was by screened natural light resulting in an average photon flux density at the canopy level of 270.0 ± 10.0 $\mu mol.m^2.s^{-1}$ in the morning, 412.1 ± 96 $\mu mol.m^2.s^{-1}$ in midday and 103.0 ± 12.0 $\mu mol.m^2.s^{-1}$ in the afternoon, measured with a quantum sensor (SK P215, Skye Llandrindod Wells, UK). Drought stress was imposed as described in Chapter 5.

2.3. Inoculum production

Soil Mozambique (SM) inoculant was produced as described before and Hannover (H) inoculant was purchased at the company Mycotec Biotechnik in Hannover, Germany. For details of inoculum production see Chapter 7.

2.4. Inoculation

The inoculant was mixed in the 20 cm top layer of the soil at a ratio of 10% of the pot volume before sowing. Non-inoculated plants served as controls. Due to insufficient amount of inoculant, the first harvest (4 weeks after planting) was not inoculated with the Hannover inoculant.

2.5. Mycorrhizal parameters

The number of spores in the soil before inoculation and the number of spores in the pots after each harvest were determined as mycorrhizal parameters. The number of spores of mycorrhizal fungi in the soil was estimated after wet sieving and decanting of 50 g soil in 3 repetitions (Daniels and Skipper 1982).
Mycorrhizal colonisation of the root was determined after clearing the roots with 5% KOH and staining with Trypan blue (Koske and Gemma 1989). Thirty root pieces of about 1 cm of each plant were mounted on slides and analysed under a compound microscope, at a magnification of 100 x (8 plants per treatment and harvest). The percentage of the root cortex colonised (M %) was estimated using the five-class system and calculated using the equation of Trouvelot *et al.* (1986), as described in Chapter 7 of this thesis.

2.7. Growth analysis

Plants were harvested at week 4, 10 and 13, corresponding to the vegetative stage, flowering and pod-setting and maturity stages, respectively, using 8 plants for each harvest. The procedure for the growth analysis was the same as used in previous Chapters.

2.8. Data analysis

Data were analysed with the SPSS statistical package, version 4.0.1. Differences in growth parameters measured between treatments were analysed by one-way ANOVA.

3. RESULTS

3.1. Phenological development

No significant differences were found in number of days to 50% flowering. Inoculated plants were larger than non-inoculated plants (results not shown).

3.2. Mycorrhizal colonisation

Four weeks after planting, cultivar Local inoculated with Soil Mozambique inoculant, non-stressed (LSM-NS) showed a significant increase in root colonisation by

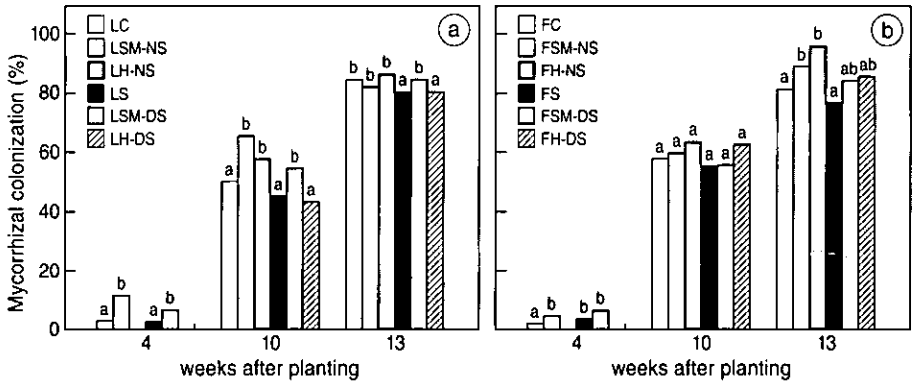

Figure 8.1 Effect of AM inoculation and drought stress on root colonisation of the two peanut cultivars Local (a) and Falcon (b). LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculamt and non-stressed; LS and FS, are the cultivars Local and Falcon non inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Different letters at a particular growth stage denote values that differed significantly at 0.05 level probability level with a least significant test (LSD).

69 % and 54 % in relation to the non inoculated, non-stressed (LC) and non-inoculated, stressed (LS) plants, respectively. Similarly, the inoculated stressed (LSM-DS) plants had higher values of mycorrhizal colonisation than the non-inoculated ones, representing an increase by 48 % and 34 % for non-stressed (LC) and stressed (LS) plants respectively (Figure 8.1a).

As at the vegetative stage, at the pod-setting stage (10 weeks after planting), the plants inoculated with the Soil Mozambique inoculant, showed a significantly higher mycorrhizal colonisation with and without drought stress. Hannover inoculant only led to a higher root colonisation with well-watered plants. At maturity stage there were no more substantial differences in root colonisation, however, under drought stress SM inoculant still lead to a significantly higher colonisation than in the other treatments.

AM inoculation in cultivar Falcon (Figure 8.1b) resulted at the vegetative stage, in a significant increase in root colonisation in the inoculated and the drought stressed treatments. In contrast to cultivar Local, in cultivar Falcon, no significant differences were found at the pod-setting stage, while at the maturity stage, watering and inoculation increased root colonisation by 9 % (FSM-NS) and 18 % (FH-NS), with Soil Mozambique and Hannover inoculants, respectively

Drought stress did not reduce root colonisation in the non-inoculated plants and in those inoculated with the Hannover inoculant.

3.3. Number of spores

The initial number of spores, counted in 50g soil before sowing was 117 ± 44. Four weeks after planting, the inoculated and non-stressed pots showed a significantly higher number of spores in both cultivars, 50 % more for cultivar Local and 79 % more for cultivar Falcon (Table 8.2).

In the pod setting stage, the number of spores showed a sharp decrease, as compared with the vegetative stage. Inoculation increased the number of spores only clearly in the non-stressed vegetative stage (LSM-NS and FSM-NS) compared to the controls (LC and FC) (Table 8.2).

Table 8.2 Effect of AM inoculation and drought stress on the number of spores of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Dap, are days after planting. Values are means of 8 determinations in 8 pots per treatment. Within column, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Number of spores	
LC	159a	38.0a	67.0a
LSM-NS	240b	51.0b	60.0b
LH-NS	-----	52.0b	45.0c
LS	140a	49.0b	43.0c
LSM-NS	132a	34.4a	60.0b
LH-NS	-----	43.4b	67.0a
FC	183a	49.0a	48.0a
FSM-NS	329b	49.3a	58.1b
FH-NS	-----	48.0a	48.0a
FS	134a	49.3a	43.3a
FSM-DS	169a	36.0b	60.4c
FH-DS	-----		61.2c

3.4. Leaf and root growth

3.4.1. Leaf growth

In general, inoculation increased or had no significant effect leaf growth and leaf growth parameters (Table 8.3).

Leaf area and leaf dry weight increased with inoculation under non-stressed conditions and with Soil Mozambique inoculant at the vegetative, pod-setting and maturity stage. Hannover inoculant did not have a strong effect. Under stressed conditions the leaf area was increased by SM inoculant at all stages, as was the leaf dry weight. Hannover inoculant had no effect.

The leaf area increased by inoculation with Soil Mozambique, both under stressed and non-stressed conditions. In cultivar Local, at the vegetative stage, the leaf parameters showed no significant differences between the non-stressed and the stressed control plants, except for leaf area to root dry weight ratio, which was significantly reduced (30 %) by drought stress. Inoculation with Soil Mozambique inoculant increased leaf area (69 % and 8 % for the control and drought-stressed treatment, respectively) and leaf number, independently of drought stress and prevented a reduction in leaf dry weight in the drought-stress treatment. The partitioning components, leaf area ratio and specific leaf area, did not differ among the treatments (Table 8.3).

At the pod-setting and maturity stages in general, drought stress reduced leaf growth in the non-inoculated treatments (50 % and 56 % reduction in leaf area at pod-setting and maturity stages), respectively. Similarly, plants inoculated with Hannover inoculant, showed a 44 % and 41 % reduction in leaf area at pod setting and maturity stages, respectively. The specific leaf area (SLA) at the pod-setting stage, did not respond to drought stress.

In cultivar Falcon at the vegetative stage (Table 8.3) the effects of drought stress in non-inoculated plants (FS) resulted in reduced leaf number (61 %) and incre-

Table 8.3 Effect of AM inoculation and drought stress on leaf growth parameters of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. LA, LN, LDW, LAR, SLA and LARDW, is leaf area, leaf number, leaf dry weight, specific leaf area and leaf area to root dry weight ratio, respectively. Dap, are days after planting. Values are means of 8 plants. Within a column, per growth stage, means followed by the same letter are not significantly different at a 0.05 probability level with a least significant test (LSD).

Treatment	LA (cm^2)	LN	LDW (g)	LAR ($cm^2.g^{-1}$)	SLA ($cm^2.g^{-1}$)	LARDWR ($g.g^{-1}$)
Vegetative stage (28 dap)						
LC	86a	6.3a	0.43a	93.4a	202a	1524a
LSM-NS	145b	10.6b	0.78b	90.5a	183a	1658a
LH-NS	-----	-----	-----	-----	-----	-----
LS	92a	6.4a	0.38a	118.0a	256a	1070b
LSM-DS	125b	8.4b	0.52a	109.0a	244a	1572a
LH-DS	-----		-----	-----		-----
Pod setting stage (77 dap)						
LC	432a	32a	2.74a	59.0a	160a	1996a
LSM-NS	739b	41a	4.37b	59.0a	168a	2386a
LH-NS	678b	38a	3.95b	64.0a	184a	1643b
LS	220c	16b	1.30c	62.5a	167a	1314bc
LSM-DS	403a	27a	2.37a	62.2a	171a	1874a
LH-DS	246c	15b	1.40c	55.0a	175a	869c
Maturity stage (91 dap)						
LC	487a	37a	3.73a	37.0a	129c	1394a
LSM-NS	761b	44a	5.46b	41.0a	140b	1673a
LH-NS	555a	40a	4.01b	39.0a	137b	1580a
LS	215c	17b	1.54c	48.0b	163a	1212a
LSM-DS	400a	33ab	2.62ab	43.0a	152a	1610a
LH-DS	288c	27ab	1.86c	48.0b	154a	1681a
Vegetative stage (28 dap)						
FC	116a	9.5a	0.60a	93.4a	191a	1195a
FSM-NS	165b	11.5a	0.86a	90.5a	189a	1871b
FH-NS	-----	-----	-----	-----	-----	-----
FS	102a	7.0b	0.48a	118.0a	218b	1126a
FSM-DS	164b	10a	0.744a	109.0a	219b	1703b
FH-DS	-----	-----	-----	-----	-----	-----
Pod setting stage (77 dap)						
FC	342a	23.4a	2.12a	51.8a	163a	1511a
FSM-NS	687b	38.0b	4.19b	51.2a	164a	2605a
FH-NS	542b	32.4b	3.33b	49.0a	162a	1885a
FS	188c	14.8c	1.18c	55.5a	158a	1255b
FSM-DS	418ab	26.7a	2.25a	58.0a	187b	2058a
FH-DS	275a	18.6ac	1.59c	54.8a	172a	869c
Maturity stage (91 dap)						
FC	423a	29.2a	2.80a	38.3a	147a	1387a
FSM-NS	609b	45a	4.60b	34.5a	131a	1989b
FH-NS	499b	32.6a	3.57a	35.3a	138a	1660b
FS	236c	16.4b	1.52c	46.0b	155a	1497a
FSM-DS	445a	29a	2.69a	43.9a	163a	1948b
FH-DS	261c	17b	1.68c	35.5a	158a	1057a

ased SLA (14 %), if compared with non-stressed, non-inoculated plants (FC), not having a significant effect on the other parameters. As with cultivar Local, at the pod-setting and maturity stages drought stress in general reduced leaf growth in the non-inoculated plants or the plants inoculated with Hannover inoculant, except for leaf area ratio (LAR) and SLA, which were significantly different.
While inoculation with Soil Mozambique under drought stress resulted in about the same leaf growth as in the non-stressed non-inoculated plants, inoculation with Hannover inoculant increased leaf growth at non-stressed conditions, similarly to Soil Mozambique inoculant, but did not or much less than Soil Mozambique compensate leaf growth reduction under drought stress.

3.4.2. Root growth

In general, drought stress reduced root growth, while inoculation, particularly with Soil Mozambique inoculant, resulted in (i) an increase in root growth in watered treatments or (ii) an increase or no growth reduction in stressed treatments (Table 8.4).
In cultivar Local at the vegetative stage, drought stress did not reduce root dry weight in all treatments, but increased root weight ratio (RWR) (81 %) and root shoot ratio (R/S) (88 %) in the non-inoculated treatment, a typical response of the peanut cultivars to drought and nutrient stress (Chapters 4 and 5). A decrease in maximum root length to leaf area ratio (MRLAR) was observed in the inoculated treatments (49 % for the control and 40 % for the drought-stressed treatment), while the non-inoculated, stressed treatment did not show any response.
At the pod-setting stage drought stress reduced root dry weight in the non-inoculated- stressed treatment (26 %) and did not affect the inoculated treatments. Similarly R/S (49 %), RWR (54 %) and MRLAR (144 %) were increased by drought stress in non-inoculated plants. The Hannover inoculant was less effective in preventing root drought stress responses, as shown by the increased RWR, R/S and MRLAR in the inoculated Local plants.

Table 8.4 Effect of AM inoculation and drought stress on root growth parameters of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Dap, are days after planting. Values are means of 8 plants. Within column, per growth stage, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Root dry weight (g)	Root weight ratio ($g.g^{-1}$)	Root shoot ratio ($g.g^{-1}$)	MRLAR ($cm.cm^{-2}$)
	Vegetative stage (28 dap)			
LC	0.057a	0.061a	0.07a	0.241a
LSM-NS	0.092b	0.058a	0.07a	0.123b
LH-NS	-----	-----	-----	-----
LS	0.091b	0.111b	0.13b	0.246a
LSM-DS	0.080b	0.072a	0.08a	0.145b
LH-DS	-----	-----	-----	-----
	Pod-setting stage (77dap)			
LC	0.228a	0.031a	0.056a	0.059a
LSM-NS	0.332b	0.027a	0.065a	0.052a
LH-NS	0.436b	0.040a	0.075a	0.037a
LS	0.168c	0.048b	0.084b	0.144b
LSM-DS	0.221a	0.035a	0.063a	0.067a
LH-DS	0.286a	0.066b	0.132b	0.101b
	Maturity stage (91 dap)			
LC	0.375a	0.028a	0.069a	0.060a
LSM-NS	0.474a	0.025a	0.058a	0.036a
LH-NS	0.371a	0.025a	0.061a	0.060a
LS	0.209b	0.040b	0.090a	0.114b
LSM-DS	0.253a	0.027a	0.063a	0.059a
LH-DS	0.180b	0.030a	0.065a	0.062a
	Vegetative stage (28 dap)			
FC	0.097a	0.079a	0.091a	0.210a
FSM-NS	0.090a	0.052b	0.057b	0.111b
FH-NS	-----	-----	-----	-----
FS	0.100a	0.091a	0.107a	0.201a
FSM-DS	0.100a	0.065a	0.074a	0.119b
FH-DS	-----	-----	-----	-----
	Pod-setting stage (77 dap)			
FC	0.242a	0.035a	0.071a	0.073a
FSM-NS	0.307a	0.022b	0.044b	0.038a
FH-NS	0.290a	0.026b	0.055b	0.036a
FS	0.174a	0.055a	0.094a	0.131b
FSM-DS	0.223a	0.025b	0.051ba	0.063a
FH-DS	0.224a	0.044a	0.093a	0.079a
	Maturity stage (91 dap)			
FC	0.303a	0.028a	0.069a	0.083a
FSM-NS	0.340a	0.018b	0.044b	0.036b
FH-NS	0.320a	0.022b	0.057b	0.044b
FS	0.170b	0.035a	0.074a	0.101c
FSM-DS	0.230b	0.022b	0.053b	0.047b
FH-DS	0.255b	0.035a	0.091a	0.062a

In cultivar Falcon (Table 8.4) the non-inoculated, stressed treatment did not show any response of root dry weight and related parameters to drought stress, at the vegetative stage, while a significant reduction in RWR (34 %) and R/S (37 %) was observed in the inoculated, non-stressed treatment. MRLAR decreased with inoculation regardless which water regime. Under drought stress conditions, inoculation resulted in the same root growth as in the non-inoculated plants and it prevented typical root drought stress responses in the vegetative stage. In the maturity stage drought stress led to a decrease of root dry weight in all treatments.

3.5. Number of pegs and pods

The yield potential of the cultivars expressed as number of pegs and pods at harvest, total sink number (TSN), and the harvest index (ratio pod dry weight to above-ground dry matter), were influenced differently by inoculation and drought stress. (Table 8.5).

Under drought stress in cultivar Local inoculation with both inoculants increased the number of pegs and pods, while the non-inoculated treatment showed a significant reduction compared with the non-stressed control plants (Table 8.5). In cultivar Falcon, both inoculants increased TSN and prevented a reduction of HI by drought-stress, at the pod-setting and maturity stages (Table 8.5).

However, under non-stress conditions, inoculation had no positive effect on TSN and HI, and Hannover inoculant had even a negative effect on TSN at the pod setting stage, reflected in 18 % reduction in TSN.

3.6. Nodulation

The number of nodules as a measure of N_2-fixation, was affected differently between the cultivars and inoculation treatments (Table 8.6).

The effect of inoculation was only evident at the pod-setting and maturity stages, but it did not prevent a reduction in the number of nodules in the drought stress treatments, showing 23 % and 47 % reduction at the pod-setting stage for plants inoculated with Soil Mozambique and non-inoculated, respectively. At the maturity stage the reduction in nodule number was at the level of 39 %, 33 % and 44 % for plants non-inoculated and inoculated with Soil Mozambique and Hannover inoculants, respectively. The Hannover inoculant was more effective in increasing nodulation of the cultivar Local, causing a 40 % and 46 % increase at the pod-setting and maturity stages, respectively. In cultivar Falcon (Table 8.6), the effect of AM inoculation was already evident at the vegetative stage, leading to a reduction in nodule number in watered plants (32 %), whereas in drought-stressed plants AM inoculation increased the nodule number. At the pod-setting stage watered and inoculated plants showed an increased number of nodules (43 %), whereas

Table 8.5 Effect of AM inoculation and drought stress on total sink number (TSN) and harvest index (HI) of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Dap, are days after planting. Values are means of 8 plants. Within column, per growth stage, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	TSN	HI ($g.g^{-1}$)
	Pod-setting stage (77 dap)	
LC	13.0a	0.36a
LSM-NS	19.0a	0.40a
LH-NS	15.1a	0.39a
LS	7.6b	0.33a
LSM-DS	10.0a	0.37a
LH-DS	10.1a	0.36a
	Maturity stage (91 dap)	
LC	16.0a	0.55a
LSM-NS	21.4a	0.52a
LH-NS	14.3a	0.53a
LS	9.2b	0.48b
LSM-DS	14.0a	0.51a
LH-DS	12.0b	0.47b
	Pod-setting stage (77 dap)	
FC	11.60a	0.41a
FSM-NS	15.70a	0.44a
FH-NS	9.42b	0.46a
FS	7.32b	0.31b
FSM-DS	11.00a	0.42a
FH-DS	9.50a	0.41a
	Maturity stage (91 dap)	
FC	13.20a	0.53a
FSM-NS	17.60a	0.53a
FH-NS	18.40a	0.55a
FS	7.70b	0.46b
FSM-DS	11.70a	0.52a
FH-DS	10.70a	0.52a

under drought stress only Hannover inoculant could compensate the decrease in nodule number. At the maturity stage drought stress reduced the number of nodules in plants inoculated with Soil Mozambique, (35 %) and non-inoculated plants (44 %). No changes were observed in the plants inoculated with Hannover, stressed and inoculated with Soil Mozambique, non-stressed. An increase in nodule number was observed in plants inoculated with the Hannover inoculant, independent of drought stress. As with cultivar Local, the Hannover inoculant was more effective in preventing the reduction of the number of nodules, due to drought stress.

3.7. Dry matter accumulation and distribution

In general, drought stress reduced dry matter in non-inoculated plants, particularly at the pod-setting and maturity stages (Table 8.7).
In cultivar Local inoculation with Soil Mozambique inoculant increased significantly the dry matter at the vegetative stage under non-stress conditions (70 %).

Table 8.6 Effect of AM inoculation and drought stress on nodulation of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Dap, are days after planting. Values are means of 8 plants.
Within column, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Number of nodules	
LC	21.9a	101.8a	133.1a
LSM-NS	24.0a	136.8b	168.0a
LH-NS	-----	143.5b	195.0b
LS	17.8a	54.5c	81.8c
LSM-DS	20.6a	79.3c	89.5c
LH-DS	-----	83.8a	74.8c
FC	24.8a	91.3a	114.8a
FSM-NS	17.0b	131.3b	122.0a
FH-NS	-----	137.3b	146.4b
FS	18.9b	51.1b	73.3b
FSM-DS	21.6a	59.8b	76.8b
FH-DS	-----	75.0a	95.2a

In this cultivar inoculation with Soil Mozambique prevented a reduction of dry matter due to drought stress from 53 % and 59 % in the non-inoculated plants to only 13 % and 28 % at the pod-setting and maturity stages, respectively.
In cultivar Falcon, a similar pattern was observed, characterised by an increase in dry matter at the vegetative stage with inoculation with Soil Mozambique and watering, no reduction of dry matter in any treatment inoculated with Soil Mozambique inoculant and a reduction of dry matter in all plants stressed, non inoculated or inoculated with Hannover inoculant. In this cultivar, inoculation prevented a reduction of dry matter due to drought stress from 51% in the non inoculated plants to only 25 % or even an increase in dry matter, when inoculated with Hannover and Soil Mozambique inoculants respectively, at the pod-setting stage, and from 53 % to 32 % and 8 %, when inoculated with Hannover and Soil Mozambique inoculants respectively, at the maturity stage.
The distribution of dry matter did not show a very clear trend, which may be

Table 8.7 Effect of AM inoculation and drought stress on dry matter accumulation (in g) of the two peanut cultivars Local and Falcon. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Dap, are days after planting. Values are means of 8 plants. Within column, means followed by the same letter are not significantly different at 0.05 probability level with a least significant test (LSD).

Treatment	Vegetative stage (28 dap)	Pod-setting stage (77 dap)	Maturity stage (91 dap)
		Dry matter (g)	
LC	0.93a	7.38a	13.0a
LSM-NS	1.60b	12.43a	18.6a
LH-NS	-----	11.00a	14.3a
LS	0.84a	3.50b	5.3b
LSM-DS	1.15a	6.50a	9.3a
LH-DS		4.40b	6.0b
FC	1.28a	6.64a	10.8a
FSM-NS	1.72b	12.53a	18.0a
FH-NS	-----	11.15a	14.1a
FS	1.05a	3.38b	5.1b
FSM-DS	1.55a	7.18a	10.0a
FH-DS	-----	4.96b	7.4b

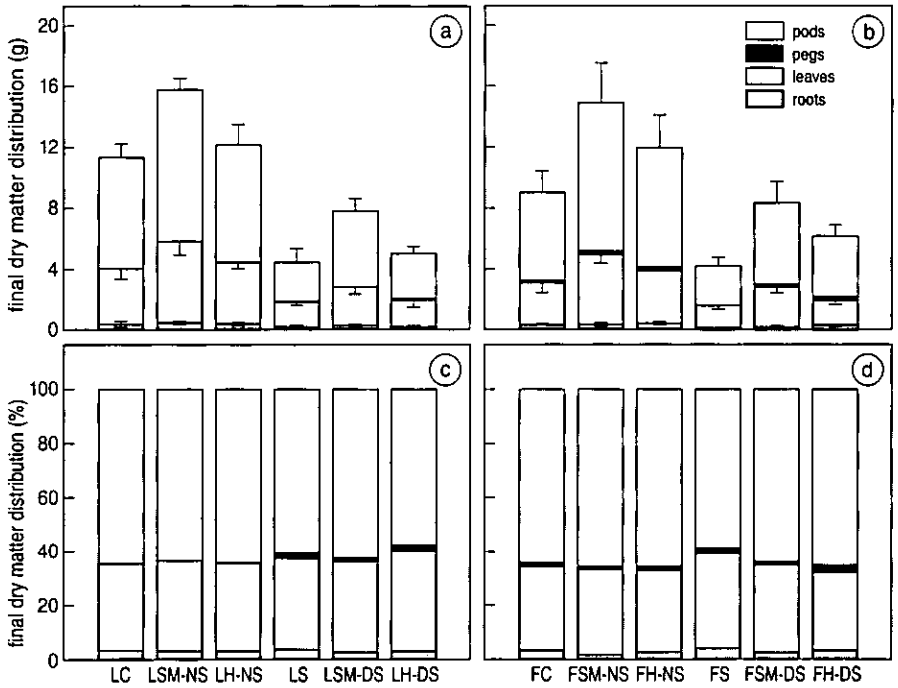

Figure 8.2 Effect of drought stress on dry matter accumulation and distribution of the two peanut cultivars Local (a and c) and Falcon (b and d) in g and percentage, respectively. LC and FC are the non-inoculated and non-stressed controls, LSM-NS and FSM-NS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and non-stressed; LH-NS and FH-NS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and non-stressed; LS and FS, are the cultivars Local and Falcon non-inoculated and stressed; LSM-DS and FSM-DS, are the cultivars Local and Falcon inoculated with Soil Mozambique inoculant and stressed, and LH-DS and FH-DS, are the cultivars Local and Falcon, inoculated with the Hannover inoculant and stressed, respectively. Values are means of 8 plants (± SD)

ascribed to either drought or inoculation (Figure 8.2a, b, c and d). In general there is a tendency of allocating more dry matter to the pods with inoculation, followed by leaves, while drought stress and non-inoculation increased dry matter allocation to the roots and delayed the formation of pods.

Non-inoculated and drought-stressed Local plants allocated 5 % of the dry matter to the roots, and 56 % to the pods, while the same treatment inoculated with Soil Mozambique and Hannover inoculants allocated 3 % of dry matter to the roots and 61 and 57 % of dry matter to the pods, respectively. In cultivar Falcon, inoculation with Soil Mozambique reduced dry matter allocation to the roots from 4 % in the non-inoculated treatment to 2.5 %, while increasing allocation of dry matter to the pods from 57 % to 63 %.

Table 8.8 Summary of the compensating effects of inoculation with Soil Mozambique inoculant and drought stress.

Growth parameters	Drought stress	Soil Mozambique inoculant
Leaf area	-	+ or no effect
Leaf number	-	+ or no effect
R/S	+	- or no effect
RWR	+	- or no effect
Leaf area to root dry weight ratio	- or no effect	+ or no effect
Maximum root length leaf area ratio	+	-

- Significant decrease
+ Significant increase

3.8. Summary of drought tolerance characteristics induced by AM inoculation

Leaf area and leaf number were significantly and constantly reduced in both cultivars under drought stress. Other parameters, which have been linked to drought sensitivity (Chapter 5 of this thesis) were constantly found to be changed under drought stress without inoculation. However, inoculation, particularly with Soil Mozambique inoculant, was found to minimise or compensate the effects of drought stress on the growth of both peanut cultivars (Table 8.8).

4. DISCUSSION

4.1. Mycorrhizal parameters

4.1.1. Root colonisation

The "background" colonisation in this study as evident from the root colonisation of the non-inoculated plants, was considerably higher than in the experiment reported in Chapter 7, particularly regarding cultivar Falcon. As the soil was collected at the same site only a year later, this shows that there is a large variation in the AM fungal community between years and seasons at the same site.

In cultivar Local, independently of the growth stage, drought stress only reduced root colonisation in plants either non-inoculated or inoculated with the Hannover inoculant (Figure 8.1a). In cultivar Falcon, drought stress did not have negative effects on root colonisation with both inoculants (Figure 8.1b). This result confirms the findings of Ruiz-Lozano *et al.* (1995b) that the root colonisation depends on the host cultivar and the AM inoculant. The reducing effects of

drought stress on root colonisation, reported by Busse and Ellis (1985) and Osonubi *et al.* (1992) or no effects, as reported by Nelsen and Safir (1982) and Allen and Boosalis (1983), were only valid when related to a specific AM endophyte, plant species/cultivar and particularly growth stage.
Both inoculants, regardless of water treatment, increased their colonisation rate with the duration of the experiment, suggesting a possible delay of the effects of inoculation with AMF to the pod-setting and maturity stages.
However, plant growth responses were already evident at the vegetative stage, when root colonisation was below 50 % (Figure 8.1 and Table 8.3). The results of this experiment, combined with those of the previous Chapter, suggest that the amount and type of inoculant may be more determinant in plant growth responses to AM inoculation than the growth stage itself.
An early growth response, despite low root colonisation may be explained by the fact that the degree of root colonisation detected by staining with Trypan blue does not always correlate with mycorrhizal efficiency (Guillemin *et al.*, 1995). However, a high mycorrhizal colonisation and efficiency at the pod-setting and maturity stages, may have more advantageous effects than at the vegetative stage, since peanut cultivars were found to be relatively more sensitive to drought stress at the pod-setting and maturity stages (Chapter 5).

4.1.2. Spore number

The increase in the number of spores as a result of inoculation is in agreement with many authors, who have reported an increase in the number of spores in different plants, including peanut, following AM inoculation (Krishna and Bagyaraj 1984; Tarafdar and Rao 1997). It contradicts, however, results reported in Chapter 7, when inoculation did not increase the number of spores at the vegetative stage.
The production of spores varies with host, endophyte species, root length (Simpson and Daft 1990a), and growth stage (Simpson and Daft 1990b). The present results do not show any evidence, linking the production of spores with one of the above indicated factors. The relatively high number of spores at the maturity stage in inoculated treatments under drought stress, indicates that the introduced inoculant can survive and compete even under drought stress conditions over a long period of time
(3 months). A long-term survival and competitiveness of an inoculant has been suggested as an important condition to be investigated before the introduction of any inoculant in the field (Simpson and Daft 1990b). Soil Mozambique and Hannover inoculants could be used in field trials under similar soil and host conditions.

4.2. Leaf and root growth

4.2.1. Leaf growth

The response of leaf growth to AM inoculation depended on the type of inoculant used (Table 8.3). While Soil Mozambique inoculant tended to increase leaf growth, even under drought stress, inoculation with Hannover inoculant did not have a positive effect despite a similar root colonisation (Figure 8.1). Thus, high root colonisation with the Hannover inoculant did not have a direct effect on drought stress, expressed as maintenance of growth under drought stress according to Safir *et al.* (1990). On the other hand, leaf area was increased before high levels of root colonisation were observed (Table 8.3), showing that root colonisation does not always correlate with leaf growth or plant growth responses. The Hannover inoculant appeared to be less suitable under drought stress conditions. Under non-stressed conditions (Chapter 7) it increased particularly leaf area and leaf dry weight as well as other growth parameters. The Soil Mozambique inoculant seemed to be more efficient in alleviating drought stress, particularly in relation to expanding leaf area. These results support those of Ruiz-Lozano and Azcón (1995), who suggested that the ability of AM fungi to protect the host plant against progressive drought stress does not seem to be linked with any specific physiological mechanism, affected by the fungus, but to physiological trends in the host according to the endophyte involved and environmental conditions, as well as to the intrinsic capacity of the fungus to resist stress. The increase in leaf area, shoot (data not shown) and total plant dry matter content by inoculation with Soil Mozambique inoculant, is not in accordance with the results of Al-Karaki and Al-Raddad (1997) in wheat, who reported that vegetative and reproductive growth were reduced by drought stress in both mycorrhizal and non-mycorrhizal plants. The present results clearly show, that AMF depending on the endophyte involved, can increase leaf drought tolerance in peanut grown in non-sterilised soil.

The leaf area responses with Soil Mozambique inoculant were clearer under drought-stress than under non-stressed conditions, despite a slightly higher mycorrhizal colonisation in non-stressed plants, suggesting a greater importance of AMF under dry conditions than under moist conditions, as proposed by Pena *et al.* (1988) and Michelsen and Rosendahl (1990).

An increase in leaf area, following inoculation under drought stress, as found in the present experiment with Soil Mozambique inoculant, has also been reported by Ellis *et al.* (1985) in wheat. Allen and Boosalis (1983) found that AM altered plant water relations and it was postulated that AM might enhance long-term drought tolerance via improved plant water relations. However, the leaf water content of the plants in this study did not show differences between the treatments (data not shown).

Expansion of leaves is very sensitive to moisture and responds more rapidly to changes in leaf water status than photosynthesis and transpiration (Hoogen-boom *et al.*, 1987). Inoculation with Soil Mozambique can be considered as an appropriate strategy of reducing the sensitivity of the peanut cultivars to drought stress.

4.2.2. Root growth

At the vegetative stage, root growth was stimulated by inoculation and drought stress in cultivar Local, while no significant differences were found in cultivar Falcon (Table 8.4). A lack of reduction in root growth at this growth stage suggests that drought stress may not yet have been sensed by the plants. Vivekanandan and Gunasena (1976) and Pallas *et al.* (1979) found that if a moderate water deficit is released within 50 days after planting, this drought is expected to have only minor effects on plant growth and yield. In fact, the drought stress tolerance characteristics, tended to be more pronounced, the longer the duration of the experiment.

Enhancement of root growth by drought stress, as found in both cultivars and with both inoculants at the vegetative stage, has also been reported by Ellis *et al.* (1985) in wheat, Graham *et al.* (1987) in citrus and Ruiz-Lozano *et al.* (1995b) in lettuce and it is considered to improve drought tolerance. An extensive root system should extract more water from a greater soil volume and should enable the plant to develop and maintain a greater leaf area during a period of drought (Bolanos *et al.*, 1993). Thus, an extensive root system is a desirable trait which allows plants to gain access to and absorb a greater volume of water, making AM a factor which may contribute to a higher performance of peanut under drought stress, as proposed earlier (Chapter 5).

Reduction of root growth has also been observed, depending on the fungus involved and such a reduction is related to a general growth reduction as a result of inoculation (Huang *et al.*, 1985). It may be argued that the duration of the stress affected the efficiency of AMF in increasing root growth as the reduction in root growth in this study only happened at the maturity stage. However, as pods were already formed, no influence on the final yield may be expected.

In Chapter 5 it was suggested that for drought tolerance it was not the root *per se* which conferred this ability, but its relation with the shoot. With inoculation, however, the root *per se* had significance in drought tolerance, supporting the findings of Yano *et al.* (1996) indicating a modification of root system morphology in a peanut seedling inoculated with AMF.

An increased root weight ratio (RWR), root shoot ratio (R/S) and maximum root length to leaf area ratio (MRLAR) as a result of drought stress, as found in the present experiment in non-inoculated treatments, reflects the resistance of the plant to drought or the ability of avoiding stress (Chapter 5). Despite the fact that inoculated plants showed a low R/S, it may be argued that the decreased root bio-

mass of the inoculated seedlings was functionally substituted by the external mycelium of the AM fungi. The length and biomass of extraradical mycelium increase under drought stress (Bethlenfalvay *et al.*, 1988) and this could be the key factor in AM mediated drought resistance. The decrease in R/S, due to mycorrhizal colonisation, has also been ascribed to a greater shoot growth of the mycorrhizal plants (Smith and Gianinazzi-Pearson 1988; Michelsen and Rosendahl 1990).
Increased RWR together with a reduction in leaf area, has the result that a unit of root dry weight supplies a small leaf area with water in drought-stressed conditions, leading to a decreased leaf area to root dry weight ratio (Table 8.3).
The lack of response of R/S, RWR and MRLAR in both cultivars under drought stress, but inoculated with Soil Mozambique at the pod-setting and maturity stages, reflects drought insensitivity. It therefore confers to AM inoculation the role of drought stress alleviator; since peanut is more sensitive to drought stress at these growth stages (Chapter 5, Pallas *et al.*, 1979; Boote and Hammonds 1981), no response of these sensitive drought-stress parameters was observed.

4.3. Nodulation

In general a high number of nodules was found in inoculated plants. The number of nodules increased from the vegetative stage towards the maturity stage, except in cultivar Local inoculated with Hannover inoculant, drought stressed (Table 8.6) and Falcon inoculated with Soil Mozambique, non-stressed (Table 8.6). A pattern of continuous increase was also found with root colonisation with AM, suggesting a close relationship between nodulation and AM root colonisation. However, a significant and positive correlation between nodule number and root colonisation was only found in cultivar Local, inoculated with Soil Mozambique, non-stressed ($r = 0.99$ at $P<0.05$) and stressed ($r = 0.99$ at $P<0.05$) and cultivar Falcon inoculated with Soil Mozambique, stressed ($r = 0.99$ at $P<0.05$). Ahiabor and Hirata (1994) reported that inoculation of peanut with *Glomus etunicatum* induced the highest dinitrogen fixation activity 80 days after planting in peanut, decreasing towards day 121, a period comprising the pod-setting and maturity stages, as found in the present experiment. Other reports indicate that nodulation of different legume species is enhanced by AM colonisation (Daniels-Hylton and Ahmad 1994; Freeden and Terry 1998). The enhancing effects of AMF on the N content in nodulated soybean for example were not necessarily only derived from N_2-fixation, but in part from a higher uptake of soil nitrogen (Azcón and Barea 1992).
The enhanced insensitivity of N_2-fixation to drought stress in AM inoculated plants particularly with Hannover inoculant, has been linked with a high degree of osmoregulation in mycorrhizal plants (Augé *et al.*, 1986a) and production of root cytokinins, which alleviate the effects to drought on nodule activity (Goicoechea *et al.*, 1996). In fact, proline, glycine betaine and proline betaine,

have been found to restore N_2-fixation in inoculated plants of *Medicago sativa* (Le Rudelier *et al.*, 1982). Root cytokinins have been found to increase under drought stress in mycorrhizal plants (Goicoechea *et al.*, 1996).

4.4. Number of pegs and pods

The total sink number (TSN) and harvest index (HI), showed a pattern related to the cultivar and inoculant involved (Table 8.5). There are few reports on the effect of AM inoculation on the number of pegs and pods, but yield has been reported as increasing in maize under well-watered conditions (Boswell *et al.*, 1998) and oil protein content in peanut under field conditions (Elsheik and Mohamedzein 1998). HI was reduced in field grown chickpea in Mediterranean plants inoculated (Weber *et al.*, 1993) or increased by inoculation with *Glomus mosseae* in field grown soybean (Ganry *et al.*, 1982) and in peanut (Ahiabor and Hirata 1994). The present results suggest that increased biomass production due to inoculation does not always represent an ability to produce also a high yield. Yield and yield components seem to be more related to cultivar than inoculant alone.

4.5. Dry matter accumulation and distribution

The increased dry matter of peanut with Soil Mozambique inoculant (Tables 8.7 and Figure 8.2) confirms reports indicating that AM colonisation confers a growth advantage to the host plant under drought stress (Wang *et al.*, 1996 and Al Karaki and Clark 1998). Differences in dry matter between inoculated and non inoculated plants have been suggested as representing the benefit derived by plants from AM fungal root associations (Al-Karaki 1998). AM inoculation induced plant insensitivity to drought stress and may have contributed to increased turgidity. Drought stress is known to reduce dry matter production through a reduction in turgidity (Munns and Cramer 1996).

5. CONCLUSIONS

Under the experimental conditions used in this study, it is concluded that (i) drought stress did not substantially reduce mycorrhizal colonisation in inoculated plants in both cultivars, (ii) AM inoculation increased growth and yield of peanut plants under drought stress conditions in comparison to non-inoculated ones, and (iii) drought stress effects could be alleviated by AM inoculation, depending on the endophyte, particularly through increased insensitivity of the host plant to reduction in leaf expansion. Therefore, peanut productivity, particularly under drought stress, may be improved by an adequate management of the AM symbiosis.

9 General discussion

1. INTRODUCTION

Peanut has been grown in Mozambique for many years, mainly in the light soils along the coastal zone. Yields are generally low due to (i) poor agricultural practices, (ii) lack of good seed quality, (iii) irregular rainfall, (iv) growing of unimproved landraces, (v) pests, diseases and weeds, and (vi) low soil fertility, reflected in deficiency in phosphorus, calcium, zinc and nitrogen (Malithano *et al.*, 1984a). Drought stress, however, has been recognised as the major constraint for crop growth in the semi-arid regions (Gibbons 1980; Kramer and Boyer 1995).

Selection for drought resistance has been recognised to be difficult because of (i) the large variation in precipitation pattern, space and time and (ii) the need of a large numbers of genotypes for screening and the soil variation over many locations. This is costly in terms of space and time, and makes a search for morphological and physiological traits contributing to a higher performance of peanut under drought and nutrient stress an essential step before conducting large field trials are planned. Some agronomic studies have been performed in Mozambique studying drought stress in peanut (e.g. Gomes 1992) and effects of fertilisers (Ramanaiah *et al.*, 1984), but no attempt has been made yet, to search for mechanisms, which regulate leaf and root growth as well as carbon allocation in stressed peanut, cultivated in Mozambique. The large increase in agricultural crop production has been basically obtained through a shift in dry matter distribution pattern within the plant (Gifford *et al.*, 1984) and several reports suggest that the target of selection should not be RGR per se, but the underlying morphological or physiological mechanisms of growth (Lambers and Dijkstra 1987; Berendse and Elberse 1990).

The present study was designed to analyse the growth of the most common peanut cultivars grown in southern Mozambique (1997, Chapter 2), the growth of selected cultivars under nitrogen limitation (Chapter 3), phosphorus limitation (Chapter 4), the growth of the cultivars under arbuscular mycorrhizal fungal (AMF) inoculation (Chapter 7) and the growth of selected cultivars under drought stress (Chapters 5, 6 and 8). In the present Chapter the main conclusions are discussed, attempting to answer the following questions: (i) does nitrogen, phosphorus and water limitation represent constraints for the growth and yield of the selected peanut cultivars? (ii) how does the presence of AMF effect these constraints?

2. EFFECTS OF NITROGEN, PHOSPHORUS, AND WATER LIMITATION AND AM INOCULATION ON GROWTH AND YIELD OF PEANUT

2.1 Effect of nitrogen limitation

N limitation resulted in plants showing symptoms of deficiency, such as yellow and abundant foliage and elongated stems only at the maturity stage (Chapter 3) as reported in other studies (Ramanaiah *et al.*, 1984; Smith *et al.*, 1984). Despite visible symptoms of N deficiency no reduction in relative growth rate (RGR) was observed, except at the vegetative stage with one of the cultivars (Falcon) and in relative leaf area expansion rate (RLAER) with both cultivars. N limitation has been found, however, to limit RGR of different crop plants (McDonald *et al.*, 1992 Gutschick and Kay 1995), and also of peanut (Malithano *et al.*, 1984) and to cause stem elongation and chlorosis (Smith *et al.*, 1984). The decrease in RGR (sensitivity to low N) in cultivar Falcon may be related to its high specific leaf area (SLA), under well-watered conditions (Chapter 5), a characteristic of a high fertility demanding crop species. The cultivar is a breeding line of high input agriculture. It can be expected that its nutrient demands are higher than those of the landrace. The lack of response to N limitation or even a reverse response of the peanut cultivars, particularly at the reproductive stage, may have resulted from different factors such as (i) growth conditions and (ii) inherent characteristics of the peanut cultivars. The homogenous substrate (vermiculite + sand + osmocote), in which the plants were grown (Chapters 2, 3 and 4), contained all the main mineral nutrients, micronutrients and approximately equal amounts of NO_3^- and NH_4^+ (6.8% for NH_4^+ and 7.2% for NO_3^-), while in the field, an imbalance may exist, due to soil or climatic characteristics. This imbalance may lead to a lower absorption of the less diffusible ion NH_4^+, or to a lower uptake of the mobile form, NO_3^-. The latter form is often present in a low concentration and its diffusion is severely restricted in dry soils (Azcón *et al.*, 1996). When present in large concentrations, NO_3^- may inhibit nodule development and nitrogenase activity, inducing the plants to depend exclusively on NO_3^- (Streeter 1988). In general, the highest growth rates and yields were obtained by combined supply of NO_3^- and NH_4^+ and any imbalance may affect the absorption of other ions, the cellular pH regulation and the rhizosphere pH, as these two forms represent 80% of the absorbed cations (Marschner 1988). On the other hand, the slow-release fertiliser osmocote, induces better plant performance and yield, compared to the conventional fertilisers or split application, since it reduces the loss of mineral nutrients and increases their uptake. The sandy soils where peanuts are grown, do not only have a low water-holding capacity, but the retention of N and also of K, is quite low (Bennet *et al.*, 1988).

In the field, where nodulation is present, this process exerts a need of extra car-

bon, compared to the non-nodulating plants of the greenhouse experiment (Chapter 3), contributing to a higher need of N than in this study, which in turn can lead to a severe N deficiency, particularly if the soil is low in N, the amount of *Bradyrhizobium* is low or both are less efficient. The poor utilisation of N by peanut (Nambiar *et al.*, 1988) is a characteristic which has been used to explain the lack of response by peanut to N limitation. If the other factors were not limiting, Cox *et al.* (1982) found that substantial amounts of N fertiliser were not needed, unless the site was extremely low in N, or no effective *Bradyrhizobium* was present. Another possible explanation could be the interactive effect of N and P (Marschner 1986), which might have resulted in increased growth in N limited plants, since according to this effect, an increase of a single nutrient (N or P) stimulates growth, resulting in deficiency of other mineral nutrients. No attempt was made during the present study to lower N and P levels simultaneously and to explore their possible interactive effects.

Symptoms of N deficiency reported in the field, may not always correspond to low N in the soil or to low yield at harvest, but they may mainly be an indirect effect of P limitation, which is known to limit N_2-fixation (Salih *et al.*, 1986; Sa 1997). In the field, N deficiency was directly related to the N level in soil and crop, but for plants, depending on symbiotic N_2-fixation, also to P deficiency: an inadequate supply of P may cause N deficiency, under these conditions arbuscular mycorrhizal fungi may be important, since they increase P, N, and K uptake (Azcón *et al.*, 1996) and indirectly nodulation (Chapters 7 and 8).

The lack of response of some growth parameters considered sensitive to N limitation, such as root/shoot ratio (Gutschik and Kay 1995) or root weight ratio (Radin and Parker 1979) in both growth stages, did not represent a total insensitivity of the peanut plants to low level of applied N. At the reproductive stage, an adaptive increased root volume in the N limited plants was still observed, suggesting a certain response of the root to N limitation. Similarly, relative leaf area expansion rate (RLAER) was reduced in both cultivars at the vegetative stage, showing a high sensitivity in comparison to photosynthesis (McDonald *et al.*, 1992) and leaf emergence rate (Uhart and Andrade 1995). A reduction in leaf expansion rate under field conditions would not only result in reduced availability of photosynthates to the whole plant, but also in their reduced export to the nodules, restricting N_2-fixation. If N_2-fixation is absent, the reduction in leaf area expansion could not exert any effect on yield. The lack of nodulation may also have exempted the plant of an extra N sink, since genetically non-nodulating peanut genotypes respond to N fertilisation. Their yields, however, are always lower than those of the nodulated lines (Sehrawat 1998). Clearly, the lack of nodulation in this study did not change the responsiveness of the cultivars towards low N. The cultivars may possess an inherently low sensitivity to low N or

may have benefited from the quality of the N supplier. In a sand culture non-nodulating *Lupinus* plants grown under low N (0.4 mM N) before floral initiation, showed a reduction in seed yield (Ma *et al.*, 1998).
Under the conditions of this experiment, low N, although reducing significantly RLAER at the vegetative stage, did not represent a constraint for the growth and yield of the peanut cultivars.

2.2. Effect of phosphorus limitation

P limitation reduced relative growth rate (RGR) on a total dry weight basis at the vegetative stage, leaf and root weight, and leaf expansion rate; no negative effects were observed at the reproductive stage. Similar results have been reported in other legumes (Ascencio 1994; Qiu and Israel 1992) and in early growth stages in maize (Rodriguez and Goudriaan 1995). This has been related to (i) reduced C availability to the leaves, (ii) increased partitioning to the roots (Lynch *et al.*, 1991; Aloni *et al.*, 1991; Ericsson *et al.*, 1992), (iii) a reduced cytokinin supply to the shoot (Horgan and Wareing 1980), or instead of a reduction of C-availability (Qiu and Israel 1992), (iv) to a more efficient utilisation of carbohydrates in the roots of P deficient plants. The low investment in the leaves was confirmed in this study by the observed reduction in relative leaf area expansion rate (RLAER) and decreased leaf area ratio (LAR), mainly resulting from a low leaf weight ratio (LWR), since specific leaf area (SLA) was increased at this growth stage. At the reproductive stage this effect was more pronounced, since LAR, LWR and SLA were all significantly decreased (Chapter 4). An increase in SLA under low P at the vegetative stage was also reported by Rodriguez *et al.* (1998) and indicated a lack of assimilates for leaf growth. The increase of SLA at the reproductive stage indicates, that the leaves had changed to storage of sugars and cell materials of low metabolic costs (Rodriguez *et al.*, 1998). This conclusion is further supported by the increased values of root weight ratio (RWR) at the vegetative stage and to the fact that no changes were observed at the reproductive stage, as well as no changes in root/shoot ratio (R/S) in both growth stages. Although R/S or its inverse has been extensively used in studies on P deficiency, this study showed that RWR was a more useful and sensitive variable as it represents the proportion of biomass allocated to the roots. In a drought stress experiment (Chapter 5), RWR was already increased at the vegetative stage when particularly no signs of stress were noticed as well as in non-inoculated treatments (Chapters 7 and 8). Therefore, RWR appeared to be a reliable indicator of nutrient and drought stress sensitivity. Despite changes in growth parameters no severe symptoms of P deficiency were observed, suggesting that P limitation was mild. Studies on P deficiency refer to symptoms such as stunted plants (Elliot *et al.*, 1997), necrosis and death of tips of the oldest blades (Grundon, 1987) and

reddening of the veins of mature leaves (Atkinson 1973). The present results showed that the absence of symptoms of deficiency may not be adequate to evaluate P deficiency since a reduced RGR and RLAER and increased RWR and SLA at the vegetative stage were observed, as possible adaptation to P limitation. Ascencio (1996) suggested that total leaf area and RGR during exponential growth could be used as physiological indicators to differentiate plants grown under P deficiency or ample P supply. In this study, reduced RGR and RLAER but not leaf area and increased RWR but not R/S and SLA, were traits related to P limitation.

Low P exerted more negative effects on growth of the groundnut cultivars, as shown by the reduction in RGR, at least at the vegetative stage, in contrast to the non response observed with a 20-fold reduction in N level (Chapter 3). This supports the findings by Ascencio (1996), suggesting that P limitation is a more important constraint in growth than low nitrogen. The reduction in RGR, only at the vegetative stage, shows the high sensitivity of peanut to P limitation at this growth stage, as found in wheat (Rodriguez and Goudriaan 1995). This supports the importance of a rapid mycorrhizal colonisation for the beneficial effects of P uptake, ascribed to arbuscular mycorrhizal fungi (AMF) (Nelsen and Safir 1982; Chapters 7 and 8).

P limitation alters N metabolism (Al-Karaki *et al.*, 1996; Sa 1997) and may mimic or affect the response of plants to drought stress (Guitierrez-Boem and Thomas 1999). It is suggested that although not affecting the final yield in this experiment, low P may represent a constraint of certain importance in peanut growth and yield under field conditions. E.g., the decrease in leaf expansion under low P was due to a decreased root hydraulic conductivity, as under drought stress (Radin and Eidenbock 1984).

2.3. Effects of drought stress and AM inoculation

The low yield of peanut under rainfed conditions as in southern Mozambique, has mainly been attributed to drought stress (Gibbons 1980). Besides the direct effect in reducing yield, it discourages the farmers to alleviate the other yield reducing constraints such as pests, diseases and weeds (Busolo-Bulafu 1992). In the present study the cultivars showed a contrasting pattern of response to drought stress, which was associated with differences in drought-acclimation strategies.

At the vegetative stage the small-seeded cultivar (Falcon) showed a higher relative growth rate (RGR), a low root weight ratio (RWR), a low maximum root length to leaf area ratio (MRLAR), and a high water content of the leaves. The large-seeded cultivar, Local, showed the opposite. However, both cultivars did not show a reduction in dry matter content (Chapter 5), suggesting that no drought stress effect on photosynthesis occurred at this stage. Low RWR and MRLAR may

lead to an early wilting under drought stress so that the absence of any signs of wilting can be associated with other mechanisms of drought tolerance. In fact, it was found that the small-seeded cultivar Falcon accumulated significantly more proline in leaves and roots at the vegetative stage, when grown under drought conditions. Proline has been found to be accumulated under several stress conditions and it correlates positively with cell membrane integrity in *Nicotiana tabacum*. Van Rensburg *et al.* (1993) suggested its use as a selection criterion for drought tolerance. Leaf water relations measured as relative water content (RWC) and relative saturation deficit (RSD) did not significantly differ between the cultivars but the cultivar Falcon showed the lowest percentage of membrane damage, measured with a polyethylen glycol (PEG)-test (Chapter 6), an indication of possessing a certain degree of tolerance to drought stress at the vegetative stage. RWC has been recommended as a suitable criterion for drought stress tolerance before anthesis and excised-leaf water loss at all growth stages (Dhanda and Sethi 1998), but in this study they were not appropriate as drought stress indicators. On the contrary, proline accumulation was an indication of drought tolerance (O'Reagan, 1993; Ali Dib *et al.*, 1994) and not an indication of sensitivity to drought (Andrade *et al.*, 1995). Cell membrane integrity, measured as electrolyte leakage, was also an indicator of drought tolerance, as reported by Premachandra *et al.* (1990).

However, despite differences in control and drought-stressed plants, proline increased continuously towards the maturity stage. This feature supported the argument that proline may not be a direct response to stress or not have an adaptive value, but may be a result (i) of the reduction in leaf expansion rate (Tardieu 1996) or (ii) a function of the growth period (Van der Mescht *et al.*, 1998).

Additionally, a significant and negative correlation was found at the maturity stage between proline content and RWC, a relation that suggests proline as indicative of drought sensitivity, as reported by Andrade *et al.* (1995).

A continuous drought stress period (91 days after planting) surpassed the response potential to drought avoidance/tolerance in both cultivars. This is a clear indication of the high metabolic costs involved in drought stress avoidance/tolerance mechanisms. Drought stress seldom occurs in isolation; it often interacts with other abiotic and biotic stresses (Cecarrelli and Grando 1996). Therefore, drought stress was found to be a major constraint for the growth and yield of the peanut cultivars.

Inoculation with Soil Mozambique arbuscular mycorrhizal inoculant under drought stress conditions (Chapter 8) did result in increased leaf and root growth and prevented the expected increase of the partitioning parameters RWR, R/S and MRLAR, a result of P deficiency (Chapter 4) and drought stress (Chapter 5). Clearly, it was possible to influence the drought tolerance of both peanut culti-

Table 9.1 Summary of the effects of N and P limitation, drought stress and AM inoculation on growth and yield of the peanut cultivars Local and Falcon. - , - -, +, ++ and * denotes no significant and negative effect, significant decrease, significant increase below 20 % and significant increase above 20 % and * highly dependent on the growth stage.

Growth parameters	Stress factor (without AM inoculation)			Soil Mozambique inoculant	
	N limitation[1]	P limitation[1]	Drought stress	No drought stress	Drought stress
Leaf area	-	- -,++ *	- -	+ +	+ + or -*
Root dry weight	-	+	- -	+ +	+ + or -
Root weight ratio	-	+	+ +	- -	-
Root shoot ratio	-	+	+ +	- -	-
Maximum root length leaf area ratio	-	+	+ +	- -	-
Yield	++	-	- -	+ +	+ or -

[1] Growth in sand/vermiculite substrate.

vars by inoculation with this multi-species AM fungal inoculant in a non sterilised field soil. The low mutualistic effectivity of the Hannover inoculant under drought stress conditions showed that there is a plant species/cultivar endophyte interaction in the ability to resist to stress (Ruiz-Lozano *et al.*, 1995a). Expansion of leaves is very sensitive to moisture and it responds more rapidly to changes in leaf water status than photosynthesis and transpiration (Hoogenboom *et al.* 1987).
Inoculation with Soil Mozambique inoculant was an appropriate measure to reduce leaf sensitivity to drought stress (Chapter 8). Indeed, the dry matter content of the inoculated plants was about equal to that of the non inoculated controls. In general, the effects of AM inoculation were higher under drought stress than under well-watered conditions, supporting the suggestion that AMF are more important in dry conditions than when moisture is adequate (Michelsen and Rosendhal 1990).
Some reports have indicated that the importance of AMF in mineral nutrition is not restricted to P uptake as indicated earlier by Nelsen and Safir (1982), but that mycorrhizas have a direct effect on absorption, translocation and assimilation of both forms of nitrogen (regardless of P content of the plant). Under drought conditions mycorrhizal plants can utilise the nitrate form more efficiently than the ammonium form, compared to non-mycorrhizal plants (Azcón and Tobar 1998). Drought stress, as indicated before, may restrict P and N uptake. Contrary to these positive effects of AMF, there are other reports indicating no significant role of AMF in alleviating drought stress in wheat (Ryan and Ash 1996), a decrease in

drought resistance in maize (Simpson and Daft 1990a) or no changes in leaf water status in safflower and wheat leaves (Bryla and Dunyway 1997). The main results of this study are summarised in Table 9.1.

Drought stress reduced root and leaf growth, and increased RWR, R/S and MRLAR. P limitation, despite not having much pronounced negative effects on leaf growth, tended to increase RWR, R/S and MRLAR. On the contrary, AM inoculation in well watered and drought stress conditions increased leaf and root growth, while decreasing RWR, R/S and MRLAR. Any factor that at the same time reduces leaf growth and increases substantially RWR, R/S and MRLAR (independent of symptoms of any deficiency), will have negative effects on final yield and *vice versa*.

3. RECOMMENDATIONS

The classical strategy for genetic improvement of plants under drought stress has mainly been the selection of genes, protecting against severe dehydration into crops of interest. Severe desiccation is however rare, due to whole plant or cellular control mechanisms of the plants e.g. increased RWR, MRLAR, reduced leaf expansion, increased proline content/osmotic adjustment (Chapters 4, 5 and 6). Severely stressed plants are seldom economically profitable (Kramer and Boyer 1995).

On the other hand, selection consists of large trials, with different cultivars and drought stress gradients, but the cultivars selected are mostly useful under similar environmental conditions. This study has shown (i) a negative correlation between ln initial seedling weight (ISW) and relative growth rate (RGR) and MRLAR, (ii) a positive correlation between RWR and ln ISW, and (iii) a negative correlation between the RGR and RWR (Chapter 2). A negative correlation was found between leaf area and RWR (Chapter 3), which support the idea that a selection of small-seeded cultivars may result in plants with a high growth rate which are able to withstand a short-term drought (Hendrix and Trapp 1992). In fact, in a drought stress experiment (Chapter 5) the small seeded cultivar Falcon showed no significant changes in drought sensitive traits such as leaf area and RWR, an indication of a certain degree of drought tolerance. Therefore, a selection for small-seeded cultivars would be an appropriate strategy, particularly for an early-season drought stress, as is mostly the case in southern Mozambique. However, the negative correlation between RWR and ln ISW and RGR makes a selection for a small-seeded cultivar a selection for less root growth, while root growth is an important parameter for drought stress and AM root colonisation. The higher the RWR, the higher the rate of photosynthesis (Farrar 1996) and the

higher the root coarseness, the higher the dependence of the plant on arbuscular mycorrhiza (AM) and the higher the root AM colonisation (Baylis 1970).
Proline accumulated to higher levels in the drought tolerant cultivar (Falcon) at the vegetative stage. This cultivar also showed a low percentage of cell membrane injury upon exposure to drought. However, as drought period developed, no differences were found between the cultivars (Chapter 4), supporting the results that resistance to drought is dependent on the time of its occurrence, duration and intensity (Nageswara Rao *et al.*, 1989).
Inoculation with Soil Mozambique inoculant at a ratio of 10 % of the pot volume, in contrast to the purchased Hannover inoculant (see Inoculum production and Inoculation, Chapters 7 and 8) did change mechanisms of coping with drought, as found in Chapter 5. Inoculation with Soil Mozambique inoculant under drought stress did increase or maintain the levels of root and leaf growth and yield in relation to the well-watered plants independently of the root, leaf growth and partitioning pattern of each cultivar.
Under low input agriculture in rainfed areas, where (i) fertiliser applications are uneconomical and unsustainable in the long run (Baligar and Bennet 1986), (ii) a large scale commercial inoculum production is still impracticable, and (iii) results of breeding/selection programmes are negligible due to the "difficult" nature of the target environment (Ceccarelli and Grando 1996), this study suggests that a correct management of the indigenous AMF may represent a most appropriate strategy for improvement of peanut yield. AMF management practices which have been recommended are (i) legume-based rotations (Lal 1997), (ii) agroforestry, where trees and annual crops are grown simultaneously as an alternative to monocultures (Boddington and Dodd 2000), (iii) reduction of root interaction in agroforestry systems, by lowering plant density (Boddington and Dodd 2000), (iv) avoidance of fire and (v) selection/breeding of genotypes responding to mycorrhization.
Under the conditions of Mozambique, where no effective AM isolates are available and occurrence and distribution are still unknown, an important step for a correct management of indigenous AMF will be the performance of controlled pot trials to determine basic characteristics, such as e.g. host-fungus specificity interaction and trials on competitiveness between AMF, effectiveness of the AMF under well-watered conditions as well as under drought stress, salinity and N and P deficiency.

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SUMMARY

Peanut is a common crop in the semi-arid tropics and for the majority of the population in southern Mozambique it is yet an irreplaceable constituent of the daily diet. The peanut yields, however, have decreased in the last years due to (i) low soil fertility, mainly low nitrogen (N) and phosphorus (P), (ii) growing of unimproved landraces, (iii) poor agricultural practices and, (iv) drought stress. Drought stress in particular has been recognised as the major constraint for the peanut production, due to the unpredictability of its occurrence, severity, timing, duration and to the interaction with other abiotic stresses, particularly extremes of temperature and variation in nutrient availability and with biotic stresses. These constraints have shifted peanut from a cash crop to a subsistence crop, so that the majority of the population has to rely on imported peanut seeds for agricultural production and human consumption. Therefore, any effort to attempt to increase peanut production is of great value, because it would bring nutritional benefits (peanut is rich in oil, protein content and vitamin B1) to the rural population, and increase their income.

A growth analysis experiment (chapter 2) showed that small-seeded cultivars (Falcon and Natal Common) had a higher relative growth rate (RGR) and root weight ratio (RWR), than the large-seeded cultivars (Local and Bebiano Branco). The dry matter allocation pattern showed substantial differences, the small seeded cultivars allocated more of their dry matter to the pegs and pods, while the large-seeded cultivars allocated more of their dry matter to the leaves and roots. Based on these differences in allocation pattern the cultivars Falcon (small-seeded) and Local (large-seeded) were used for subsequent studies on nutrient deficiency, drought stress and symbiotic associations.

This thesis deals with growth responses of selected peanut cultivars to controlled changes in soil fertility, especially low N and low P (chapters 3 and 4); drought stress (chapters 5 and 6) and symbiotic interaction between arbuscular mycorrhizal fungi (AMF) and peanut, under well-watered conditions (chapter 7) and under drought stress conditions (chapter 8).

Limitation of mineral nutrients was achieved by reducing the amount of the slow

release fertiliser osmocote, while maintaining a constant amount of the other nutrients in the sand/vermiculite substrate. Drought stress was imposed by withholding irrigation until the moisture content of the pot soil reached 3 % (near the wilting point of the peanut cultivars).

N limitation resulted in symptoms of deficiency only at the reproductive stage. At the vegetative stage RGR was significantly reduced in cultivar Falcon, while relative leaf expansion rate (RLAER) was reduced in both cultivars at this growth stage (chapter 3). It was concluded that leaf expansion rate was more sensitive to N limitation than the other growth parameters. The high sensitivity of the cultivar Falcon, was ascribed to its high specific leaf area (SLA), since high SLA has been associated with high fertility demanding species, and this cultivar is originating from a high input breeding system, in Zimbabwe. Symptoms of N deficiency alone were found to be not a direct and adequate indication of the N requirement of the peanut cultivars, as these symptoms may result from other factors, such as low P or drought stress. Only small amounts of N are sufficient to allow normal growth of the peanut cultivars if nodulation is adequately present or completely absent as in the N limitation experiment, since peanut is a poor utiliser of fertiliser N.

P limitation (chapter 4) resulted in a reduced leaf area, leaf area ratio (LAR), SLA and leaf area to root dry weight ratio at the reproductive stage, in both cultivars. Similarly, root dry weight and root volume were increased at this stage. RGR was only reduced at the vegetative stage, but relative leaf expansion rate (RLAER), was reduced in both cultivars at both growth stages. It was therefore concluded that, P limitation although not reducing yield, represents, particularly under field conditions, a constraint of certain importance for the growth and production of the peanut cultivars. RLAER and RWR were very sensitive growth parameters under low P.

Drought stress (chapter 5) resulted in differentiated responses at the vegetative stage. While cultivar Falcon did not show a significant growth reduction, cultivar Local reduced its leaf area, LAR and SLA, while increasing its RWR, maximum root length to leaf area ratio (MRLAR) and root shoot ratio (R/S). Dry matter content of both cultivars was not significantly reduced, suggesting a lower sensitivity of photosynthesis to drought stress than leaf expansion. N_2-fixation measured as the number of nodules was not reduced by drought stress at the vegetative stage in both cultivars.

It was concluded that the peanut cultivar Falcon possessed an osmotic adjustment mechanism which enables it to withstand short-term drought stress. A measurement of the cell membrane integrity, with polyethylene glycol test (PEG)-test, showed that the membranes of the cultivar Falcon were less injured, compared to those of the cultivar Local, under drought stress.

Additionally, proline was substantially more accumulated in this cultivar, than in

cultivar Local (chapter 6). Therefore, cultivar Falcon was classified as drought-tolerator and cultivar Local as drought-avoider. A continuous drought stress, however, resulted in a substantial reduction in leaf and root growth, and contrary to the vegetative stage also in reduction in dry matter content. Additionally and contrary to the low N and P experiments, yields were significantly reduced.

Drought stress, in this study, was found to be the major constraint for the production and yield of the peanut cultivars. Relative water content, cell membrane integrity, proline content, RWR, MRLAR and R/S were found to be drought stress indicators, loosing their significance as drought stress developed.

Inoculation (at a ratio of 5 % of the pot volume) of the peanut cultivars with self-produced inoculant (Soil Mozambique) and purchased inoculant (Hannover), resulted in increased root colonisation and growth, particularly for leaf area and leaf dry weight at the vegetative stage (chapter 7). At the reproductive stage, particularly in cultivar Falcon, an increased leaf area andleaf number was observed, while RWR was significantly reduced. The cultivar Falcon, a product of a high input agriculture system showed a higher dependence on AM inoculation and the cultivar Local, a landrace, was more compatible with the indigenous (Soil Mozambique) inoculant. This confirmed the high fertility demanding characteristic of cultivar Falcon, expressed by the high sensitivity to low N (chapter 3).

Inoculation (at a ratio of 10 % of the pot volume) under drought stress, particularly with the Soil Mozambique inoculant, resulted in the same growth as the control plants (chapter 8). An increase in leaf area, leaf number and a reduced RWR and R/S was observed in drought-stressed and inoculated plants of both cultivars. Clearly Soil Mozambique inoculant could alleviate drought stress effects in a non-sterilised soil. The indigenous inoculant was more adapted to the drought stress conditions than the purchased inoculant.

The present results have shown that under low input agriculture, the correct management of indigenous AMF, represent a real alternative to minimise the constraints in peanut production, since they are known not only to increase drought tolerance, as found in present study, but also to increase the uptake of other mineral nutrients such as N, P, potassium, sulphate, copper and zinc. Mycorrizal symbiosis could also help improving not only grain yield, but also soil physical, chemical and biological factors, enhancing the growth environment for other non-legumes.

Further research is needed to optimise the beneficial effects of the mycorrhizal symbiosis, under the Mozambican conditions, which include the determination of the host-fungus interactions, the specificity of the AMF, the competitiveness and mutualistic effectiveness of the AMF, under optimal and stress conditions, particularly under low N, low P, salinity and drought stresses.

SAMENVATTING

Pinda is een veel voorkomend gewas in de halfdroge tropen. Voor het merendeel van de bevolking in het zuiden van Mozambique is het een momenteel nog niet te vervangen bestanddeel van het dagelijkse dieet. De pindaopbrengsten zijn de laatste jaren echter gedaald door (i) lage bodemvruchtbaarheid, vooral stikstof en fosfaat, (ii) het gebruik van niet verbeterde locale variëteiten, (iii) slechte landbouwpraktijken en (iv) droogtestress. Met name droogtestress vormt de belangrijkste beperking voor de productie van pindas, vanwege de onvoorspelbaarheid van het tijdstip waarop droogtestress optreedt. De ernst ervan hangt samen met andere biotische stressfactoren als extreme temperaturen en variatie in beschikbaarheid van voedingsstoffen, en biotische stressfactoren. Deze beperkingen hebben ertoe geleid dat pindas in plaats van een winstgevend nu een marginaal product geworden zijn, zodat het merendeel van de bevolking afhankelijk is van geïmporteerde pindas voor landbouw en menselijke consumptie. Daarom is iedere poging om de productie van pindas, die rijk zijn aan olie, eiwitten en vitamine B1, te verhogen van groot belang, omdat dit niet alleen bijdraagt aan het dieet van de plattelandsbevolking, maar ook aan de verhoging van hun inkomen.

Uit de resultaten van een groeianalyse met vier pindacultivars (hoofdstuk 2) bleek dat de cultivars met kleine zaden (Falcon en Natal Common) een hogere relatieve groeisnelheid en een grotere wortel/spruit verhouding hadden dan cultivars met grote zaden (Local en Bebiano Branco). De verdeling van droge stof over de plant was eveneens verschillend. De kleinzadige cultivars investeerden relatief meer droge stof in zaadsteel en zaden, terwijl de grootzadige cultivars meer investeerden in blad en wortel. Gebaseerd op deze resultaten werden de overige experimenten uitgevoerd met de cultivars Falcon, met kleine zaden en Local, met grote zaden.

Dit proefschrift behandelt de invloed van veranderingen in bodemvruchtbaarheid, met name lage stikstof- en fosfaatbeschikbaarheid (hoofdstuk 3 en 4), droogtestress (hoofdstuk 5 en 6) en symbiose met arbusculaire mycorrhizae zonder (hoofdstuk 7) en met waterbeperking (hoofdstuk 8) op de groei van een aantal pindacultivars.

De pindacultivars werden gekweekt in potten in de kas in een zand-vermiculiet mengel. In de experimenten werd beperking van een bepaald mineraal gerealiseerd door de hoeveelheid osmocote, een product dat voedingsstoffen langzaam afgeeft, te verminderen. Door osmocote van verschillende samenstelling te gebruiken werd ervoor gezorgd dat de andere mineralen niet beperkend waren. Droogtestress werd opgelegd door geen water te geven, tot een vochtgehalte van 3 %, dichtbij het verwelkingpunt van de pindacultivars, bereikt was.

Symptomen van stikstofbeperking werden alleen in de reproductieve fase gevonden. Gedurende het vegetatieve stadium was de relatieve groeisnelheid significant lager in de cultivar Falcon, terwijl de relatieve strekkingssnelheid van het blad in dit groeistadium in beide cultivars lager was bij stikstofbeperking (hoofdstuk 3). Hieruit werd de conclusie getrokken dat de relatieve strekkingssnelheid van het blad meer gevoelig was voor stikstofbeperking dan andere groeiparameters. De grote gevoeligheid van Falcon werd toegeschreven aan het grote specifiek bladoppervlak van deze cultivar. Een groot specifiek bladoppervlak wordt algemeen gevonden bij soorten die een hoge bodemvruchtbaarheid verlangen. De cultivar Falcon is voortgekomen uit een hoog input teeltprogramma in Zimbabwe. Symptomen van stikstofbeperking op zich bleken geen directe en adequate indicatie voor de stikstofbehoefte van de pindacultivars te zijn, aangezien deze symptomen ook veroorzaakt kunnen worden door andere factoren, zoals lage fosfaatbeschikbaarheid en droogtestress. Als planten wortelknolletjes hebben om stikstof uit de lucht te kunnen fixeren, of in afwezigheid daarvan, zoals de planten uit de in dit hoofdstuk beschreven experimenten, zijn kleine hoeveelheden stikstof in de bodem voldoende voor normale groei.

Fosfaatbeperking (hoofdstuk 4) resulteerde in beide cultivars in een verlaging van totaal bladoppervlak en specifiek bladoppervlak tijdens de reproductieve fase. Gelijktijdig waren drooggewicht en volume van de wortel hoger tijdens deze fase. De relatieve groeisnelheid werd alleen verlaagd tijdens de vegetatieve fase, terwijl de relatieve bladstrekkingsnelheid in beide cultivars tijdens beide fases lager was. Hieruit werd de conclusie getrokken dat, hoewel fosfaatbeperking op zich de opbrengst niet verlaagt, dit toch een zekere beperking van groei en productie van pindas onder veldomstandigheden met zich mee brengt. Met name de bladstrekkingsnelheid en wortel/spruit verhouding bleken gevoelige parameters voor een lage fosfaatbeschikbaarheid te zijn.

Droogtestress (hoofdstuk 5) resulteerde in een verschillende respons voor de beide cultivars gedurende de vegetatieve fase. De groei van cultivar Falcon werd niet verlaagd, terwijl cultivar Local bladoppervlak en specifiek bladoppervlak verlaagde en maximale wortellengte en wortel/spruit verhouding verhoogde. De hoeveelheid droge stof van beide cultivars werd niet significant verlaagd, hetgeen suggereert dat fotosynthese minder gevoelig is voor droogtestress dan bladstrek-

king. De mogelijkheid tot binding van stikstof uit de lucht, gemeten als het aantal wortelknolletjes, werd in geen van beide cultivars gedurende de vegetatieve fase verlaagd door droogtestress.
Uit verdere experimenten kon geconcludeerd worden dat cultivar Falcon kortdurende droogtestress kan doorstaan door osmotische aanpassingen. Bepaling van de integriteit van de celmembraan, gemeten met de polyethyleen glycol test, toonde aan dat de membranen van Falcon bij droogtestress minder beschadigd werden dan die van Local. Proline kwam in grotere hoeveelheden voor in Falcon dan in Local (hoofdstuk 6). Op grond hiervan werd cultivar Falcon geclassificeerd als een droogtegedoger en cultivar Local als een droogtevermijder. In tegenstelling tot de tijdens de vegetatieve fase gevonden reacties, resulteerde aanhoudende droogtestress in aanzienlijke reductie van blad- en wortelgroei en hoeveelheid droge stof. Ook werd de opbrengst aanzienlijk gereduceerd, in tegenstelling tot de reactie hiervan op stikstof- en fosfaatbeperking. Uit de in dit proefschrift beschreven experimenten bleek dat droogtestress de belangrijkste beperkende factor was voor productie en opbrengst van pindas. Relatief watergehalte, celmembraan integriteit, proline gehalte en wortel/spruit ratio bleken goede parameters voor droogtestress, hoewel ze aan belangrijkheid inboetten bij aanhoudende droogtestress.
Experimenten waarin het effect van de aanwezigheid van arbusculaire mycorrhizae onderzocht werd toonden aan dat enten van de cultivars met zelf geproduceerd inoculum (Soil Mozambique) en een commercieel verkregen inoculum (Hannover) resulteerde in toegenomen kolonisatie van de wortel en groei, van vooral bladoppervlak en blad drooggewicht, gedurende de vegetatieve fase (hoofdstuk 7). Tijdens de reproductieve fase werd een toename van bladoppervlak en aantal bladeren gevonden, terwijl de wortel/spruit verhouding significant gereduceerd werd, vooral bij cultivar Falcon. Cultivar Falcon, het resultaat van een hoog input teeltprogramma was relatief meer afhankelijk van inoculatie, terwijl de plaatselijk gebruikte cultivar Local meer compatibel was met het uit de bodem van Mozambique verkregen inoculum (Soil Mozambique). De resultaten bevestigden dat cultivar Falcon een hoge bodemvruchtbaarheid verlangt, hetgeen ook bleek uit de grote gevoeligheid voor stikstofbeperking (hoofdstuk 3).
Inoculatie, vooral met het inoculum "Soil Mozambique", in combinatie met droogtestress, resulteerde in een even grote groei als die van de controleplanten. Tegelijkertijd werd voor beide cultivars een toename in bladoppervlak, aantal bladeren en wortel/spruit verhouding gevonden (hoofdstuk 8). Geconcludeerd werd dat inoculum "Soil Mozambique" de effecten van droogtestress duidelijk kan verlichten. Bij droogtestress had dit inoculum meer effect dan het commercieel verkregen inoculum "Hannover".
Uit de in dit proefschrift verkregen resultaten kan geconcludeerd worden dat voor

lage input landbouw een goed beheer van locale mycorrhizae een reëel alternatief biedt om de beperkingen in pindaproductie op te heffen vanwege het feit dat droogtetolerantie verhoogd wordt. Verder is bekend dat arbusculaire mycorrhizae ook de opname van mineralen als stikstof, fosfaat, sulfaat, koper en zink verhogen. Symbiose met mycorrhizae kan daarom niet alleen de opbrengst van gewassen verhogen, maar ook leiden tot verbetering van bodemfysische, bodemchemische en biologische factoren.

Verder onderzoek is nodig om de gunstige effecten van symbiose met arbusculaire mycorrhizae te optimaliseren onder de in Mozambique heersende omstandigheden, wat betreft gastheer-schimmel interactie en specificiteit en effectiviteit van de mycorrhizae, zowel onder optimale- als stresscondities, in het bijzonder lage stikstof en fosfaat beschikbaarheid, zout-en droogtestress.

SUMÁRIO

O amendoim é uma cultura comum da região semi-árida tropical e para a maioria da população do sul de Moçambique, um elemento insubstituível na alimentação diária. Contudo, os níveis de produção do amendoim baixaram nos últimos anos devido a factores como (i) baixa fertilidade dos solos, particularmente baixos níveis de nitrogénio (N) and fósforo (F) (ii) utilização de variedades indígenas com baixo potencial produtivo (iii) práticas agrícolas arcaicas e (iv) seca. A seca é hoje reconhecida como o principal factor na redução da produção do amendoim, devido á imprevisibilidade da sua ocorrência, da sua severidade, do seu período de ocorrência, da sua duração e devido fundamentalmente á sua interacção com outros factores abióticos, particularmente temperaturas extremas e variaçãcao na disponibilidade de nutrientes e também á sua interacção com factores bióticos. Estes constrangimentos levaram a que o amendoim passasse de uma cultura de rendimento para uma cultura de subsistência. As populações rurais, têm hoje, de depender de semente adquirida na rede comercial ou importada tanto para o consumo como para a produção agrícola. Assim, qualquer esforço tendente a aumentar a produção/ rendimento do amendoim, revestese de grande importância, uma vez que uma maior produção melhoraria nao só a dieta das populações rurais (o amendoim é rico em proteínas, óleos e vitamina B1), mas contribuiria também para a melhoria do seu rendimento económico.

Uma experiência de análise da taxa de crescimento (capítulo 2) indicou que as variedades com semente pequena (Falcon e Natal Comum) possuiam uma taxa de crescimento relativo (TCR) maior e um rácio peso seco da raíz / peso total da planta maiores em relação ás variedades de semente grande (Bebiano Branco e Local). O padrão de alocação de matéria seca, por seu lado, indicou que as variedades de amendoim com semente pequena, alocavam maior quantidade de matéria seca nos órgãos generativos, ginóforos e vagens, enquanto as variedades de semente grande, nas raízes e nas folhas. Estas diferenças extremas levaram a que fossem seleccionadas as variedades Falcon (semente pequena) e Local (semente grande) para estudos posteriores sobre a resposta á deficiência de nutrientes, stress hídrico e interacções simbióticas.

Esta tese trata da resposta de variedades seleccionadas de amendoim a mudanças controladas na fertilidade do solo, especialmente, baixos níveis de N e F (capítulos 3 e 4); stress hídrico (capítulos 5 e 6) e interacções simbióticas entre o amendoim e fungos arbúsculo-micorrízicos (FAM), em condições normais de rega (capítulo 7) em condições de stress hídrico (capítulo 8).

Um nível baixo de nutrientes no solo, foi obtido por redução da quantidade do fertilizante de libertação lenta osmocote, mantendo simultânemente constantes os níveis de restantes nutrientes, no substrato areia/vermiculite. O stress hídrico foi conseguido através da interrupção da rega, até que os níveis do conteúdo de água no vaso estivessem a 3 % (ponto de emurchecimento permanente, para estas variedades).

Baixo nível de N, resultou em sintomas de deficiência, apenas na fase reprodutiva. Na fase vegetativa foi registada uma redução na TCR apenas na variedade Falcon e redução na taxa relativa de expansão foliar (TREF) em ambas as variedades, nesta fase de crescimento. Concluiu-se que a expansão foliar era mais sensível a baixos níveis de N, que os restantes parâmeteros de crescimento. A alta sensibilidade da variedade Falcon, foi interpretada como sendo resultado da sua elevada área específica foliar (AEF), uma vez que este parâmetro está associado a espécies com altas exigências nutricionais e a variedade Falcon, é resultado dum programa de melhoramento em condições de agricultura de rendimento. Foi concluído igualmente que sintomas de deficiência, não são uma indicação directa e adequada das necessidades da planta em N, pois eles podem resultar de outros factores tais como baixos níveis de F e stress hídrico. Sabido que o amendoim é um utilizador fraco de N, se a nodulação for adequada ou for inexistente, como na presente experiência, pequenas quantidades de N, são suficientes para garantir um crescimento normal do amendoim.

Baixo nível de F (capítulo 4) resultou na redução da área foliar, peso seco da folha (nem sempre estatísticamente significativa) e uma redução significativa do rácio área da folha/peso seco total da planta, AEF e do rácio área da folha/ peso seco da raiz, na fase vegetativa em ambas as variedades. Da mesma maneira, o peso seco da raíz e o volume da raíz, mostraram um aumento significativo, nesta fase de crescimento. A TCR registou uma redução apenas na fase vegetativa, mas a TREF, registou uma redução significativa nas 2 fases e em ambas as variedades. Concluiu-se assim que a limitação dos níveis de F, mesmo que nao reduzam a produção/rendimento do amendoim, representam, particularmente em condições de campo, um constrangimento de certa importância. A TREF e o rácio peso seco da raíz/ peso seco total da planta, sao parâmetros de crescimento sensíveis a baixos níveis de F no substrato.

O stress hídrico (capítulo 5) resultou em respostas diferenciadas na fase vegetativa. Enquanto a variedade Falcon, não mostrou qualquer redução significativa

no crescimento, a variedade Local, reduziu a sua área foliar, o rácio área foliar/ peso seco total da planta, a AEF, enquanto aumentava significativamente o rácio peso seco da raíz/peso seco total da planta, o rácio comprimento máximo da raíz/área foliar e o rácio peso seco da raíz/peso seco da parte aérea. A matéria seca, não foi, contudo, significativamente afectada, nesta fase de crescimento, sugerindo, certa insensibilidade da fotossíntese ao stress hídrico quando comparada com a expansão foliar. A fixação de N, medida pelo número de nódulos formados, mostrou também certa insensibilidade ao stress hídrico, na fase vegetativa de crescimento.

Concluiu-se que a variedade Falcon, possuia um mecanismo de ajuste osmótico que a ajudava a resistir ao stress hídrico de curta duração. A determinação da integridade das membranas através do teste de polyethylene glycol, indicou que, de facto, as membranas da variedade Falcon eram menos afectadas em relaçãco as da variedade Local, em condições de stress hídrico. Por outro lado, os níveis de prolina foram mais altos na variedade Falcon, que na variedade Local (capítulo 6). Com base nestes resultados a variedade Falcon foi classificada como tolerante ao stress hídrico e a variedade Local como uma variedade capaz de evitar o stress hídrico através de mecanismos de reduçãcao da área foliar e aumento do peso da raíz. Contudo, um stress hídrico contínuo, resultou numa redução significativa do crescimento foliar e radicular, e ao contrário do que acontecera na fase vegetativa, resultou também numa redução do peso da matéria seca em ambas variedades. Por outro lado, e ao contrário das experiências com baixos níveis de F e N, o rendimento/produção foram significativamente reduzidos, nas duas variedades.

O stress hídrico foi considerado por isso, o factor principal na redução da produçãcao/rendimento das variedades de amendoim utilizadas. O conteúdo relativo de água, a integridade das membranas, o conteúdo de prolina, o rácio peso seco da raíz/peso seco total da planta, rácio comprimento máximo da raíz/área foliar e o rácio peso seco da raíz/peso seco total da panta, são parâmetros úteis e indicadores do stress, apesar de o seu valor depender da duração do stress e da fase de crescimento das plantas.

A inoculação das variedades (rácio de 5 % do volume do vaso) com um inoculante de fungos micorrízicos de produção local (inoculante solo de Moçambique) e com um inoculante adquirido no Instituto de Contrôle de Doenças e Protecção de Plantas de Hannover-Alemanha (inoculante Hannover), resultou num aumento significativo da colonização radicular e do crescimento, particularmente da área foliar e peso seco da folha, na fase vegetativa (capítulo 7). A variedade Falcon, produto de melhoramento em condições de agricultura de rendimento, mostrou uma alta dependência da

colonização com fungos micorrízicos enquanto a variedade Local, uma variedade indígena, mostrou maior compatibilidade com o inoculante solo de Moçambique,

contendo fungos indígenas, do que com o inoculante Hannover. Este resultado confirmou a alta exigência nutricional da variedade Falcon, demonstrada pela sua alta sensibilidade a níveis baixos de N, como descrito no capítulo 3.
A inoculação (rácio de 10 % do volume do vaso) e em condições de stress hídrico, particularmente com o inoculante solo de Mocambique, resultou num crescimento quase igual ao das plantas normalmente irrigadas, as plantas de contrôle (capítulo 8). Igualmente, observou-se um aumento significativo da área foliar, número de folhas e uma redução significativa do rácio peso seco da raíz/peso seco total da planta e do rácio peso seco da raíz/peso seco total da parte aérea. Este resultado provou claramente que a inoculação com fungos micorrízicos alivia o stress hídrico, mesmo em solos não esterilizados e que o inoculante indígena é o mais apropridao em condições de stress, quando comparado com o inoculante Hannover.
Os resultados do presente estudo indicam que em condições de agricultura de subsistência, um maneio correcto dos fungos micorrízicos indígenas, representa uma alternativa real, para minimizar os constrangimentos na produção/rendimento do amendoim, pois além de permitirem uma elevada resistência á seca, como resultou do presente estudo, eles também melhoram a absorção de outros nutrientes minerais, tais como, o N, F, potássio, sulfato, cobre, zinco e ferro. A simbiose micorrízica pode igualmente ajudar a aumentar não só a produção/rendimento desta cultura, mas também a optimização dos factores físicos, químicos e biológicos do solo, melhorando o ambiente para o crescimento de outras culturas, incluindo não leguminosas.
Deste modo, recomenda-se que nas condições de Moçambique em que (i) ainda não existem fungos micorrízicos efectivos isolados e (ii) a ocorrência e distribuição dos fungos micorrízicos é ainda desconhecida, sejam iniciados estudos tendentes a optimizar os benefícios da simbiose micorrízica e que incluam a determinação das interacções fungo-hospedeiro, a especificidade, a competitividade e eficiência mutualística dos fungos em condições de rega e nutrição mineral óptimas e em condições de stress nutricional (especialmente N e P), salinidade e seca.

Curriculum vitae

Orlando António Quilambo was born on July 9, 1959
in Zavala-Inhambane, Mozambique.
He studied Biology at the PH Güstrow, University of Rostock,
in Germany, where he was graduated as diplom in 1987.
After graduation he worked as Assistant in Plant Physiology
at the Department of Biological Sciences of the Universidade
Eduardo Mondlane in Maputo, Mozambique, a position he
still holds at the present.
In 1997, he started his Ph.D. project at the Department of
Plant Biology, Biological Centre of the University of Groningen,
The Netherlands, sponsored by the DEIBI-Project,
in the framework of the MHO-program.