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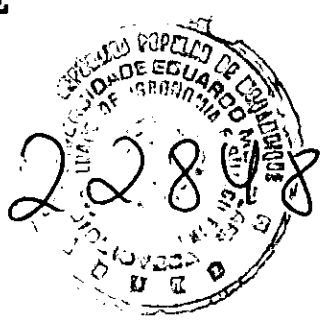
GROWTH, DEVELOPMENT AND NUTRITIONAL VALUE OF *Amaranthus tricolor* L. AS AFFECTED BY SALINITY AND HARVESTING PROCEDURE



by

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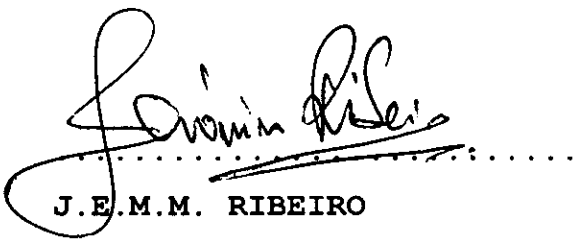
DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.



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ABSTRACT

Low crop productivity in arid and semi-arid regions is a problem caused by water stress as well as associated high levels of soil and water salinity. An increased demand for salt tolerant crops is experienced in these regions. Amaranth is a glycophyte and C_4 dicotyledonous crop, well adapted to arid and semi-arid regions. Previous studies on the physiological response of salt-stressed amaranths have indicated that this crop is salt tolerant. As vegetable, amaranths can be harvested by uprooting and by topping. The most common harvesting method is by topping, allowing repeated harvesting. When harvested by topping, the cutting height is an important parameter that may be manipulated to optimise growth rates. In this study, plants were exposed to different salt stress levels and harvesting procedures while yield and quality of *Amaranthus tricolor* were investigated. Nutrient solutions at four different electrical conductivity (EC) levels were used to fertigate the plants. At high EC levels (4 mS cm^{-1} and 8 mS cm^{-1}), the length and diameter of main stems, internode lengths, stem weights as well as root weights were reduced, especially with a longer growth period. However, the shoot:root ratio and leaf protein yields increased and flowering was delayed. The best leaf yield was obtained where plants were fertigated at an EC of 4 mS cm^{-1} for 45 days. The cutting height did not affect leaf yield, growth rates and leaf protein yield in plants fertigated at EC levels of 1, 2 and 4 mS cm^{-1}. At an EC of 8 mS cm^{-1}, the growth rate recovered to a value similar to that of plants fertigated with an EC of 2 mS cm^{-1}, only where plants were topped at 25%. With this less destructive cutting height (topped at 25%), leaf yields, growth rates and leaf calcium and protein yields at an EC of 8 mS cm^{-1} were superior to that of plants topped at 50%. In plants topped at 25%, the recovered growth rates at EC 8 mS cm^{-1} was probably due to more photosynthetic active tissue left after cuttings,

resulting in the accumulation of compatible solutes for osmotic adjustment.

UITTREKSEL

Die invloed van soutstremmings en oesprosedures op die groei, ontwikkeling en blaarkwaliteit van *Amaranthus tricolor* L.

Lae produksie van gewasse in ariede en semi-ariede gebiede is 'n probleem wat veroorsaak word deur watertekorte asook geassosieerde hoë peile van grondverbrakking en soute in water. 'n Verhoogde vraag na soutverdraagsame gewasse word in hierdie gebiede ervaar. Amaranthus is 'n glikofiet en dikotiele C_4 gewas wat goed in ariede en semi-ariede streke aangepas is. Vorige ondersoeke oor die fisiologiese reaksie van Amaranthus op soutstremmings het daarop gedui dat die gewas soutverdraagsaam is. As groentegewas word dit ge-oes deur dit uit te trek of deur dit gereeld te top. Waar dit getop word is die oes-tophoogte 'n belangrike parameter wat gemanipuleer kan word om die groeitempo te optimaliseer. In hierdie ondersoek is plante aan verskillende peile van soutstremmings en oesprosedures blootgestel terwyl die opbrengs en kwaliteit van *Amaranthus tricolor* ondersoek is. Voedingsoplossings is teen vier elektriese geleidingspeile (EC) gebruik om plante te voedsproei. Teen hoë EC peile (4 mS cm^{-1} en 8 mS cm^{-1}), het lengtes en die deursnit van hoofstamme, internode lengtes, stam massas en wortelmassas afgeneem, veral met lang groeiperiodes. Die bogroei:wortel verhouding en blaarproteien opbrengs het egter toegeneem terwyl blomvorming vertraag is. Die beste blaar opbrengs is na 45 dae verkry waar plante teen 'n EC van 4 mS cm^{-1} gevoedsproei is. Oes-tophoogte het nie blaar opbrengs, groeitempo of blaar proteien opbrengs beïnvloed met EC waardes in voedingsoplossings van 1, 2 en 4 mS cm^{-1} nie. Met 'n EC van 8 mS cm^{-1} het die groeitempo herstel tot 'n vlak, gelykwaardig aan wat by 'n EC van 2 mS cm^{-1} verkry is, slegs waar teen 25% tophoogtes ge-oes is. Met hierdie minder destruktiewe oesmetode (oes-tophoogte 25%), was blaar opbrengs, groei tempo en blaar kalsium en -proteien opbrengste by 'n EC van 8 mS cm^{-1} betekenisvol beter as waar die oes-topdiepte 50% was. Plante wat

met 25% topdieptes ge-oes is se herstel in groetempo teen EC 8 mS cm^{-1} was waarskynlik te danke aan meer fotosinteties aktiewe materiaal wat na oes op plante gelaat is. Dit kon tot die akkumulasie van oplosbare stowwe en osmotiese aanpassings aanleiding gegee het.

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CONTENTS

CHAPTER 4

ADDENDUM

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

AMARANTH AS VEGETABLE

Main species and their importance

Amaranth is a glycophyte and C_4 dicotyledonous crop, widely distributed in subtropical and tropical zones and well adapted to different growing conditions. It is well adapted to arid and semi-arid regions and is grown as a green leafy vegetable or as a grain crop in Africa, Asia and Central America. There are three main species that appear to be superior for use as vegetable: *Amaranthus cruentus, Amaranthus dubius* and *Amaranthus tricolor* (Daloz & Munger, 1980).

A. cruentus, originally developed as a grain type, is also used as leafy vegetable. This specie is an important crop in tropical Africa, as corn, sweet potato and peanuts (Anon., 1984). This plant has long stems and high dry matter concentrations in their leaves (Oomen & Grubben, 1978) and is less affected by cool and wet conditions than *A. dubius* and *A. tricolor* (Campbell & Abbott, 1982). *A. dubius* and *A. tricolor*, also known as African spinach, are used as vegetable in West Africa, the Caribbean and Asia. Their growth habits are similar to spinach, a C_3 plant, with short stem and succulent leaves (Oomen & Grubben, 1978). Previous studies have shown that *A. dubius* produced higher yields when grown at high temperatures than *A. cruentus* and *A. tricolor* (Campbell & Abbott, 1982; Olufolaji, 1989). Although the total yield of *A. tricolor* is usually lower than *A. dubius* and *A. cruentus*, its yield is more stable with repeated harvests (Allemann *et al.*, 1996).

These amaranth species can be produced during the hot seasons when other leafy vegetables are unavailable (Anon., 1984). The leaves are particularly rich in iron, calcium, vitamin A and C and protein, providing a good source of nutrition for people in

areas with hot and dry climates (Allemann *et al.*, 1996). Calcium is an important mineral for children growing. The best source of calcium is milk but since it is rarely available in developing tropical countries, calcium may be provided with green leafy vegetables (Chweya, 1985). The leaf calcium content of amaranths is higher than exotic leaf vegetables such as spinach, lettuce and cabbage as well as other tropical leaf vegetables such as cassava, cowpea and sweet potato (Schmidt, 1971; Grubben, 1976; Oomen & Grubben, 1978; Chweya, 1985). Calcium concentrations may vary from 2.45 to 3.88 mg 100 g^{-1}, depending on the species (Allemann *et al.*, 1996). Protein is often deficient in diets of people in rural areas of developing countries (Chweya, 1985). Amaranth species are good sources of protein because leaf crude protein content has been reported to vary between 26% and 30% on a dry matter base (Allemann *et al.*, 1996; Auwalu & Tenebe, 1997).

Harvesting procedure

As vegetable, amaranths can be harvested by uprooting and by topping. Due to its regeneration capacity, *A. cruentus* may economically be harvested by uprooting while *A. dubius* can be topped with repeated cuttings (Olufolaji, 1989). The most common harvesting method is by topping, allowing repeated harvesting. By uprooting, Grubben (1976) states that the best yield is obtained when amaranths are harvested at six weeks after transplanting (WAT). By topping, the time of the first cut, the frequency of cutting and cutting heights are important parameters that may be manipulated to obtain good growth rates.

The first cut should be done three to five WAT, when the plants are very vigorous (Grubben, 1976; Norman & Shongwe, 1993). Grubben (1976) found that cutting every three weeks produced higher yields than every two weeks, however, Norman & Shongwe (1993) found that harvesting at two-week intervals produced better total yields and leaf:stem ratios than three-week intervals. Harvesting at two-week intervals allowed more

harvests, resulting in higher total yields (Norman & Shongwe, 1993). Repeated cuttings, depending on cutting height, may affect growth rate as well as flowering time. The inflorescence appeared about two weeks later in plants, cut at 15 cm above ground level than those cut at 25 cm (Grubben, 1976). On the other hand, the growth rate was better at 20 and 25 cm cutting heights (Grubben, 1976; Mnzava & Masam, 1985; Norman & Shongwe, 1993). The poor growth rate following vigorous cutting can be due to complete removal of active photosynthetic young leaves as found by Auwalu & Tenebe (1997), and low reserve levels for re-growth in the remaining plant (Mnzava & Masam, 1985).

All plants have specific shoot:root ratios. After removal of the first leaves, the allocation of photosynthates change, associated with a reduction in dry matter accumulation (Poljakoff-Mayber & Lerner, 1994). Klepper (1991) found that after a certain time, the ratio might be restored, probably due to an increased growth of damaged shoots (leaves and stems). With repeated cutting, Mnzava & Masam (1985) found a decrease in shoot:root ratio to a minimum at the third harvest and then increased to a maximum at the fifth harvest. They also found higher shoot:root ratio with cutting height at 20 cm than at 10 and 15 cm.

Potential photosynthesis

C_4 plants, as amaranths, are well adapted to hot climates due to higher CO_2 assimilation rates at temperatures beyond 30°C when compared to C_3 crops in full sunlight (Brown, 1999). The temperature range for C_4 crops lies between 15 and 40°C while C_3 crops can tolerate temperatures from 5 to 30°C (Lawlor, 1979). Under optimum field conditions, the growth rate of C_4 plants may reach 50 to 55 g m^{-2} day^{-1} compared to about 40 g m^{-2} day^{-1} of C_3 plants (Ludlow, 1985). Higher growth rates of C_4 plants are related to three factors: nitrogen, solar radiation and water. C_4

plants are usually better equipped to make efficient use of nitrogen, radiation and water than C_3 plants (Brown, 1999).

Other important characteristics that differ between C_4 and C_3 plants are the lower transpiration coefficient and higher dry matter (DM) production of C_4 plants (Black, 1973). Grubben (1976), found at 35°C that the transpiration of *A. cruentus* was less than *Celosia argentea*, a C_3 plant. He also found that at day/night temperatures of 30/20°C, the total DM of leaves was 11.53 g $plant^{-1}$ for *A. cruentus* compared to 8.06 g $plant^{-1}$ for *C. argentea*. In general, the maximum dry matter production of C_4 plants is 303 ± 138 kg ha^{-1} day^{-1} compared to 195 ± 39 kg ha^{-1} day^{-1} for C_3 plants (Black, 1973).

SALINITY

Physiological responses

Previous studies on physiological response of salt-stressed amaranths have shown that glycinebetaine, an osmoprotectant, accumulated in *Amaranthus caudatus* (Russell *et al.*, 1998) and *A. tricolor* leaves (Wang *et al.*, 1999; Wang & Nii, 2000). This osmotic agent is a good indicator of plant salt tolerance (Hayashi *et al.*, 1997; Takabe *et al.*, 1998). The decrease in the ratio of absorbed potassium to sodium in salt-stressed *A. tricolor* was also considered as a good indicator of salt tolerance (Shimose *et al.*, 1991).

Salinity adversely affects photosynthesis, primarily due to reduction in stomatal conductance (closure of stomata), causing a decreased CO_2 availability (Dubey, 1997). With a short-term salt stress, a reduction in stomatal conductance of amaranth leaves reduced photosynthesis and transpiration rates (Wang & Nii, 2000). According to Plaut (1995) the reduction in stomatal conductance can be caused by a combination of osmotic and specific Na^+ effects. On the other hand, sodium can stimulate

photosynthesis in dicotyledonous C_4 plants such as *A. tricolor* (Murata *et al.*, 1992).

Growth responses

Most leafy vegetables are sensitive to saline root media which may reduce their market value. Salinity effects include reduced plant growth and stunted plants due to shorter and fewer internodes (Shannon *et al.*, 1993; Shannon *et al.*, 1994), changes in stem diameter (Poljakoff-Mayber, 1975), smaller and fewer leaves, and changes in root thickness and shoot:root ratios (Shannon & Grieve, 1999). The effect of salinity on flowering depends on the species and nutritional level. This can be either delayed or promoted (Shannon *et al.*, 1994). These effects are quantitatively dependent on the concentration and type of salt involved, the duration of exposure to the stress and on environmental conditions (Shannon *et al.*, 1994; Bernstein & Kafkafi, 2002).

Under salinity conditions, at low salt concentration, plant growth decreases and the detrimental effect becomes lethal at high salt concentrations (Shannon *et al.*, 1993; Shannon *et al.*, 1994). Strogonov, 1962 cited by Poljakoff-Mayber (1975) found that cotton stems were thicker when chloride (Cl^-) was the dominant ion and when sulphate (SO_4^{-2}) was the dominant ion, the stems were thinner. Salt injury symptoms can develop when high concentrations of Na^+ or Cl^- accumulate in leaves, resulting in scorching or firing of leaves (Shannon & Grieve, 1999; Munns, 2002). Lettuce leaves may develop calcium deficiency symptoms under high SO_4^{-2} levels in nutrient solutions (Shannon *et al.*, 1994).

The time period that plants can withstand a salt stress without a significant reduction in quality and yield is an important parameter. The earliest response of a glycophyte to salinity is reduced leaf growth; this is due to a short-term water stress after which the plants adjust osmotically (Munns & Termaat, 1986). On long-term exposure to salinity conditions,

the growth rate declines and leaf mortality may increase (Yeo *et al.*, 1991). These adverse effects are associated with excessive salt concentrations in the leaves, as well as leaf death rate higher than the new leaf development rate, resulting in a reduced photosynthetic area and inadequate support for continued growth (Munns & Termaat, 1986).

Environmental conditions also affect salt tolerance. With cool and humid conditions plants are more tolerant than with hot and dry conditions (Shannon *et al.*, 1994; Shannon & Grieve, 1999). Moreover, the reduction in plant growth under saline conditions is aggravated at high temperatures, low relative humidity and high irradiance due to high transpiration rates (Shannon *et al.*, 1994).

The shoot:root ratio is generally used to indicate the allocation and partitioning of photosynthates in plants. The shoot:root ratio often decreases when plants grow under saline conditions because root growth is usually less sensitive to salt stress than shoot growth (Rawson *et al.*, 1988). Under high illumination conditions, the shoot:root ratio of a halophytic C_4 grass was not affected by NaCl stress (Kemp & Cunningham, 1981). The shoot:root ratio of *Sorghum bicolour* (C_4 plant) increased in response to an increased salt stress (Yang *et al.*, 1990). In many glycophytes, the dry:fresh-weight ratio may also increase after osmotic adjustment (Shannon *et al.*, 1994).

Calcium and protein response

Leaf calcium may be adversely affected under saline conditions, especially with high Na:Ca ratios (Shannon & Grieve, 1999). High concentrations of Na^+ in nutrient solutions may limit Ca^{+2} supply to roots (Bernstein & Kafkafi, 2002), consequently lowering calcium levels in leaves. Ho & Adams (1994) found a reduction in Ca uptake in cucumber plants with an increase in salinity over a range of 3 to 8 mS cm^{-1}.

Salinity may adversely affect protein synthesis (Dubey, 1994). In salt stressed plants, the total protein level may

decrease or increase, depending on the part of the plant (Dubey, 1994). In general, protein levels drop due to a decreased synthesis of protein as well as an increased metabolic activity (Dubey, 1994). The synthesis of new salt-induced protein increased with a concomitant decrease in the level of existent proteins. These proteins appear to act as osmoprotectants, providing tolerance or adaptation to the plant (Dubey, 1994).

Adaptation to salinity

By exposing plants to saline growing conditions, they may adapt to these conditions. Amzallag *et al.* (1990) state that adaptation is achieved when the growth rate of an exposed plant is restored to a value similar to the growth rate of a control plant or when it has acquired the capacity to complete its life cycle. The adaptation to salinity involves complex mechanisms such as osmotic adjustment by accumulation of compatible solutes such as glycinebetaine, proline and polyols (Yeo, 1998). Ghoulam *et al.* (2002) found an accumulation of inorganic ions such as Na^+, K^+ and Cl^- in leaves involved in the osmotic adjustment in sugar beet cultivars under saline conditions.

The process of photosynthesis plays an important role in the plant's adaptation to salinity. C_4 plants have an advantage due to their greater CO_2 fixation capacities, allowing the production of compatible solutes used for osmotic adjustment (Cushman *et al.*, 1990). The adaptation to salt stress involves changes in the plant's behaviour (Amzallag *et al.*, 1990) mainly in morphological characteristics and biomass allocation (Heuer, 1997). The allocation and partitioning of photosynthases provide resources for adaptation to stress (McCree, 1986).

Evaluation of the problem

Soils in arid and semi-arid regions tend to be saline containing dominantly sodium sulphate (Na_2SO_4) and NaCl (Szabolcs, 1994). Soil salinity may drastically affect crop productivity (Dubey, 1994). Yield reductions are mainly caused by Na salts, particularly NaCl (Plaut, 1995). It may be difficult to satisfy the demand for food in affected areas because of the detrimental effect on the productivity of crops. Due to the allocation of quality water for urban and industrial use, there is a decline in the availability of good quality water for agriculture and food production. Pasternak & De Malach (1994) suggested that saline water with an appropriate ionic composition and EC of up to 10 dS m^{-1} be used to irrigate a range of saline tolerant crops as a solution to minimise this problem.

To achieve good food production in arid and semi-arid regions, the priority should be to select salt tolerant crops, best suited for hot climates. Amaranths may be salt tolerant (Wang *et al.*, 1999; Wang & Nii, 2000) and a good choice for crop production in warm regions. This study was planned to gather more information in this field since the performance of amaranths under saline conditions has not been properly investigated yet.

Object of this study

The aim of this study was to test the tolerance of *Amaranthus tricolor* to increasing salinity levels and to adopt harvesting procedures in order to optimise yield and quality under these conditions.

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CHAPTER 2

EFFECTS OF SALINITY AND HARVESTING STAGE ON THE GROWTH AND DEVELOPMENT OF *Amaranthus tricolor* L.

ABSTRACT

Seedlings of *Amaranthus tricolor*, also known as African spinach, were raised in seedling trays in a greenhouse. After four weeks, single seedlings were transplanted into containers filled with five litres of river sand and placed outdoors, using a "drain to waste" hydroponics system. Plants were fertigated 4 to 6 times per day, using pressure-compensating drippers (2 *l* h^{-1}). Four tanks were used to supply nutrient solutions at electrical conductivity (EC) levels of 1, 2, 4 and 8 mS cm^{-1}. The plants were harvested by uprooting at two harvesting stages, 30 and 45 days after transplanting (DAT). This 2x4 factorial experiment was replicated seven times using a fully randomised block design. The number of leaves and side-shoots, leaf moisture content, dry mass of leaves and side-shoots as well as leaf calcium and protein contents were monitored. Interactions between EC levels and harvesting dates affected most of the parameters. The optimum EC level for a high shoot:root ratio was 4 mS cm^{-1} at 30 DAT compared to an EC of 8 mS cm^{-1} which improved the shoot:root ratio at 45 DAT. Increased EC levels lowered leaf calcium concentrations and delayed flowering, but increased leaf protein contents.

INTRODUCTION

Since problems with saline soils have increased in arid and semi-arid regions (Szabolcs, 1994), coinciding with a decline in the availability of good quality water for agriculture, more information is needed about salt tolerant crops. *Amaranthus tricolor* L. is a glycophyte and a C_4 dicotyledonous crop, well adapted to arid and semi-arid regions. Salt tolerance has been

reported for *A. tricolor* (Shimose *et al.*, 1991; Wang *et al.*, 1999; Wang & Nii, 2000) and may thus be one of the best crops for production in salt affected regions. Very little is known regarding this crop's tolerance to saline conditions.

The period that glycophyte plants are exposed to salt stress is very important because they have different responses to adapt to salinity. Short-term exposure to salinity reduces growth rate, which can recover gradually to a new reduced growth rate (Munns, 2002). On long-term exposure, the plant may die due to excessive salt accumulation in the oldest leaves, causing older leaves to die more rapidly than new leaves are formed (Munns & Termaat, 1986; Munns, 2002). Species, salt concentration and type of salt as well as climatic conditions may affect the adaptation rate of saline tolerant crops (Shannon *et al.*, 1994; Bernstein & Kafkafi, 2002). This may happen after days, weeks or months.

Grubben (1976) states that where amaranths are harvested by uprooting, it should be done at 42 days after transplanting (DAT) to obtain the best yield under normal conditions. This study was done to evaluate how *A. tricolor* respond to increasing salinity at two growth stages and how salinity levels can be affected by harvesting time.

MATERIALS AND METHODS

Plant material

Seeds of *A. tricolor,* obtained from the ARC-Roodeplaat, were sown (1 October 2002) in seedling trays filled with a 1:1:1 mix of vermiculite, composted pine bark and Hygrotech seedling mix. Seedlings were grown in a plastic-covered seedling house at a mean temperature of 20.6°C. The minimum night temperatures ranged from 10.0°C to 18.0°C and maximum day temperatures ranged from 24.0°C to 40.0°C. The relative humidity varied from 50% to 90%. Transplanting was done 21 days after more than 50% of the

seedlings had emerged (31 October 2002). One seedling was transplanted into 5 litres plastic bags, filled with washed river sand, with 12 mm drainage holes, 25 mm from the base. The sand was sterilised with methyl bromide before bags were filled. At transplanting the seedlings had two pairs of open and one pair of closed true leaves. The plastic bags were placed in double rows, spaced at 25 x 35 cm. The experiment was done outdoors, using a "drain to waste" hydroponics system. The plants grew at a mean temperature of 21.1°C. The minimum night and maximum day temperatures ranged from 8.0°C to 23.0°C and 20.0°C to 39.0°C, respectively.

Treatments

Four levels of electrical conductivity; 1, 2, 4 and 8 mS cm^{-1} were combined with two growth periods. Plants were harvested at 30 days after transplanting (30DAT) and at 45 days after transplanting (45DAT). The concentrations of macro-elements in the nutrient solutions were prepared, based on the formulation of Steiner (1984). Sodium chloride (NaCl) was included at the two high EC levels (Table 2.1). The plants were subjected to these treatments from transplanting to harvesting.

Table 2.1. Macro element concentrations (meq l^{-1}) in nutrient solutions used for the different electrical conductivity (EC) treatments

EC (mS cm^{-1})	Na^+	K^+	Ca^{++}	Mg^{++}	NO_3^-	$H_2PO_4^-$	$SO_4^{=}$	Cl^-
1.0	0.0	3.5	4.5	2.0	6.0	0.5	3.5	0.0
2.0	0.0	7.0	9.0	4.0	12.0	1.0	7.0	0.0
4.0	10.0	10.5	13.5	6.0	18.0	1.5	10.5	10.0
8.0	35.0	15.8	20.2	9.0	27.0	2.2	15.8	35.0

Fertigation

The plants received nutrient solution through an irrigation system, using pressure-compensating drippers (2 l h^{-1}). The schedule of irrigation from transplanting up to 11 DAT was 4 times per day for 2 minutes each, corresponding to 267 ml $plant^{-1}$

day^{-1}. This was increased to 2.5, 3.0 and 4.5 minutes, corresponding to 333, 400 and 900 ml plant^{-1} day^{-1} at 12, 27 and 35 DAT, respectively. Irrigation frequency was increased to 6 times per day at 35 DAT. The pH of the solutions varied from 5.3 to 5.7.

Experimental design

Two growth periods and four salt concentrations in nutrient solutions were used as treatments. The experimental design was a randomised complete block with a 2x4 factorial arrangement with seven replicates. An experimental unit consisted of one plant. Duncan´s New Multiple Range Test (DMRT) was used to compare treatment means. The data were analysed using the MSTAT-C Version 1.2 computer program.

Measurements

Length, diameter and numbers

At harvesting the length and diameter of the main stem were measured and the number of leaves as well as all the side-shoots longer than 2 cm were counted. The number of internodes was also counted on the main stem and the average internode length was calculated.

Moisture content and yield

The fresh weight (FW) of leaves and stems (main stem and side-shoots) was measured and dry mass (DM) was determined at harvesting time after drying at 80°C for 48 hours. Using the difference between FW and DM of leaves, the percentage (%) leaf moisture content was calculated. The yield of leaves and stems were calculated per plant (g plant^{-1}). The ratio between leaf and stem weights (DM basis) was calculated to determine the leaf:stem ratio (LSR).

Biomass and growth rate

The DM of shoots (leaves and stems) was calculated by adding leaf and stem weights on a DM basis. Since the DM of roots was determined, the allocation and partitioning of photosynthates in the plant was monitored with the calculated shoot:root ratio (SRR). The growth rate was calculated, using the following equation (Greenwood *et al.*, 1977):

$$kt = \ln(w) + w - \ln(w_0) - w_0$$

where:

k = growth rate coefficient in t ha^{-1} day^{-1};

t = time in days;

w = total dry weight of plant in t ha^{-1};

w_o = weight of seeds sown;

ln = natural logarithm.

Mineral analyses

Leaf calcium and nitrogen contents were analysed at the laboratory of the Department of Agriculture, Western Cape at Elsenburg (RSA). The leaf crude protein content was calculated by multiplying nitrogen content with 6.25. By multiplying leaf yield with leaf calcium and crude protein content, the leaf calcium and protein yields were calculated, respectively.

Flowering

The first flowers appeared on some of the plants about 35 DAT. Index values were used to determine flowering at 45 DAT. The following index values were used: 1 = flower signs; 2 = some flowers; 3 = many flowers. The flower weights were included in stem weights at 45DAT.

RESULTS AND DISCUSSION

General results

The growth and yield of plants fertigated with the EC1 nutrient solution were inferior to all other EC treatments tested. It is therefore clear that a low EC is not suitable to produce *A. tricolor* in a hydroponics system. Although lettuce is grown at an EC of 1 mS cm^{-1}, most crops grow well in nutrient solutions at a concentration of around 2 mS cm^{-1} (Kreij *et al.*, 1999).

At 20 DAT, the plants fertigated with the EC4 and EC8 treatments started to develop stress symptoms on leaves in the afternoon. This happened at air temperatures higher than 30°C, but the plants recovered during the nights. These symptoms could have been caused by an osmotic effect or by the accumulation of Na^+ and/or Cl^- ions in leaves at high temperature (Shannon *et al.*, 1994; Shannon & Grieve, 1999).

Significant interactions were found between electrical conductivity (EC) and growing period for all variables studied, except leaf calcium and leaf crude protein contents (See ANOVA; Table 1 in Addendum).

Length and diameter of main stem

Main stem length was significantly reduced with EC levels beyond EC2 at 45DAT only (Figure 2.1). No significant differences were observed between EC1 and EC2 or between EC4 and EC8. A significant reduction in plant height was also found in pepper (Chartzoulakis & Klapaki, 2000) and tomato plants (Shannon *et al.*, 1993b) with an increase in salinity. The short stems observed at 30DAT are common for this specie (Oomen & Grubben, 1978). At 30DAT, the largest main stem diameter was found at EC4 (Figure 2.2), but at 45DAT, the largest main stem diameter was achieved at EC2. Poljakoff-Mayber (1975) is of the opinion that more information is needed to describe the effect of salinity on the structure of the stem.

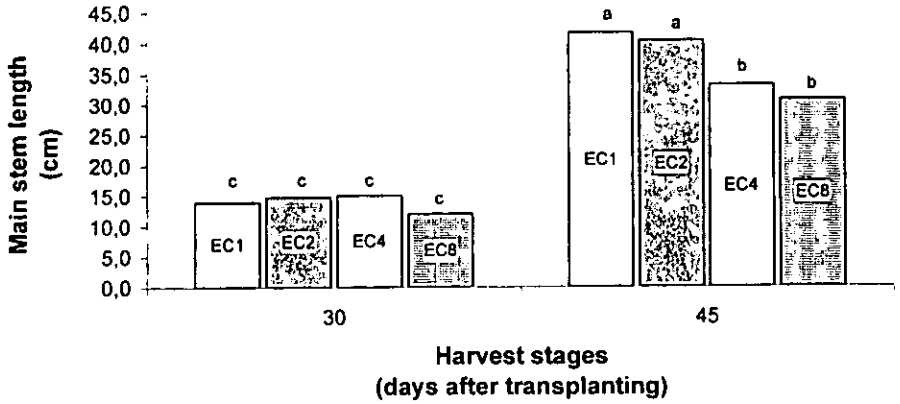

Figure 2.1. Main stem lengths of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 12.45%

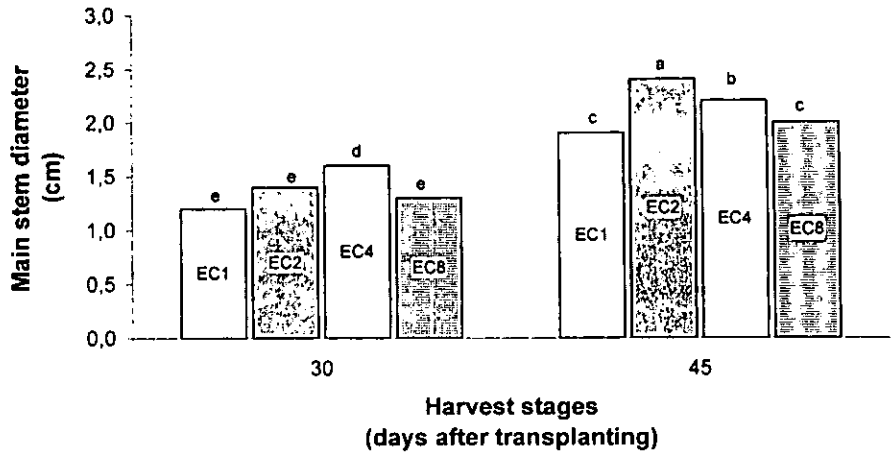

Figure 2.2. Main stem diameters of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 10.68%

Number of leaves, side-shoots and internodes

With both growth periods, the number of leaves decreased with EC levels higher than EC4 (Figure 2.3) but no significant difference was observed between EC2 and EC4. The fact that EC8 did not produce more leaves than EC1 at 30DAT compared to significantly more leaves with EC8 at 45DAT, may be an indication that it took longer than 30 days for the plants to

adapt to the high salinity level. The low leaf numbers at the EC8 level compared to EC2 and EC4, for both growth periods, is a typical plant response to salinity (Shannon & Grieve, 1999; Munns, 2002). According to Heuer (1997) the reduction in the number of leaves may be a mechanism used by plants to adapt to salinity. However, with an increase in NaCl concentrations up to 200 mM, the leaf number in five cultivars of sugar beet, considered to be relatively salt tolerant, was less affected than leaf area, FW and DW of leaves and roots (Ghoulam *et al.*, 2002).

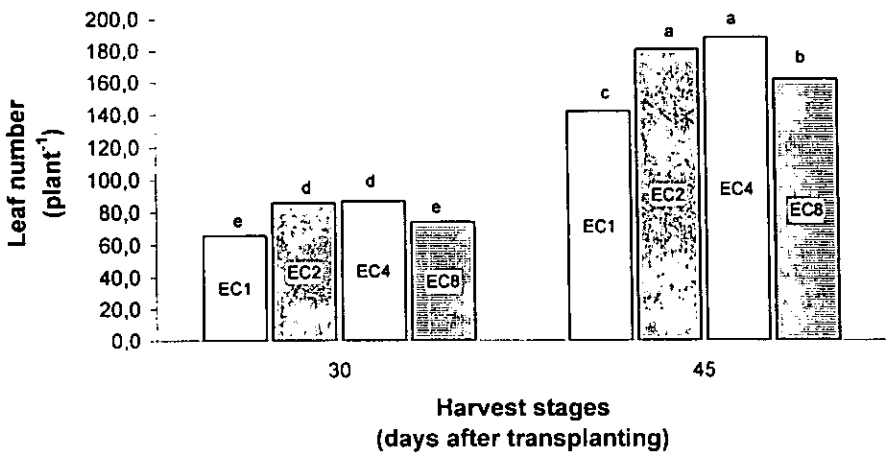

Figure 2.3. Leaf numbers of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 8.47%

With the long growth period (45DAT), the highest number of side-shoots was found at EC2 and EC4 (Figure 2.4). By comparing the EC1 and EC8 treatments, it is clear that side-shoot development did not differ at 30DAT, but significantly more side-shoots developed at EC8 than at EC1 with the longer growth period (Figure 2.4). The poor side-shoot development at EC1 could be attributed to the associated low nutrient levels. Side-shoot development was reduced at EC levels higher than EC4 at 45DAT. According to Munns (2002) salt stressed plants produce less side-shoots during long-term exposure. On the other hand,

the low side-shoot number at EC1 may be due to the long main stem at 45DAT (Figure 2.1). This is in agreement with the "Nutritive Theory" of apical dominance (Wareing & Phillips, 1981). At low mineral (particularly nitrogen) nutritional levels, one may expect apical dominance, causing an increase in stem length and a reduction in side-shoot numbers.

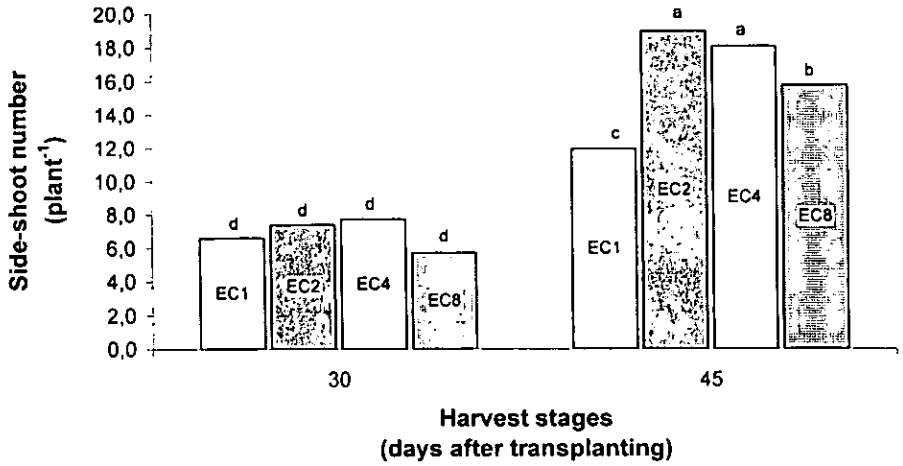

Figure 2.4. Side-shoot numbers of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 15.68%

At 45DAT, the number of internodes decreased slightly at EC levels higher than EC1 (Figure 2.5). At 30DAT, the length of internodes hardly differed, although the EC8 treatment produced shorter internodes than EC1 (Figure 2.6). However, at 45DAT, internode lengths were shorter with EC4 and EC8 compared to EC1 and EC2. Shorter internodes are typical salinity effects on crops (Shannon *et al.*, 1993a; Shannon *et al.*, 1994).

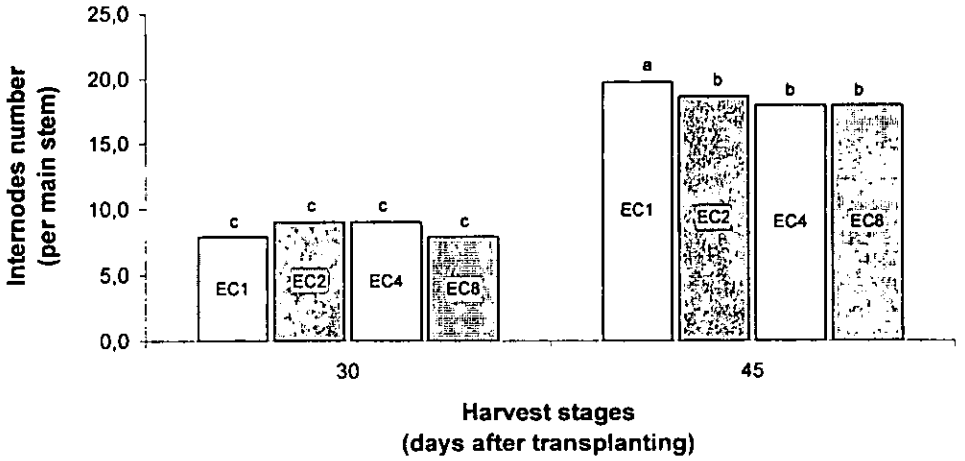

Figure 2.5. Internode numbers on the main stem of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 7.81%

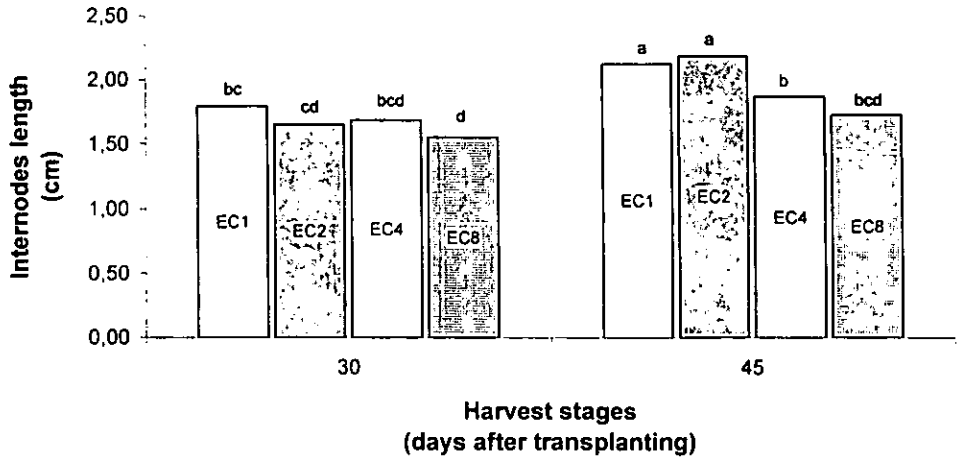

Figure 2.6. Internode lengths of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 9.03%

Leaf moisture content, leaf and stem dry yields and leaf:stem ratio

The leaf moisture content decreased with a longer growth period (45DAT). No significant difference was found among EC treatments at 30DAT, but at 45DAT, the highest leaf moisture content was found at EC4 and EC8 (Figure 2.7). This increase in

leaf water content at high EC levels is a typical morphological response to salinity in dicotyledonous species (Shannon *et al.*, 1994). According to them, this is an adaptation to plants that minimizes the negative effects of high salt concentrations in leaves.

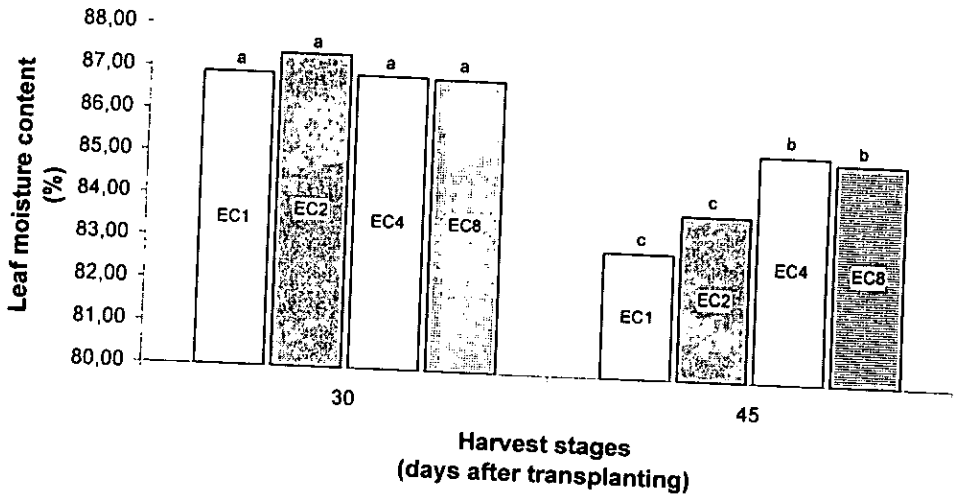

Figure 2.7. Leaf moisture content of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 1.06%

With both growth periods, the leaf dry yield increased with increases in EC, up to EC4, but decreased with further increases in EC to EC8 (Figure 2.8). However, at the EC8 level, the relative leaf dry yield gain from 30DAT to 45DAT was higher than at EC2. The gain in leaf dry yield from 30DAT to 45DAT (Figure 2.8) may be due to an increase in the production of compatible solutes for osmotic adjustment (Yeo, 1998) associated with a restriction in the production of leaves (Figure 2.3). This result suggests that *A. tricolor* needs at least 45 days to adapt to salinity.

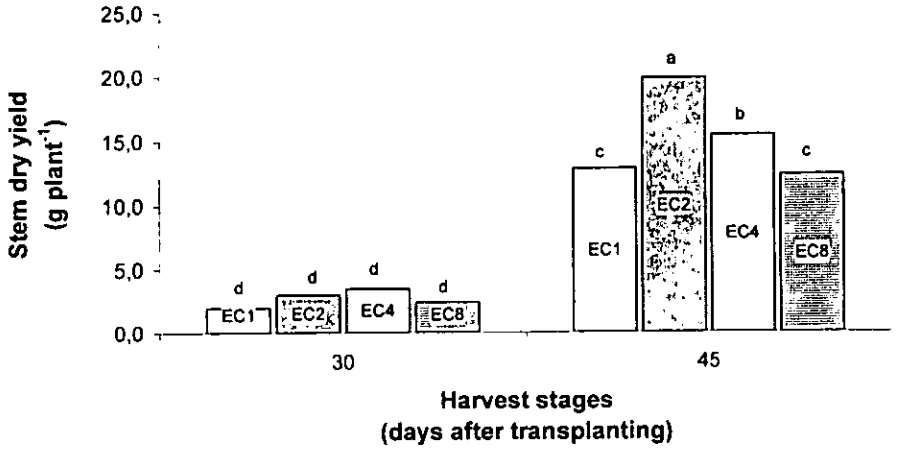

Figure 2.9. Stem dry yield of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 18.31%

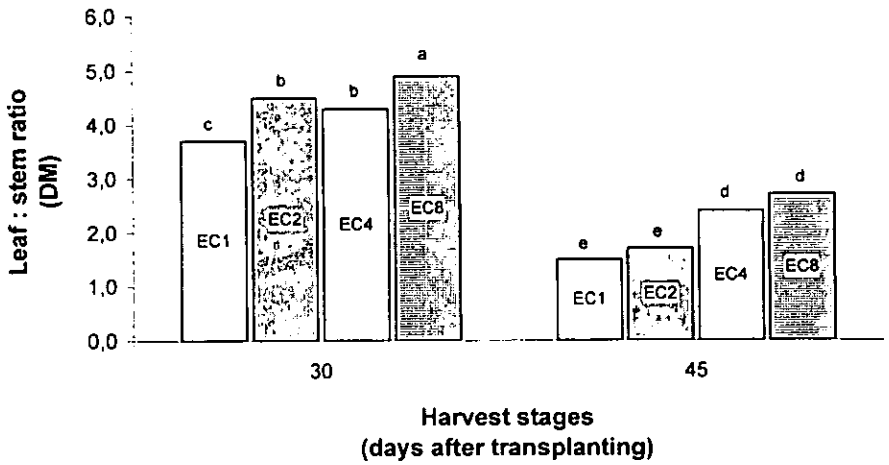

Figure 2.10. Leaf:stem ratios (DM-basis) of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 11.40%

Shoot dry mass, root dry mass, shoot:root ratio and growth rate

Apart from poor growth at the lowest EC level (EC = 1 mS cm^{-1}), found at 30DAT and 45DAT, dry mass of shoots (leaves and stems) decreased when EC levels exceeded EC4 (Figure 2.11). No significant difference was found between EC2 and EC4 levels at both growth stages. A different response was found with root dry

mass. Only at 45DAT, the root dry mass decreased significantly with each increase in EC level (Figure 2.12). It is possible that low nutrient levels may stimulate root growth to the detriment of shoot growth. This is illustrated by the decrease in root dry mass with an increase in EC from EC1 to EC2 (Figure 2.12) while shoot dry mass increased at both growth stages (Figure 2.11).

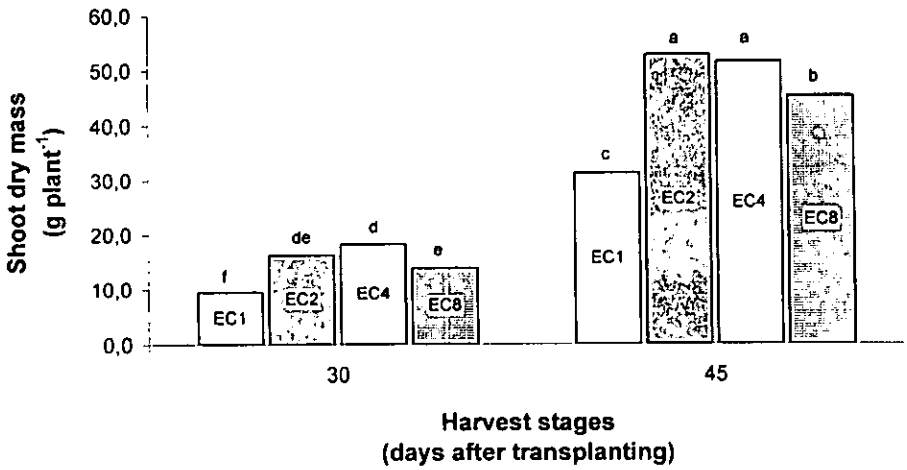

Figure 2.11. Shoot dry mass (leaves and stems) of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 8.29%

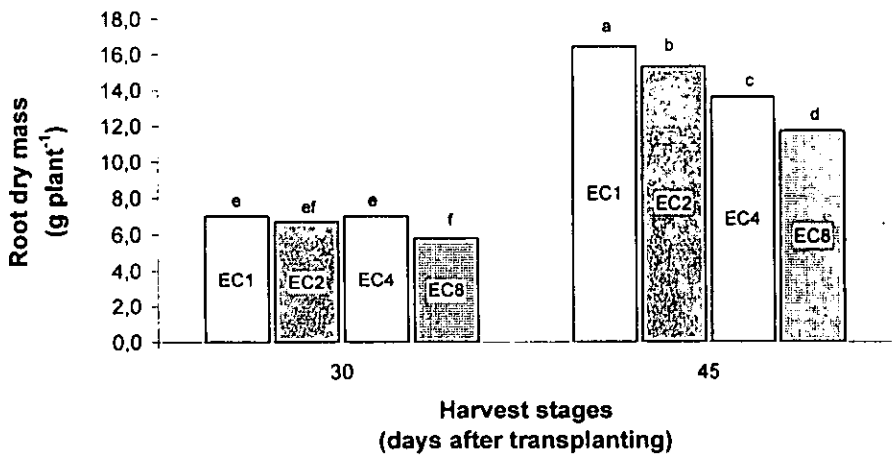

Figure 2.12. Root dry mass of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 9.02%

The shoot:root ratio increased at both growth stages with an increase in EC up to the EC4 level (Figure 2.13). At 30DAT a further increase up to EC8 significantly lowered the shoot:root ratio but the ratio was unaffected at 45DAT. The allocation of photosynthates to the shoot, rather than to roots, improved at EC8 with a longer growth period. Yang *et al.* (1990) found an increase in shoot:root ratio in response to an increased salt stress with *Sorghum bicolor*, a C_4 plant. In contrast Rawson *et al.* (1988) found that the shoot:root ratio often decreases under saline conditions because root growth is usually less sensitive to salt stress than shoot growth.

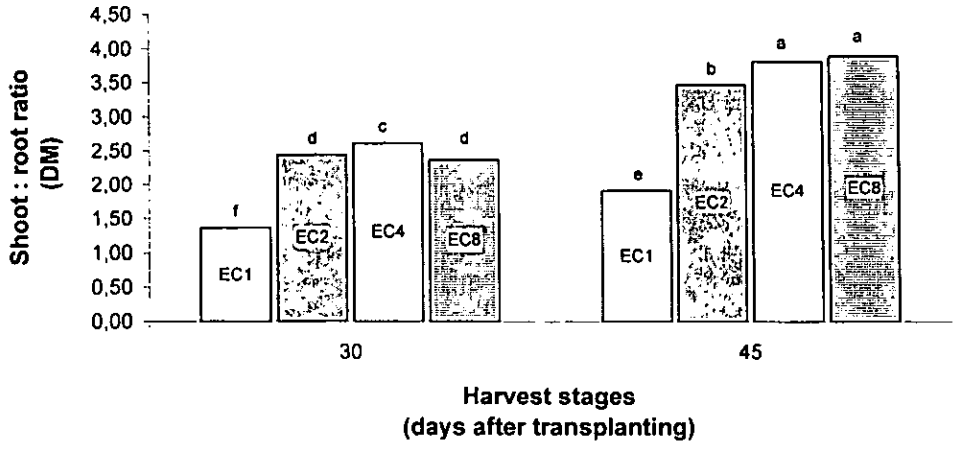

Figure 2.13. Shoot:root ratio (DM-basis) of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 5,64%

With both growth periods, growth rates were not significantly affected by increases in EC from EC2 to EC4 (Figure 2.14). However, a reduction in growth rate occurred at EC levels higher than EC4 in both cases. This reduction is a typical plant response to salinity (Munns, 2002).

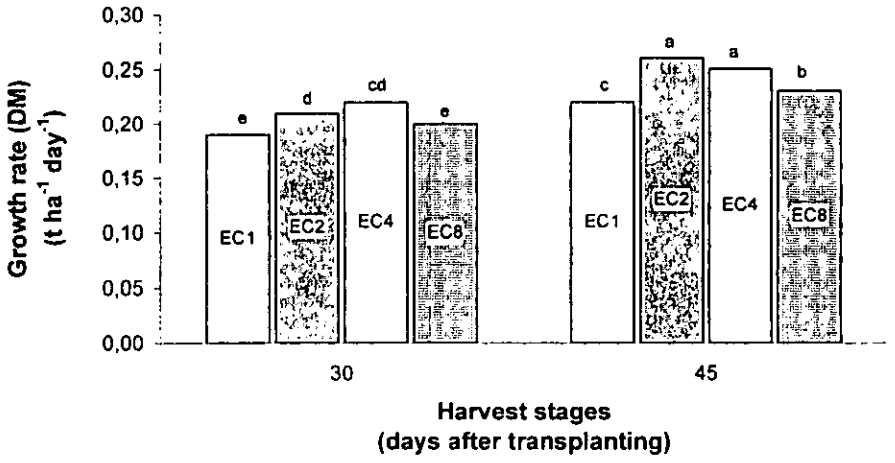

Figure 2.14. Growth rate of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 3.03%

Leaf composition and leaf crude protein

Only the main effects of EC and growth period were significant for leaf calcium and crude protein concentrations (Table 2.2). The leaf calcium content decreased with EC levels higher than 2 mS cm^{-1}, most probably due to the increase in Na^{+} concentration in the nutrient solutions (Table 2.1) and insufficient Ca^{+2} supply to the roots (Bernstein & Kafkafi, 2002). Ho & Adams (1994) also found a reduction in Ca uptake in cucumber with an increase in EC over a range of 3-8 mS cm^{-1}. An increase in leaf calcium content was found with the longer growth period.

Increased levels of salinity did not affect leaf protein content negatively (Table 2.2). Leaf crude protein content improved with an increase in EC, probably due to an increase in the production of osmoprotectants in leaves (Wang *et al.*, 1999; Wang & Nii, 2000) associated with an increased nitrogen concentration in the nutrient solutions (Table 2.1). According to Dubey (1994) some proteins appear to act as osmoprotectants, providing tolerance to salinity or enabling the plant to adapt. High leaf crude protein contents were found at 30DAT (Table

2.2). The protein level was lower at 45DAT, probably due to a decrease in protein synthesis as well as an increased metabolism (Dubey, 1994).

Table 2.2. The effects of electrical conductivity (EC) of nutrient solutions and growing period on leaf calcium and crude protein contents of *Amaranthus tricolor*.

Treatments	Leaf composition	
	Calcium (%)	**Crude protein (%)**
EC (mS cm^{-1})		
EC1	2.99 ab	25.78 d
EC2	3.12 a	28.15 c
EC4	2.88 b	31.28 b
EC8	2.40 c	33.31 a
LSD (P = 5%)	0.220	1.09
Growth period		
30 days	2.58 B	30.57 A
45 days	3.11 A	28.69 B
Probability [z]	**	**
CV (%)	10.14	4.82

Means followed by the same letters are not significantly different at 5% level probability (DMRT)
z ** = P<0.01

The leaf calcium yield increased for both growth periods with an increase in EC from 1 to 4 mS cm^{-1}, but decreased with a further increase to EC8 (Figure 2.15). At EC8, the leaf calcium yield was higher than at EC1 but only at 45DAT. Leaf calcium yield did not differ between EC2 and EC4 for both growth periods. This was due to a higher leaf dry weight but lower calcium concentration at EC4 than at EC2 (Figure 2.8 and Table 2.2).

At 30DAT the leaf protein yield increased with an increase in EC up to EC4 and then decreased significantly at EC8 (Figure 2.16). With the longer growth period, leaf protein yields were higher and did not differ significantly between the EC4 and the EC8 treatments at 45 DAT. Since leaf dry yield was higher at EC4

than at EC8 (Figure 2.8), the relatively high leaf protein yield at 45DAT was probably due to the higher protein concentration (Table 2.2).

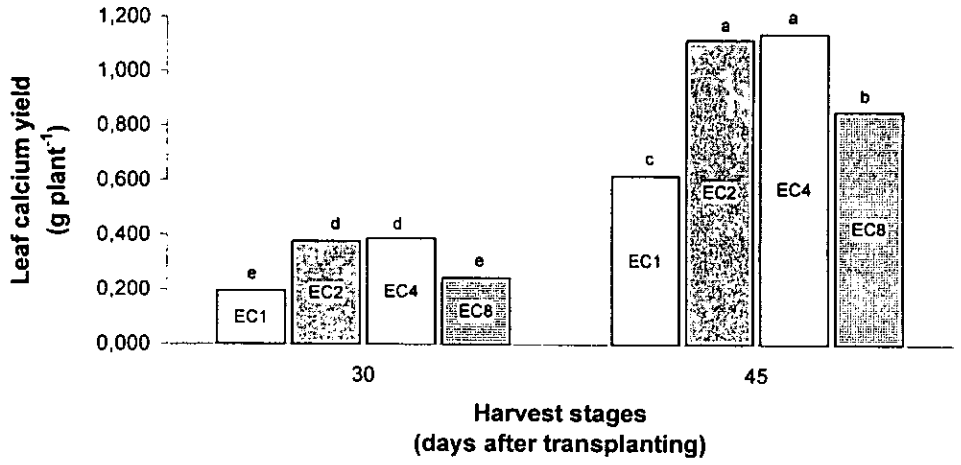

Figure 2.15. Leaf calcium yield of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 8.58%

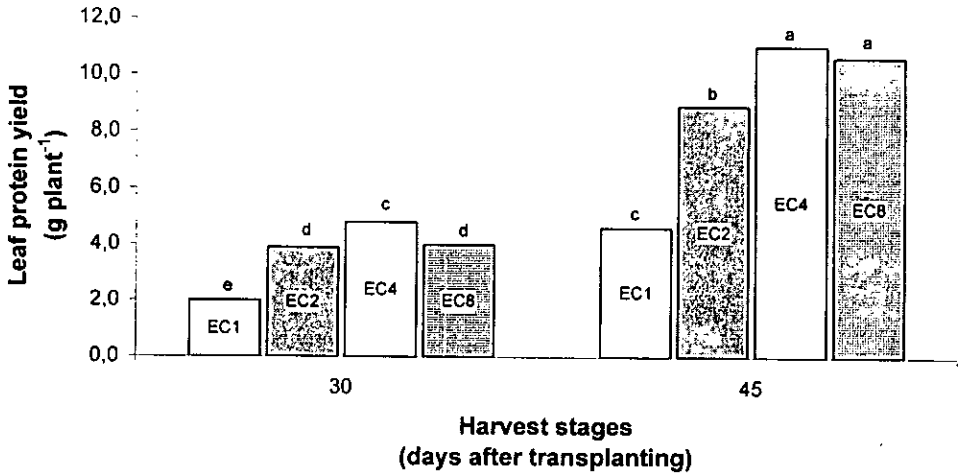

Figure 2.16. Leaf protein yield of *Amaranthus tricolor* in response to electrical conductivity (EC) of nutrient solutions and growth period.
Means with a common letter are not significantly different at the 5% level.
EC treatments shown in Table 1.
CV = 6.83%

Flowering

Flowering started about 35DAT. Most flowers developed on the plants grown with low EC nutrient solutions (Table 2.3). Saline

conditions therefore delayed flowering in *A. tricolor*. This delayed flower formation with an increase in EC appears to be a typical response of a salt-tolerant plant to salinity (Munns, 2002). The delayed flowering could have been caused by nitrogen concentrations that increased in nutrient solutions at higher EC levels (Table 2.1). According to Wareing & Phillips (1981) high levels of nitrogen tend to promote vegetative growth.

Table 2.3. Flowering index at 45 days after transplanting.

EC (mS cm^{-1})	Flowering index [y]
EC1	3
EC2	2
EC4	1
EC8	1

y 1. flower signs; 2. some flowers; 3. many flowers

CONCLUSIONS

Although values for most of the measured variables increased with an increase in growth period, the effects of the electrical conductivity of nutrient solutions used to fertigate *Amaranthus tricolor*, differed among variables and between harvesting stages. As potential salt tolerant leafy vegetable, it was encouraging that the best leaf yield was produced with a nutrient solution at an EC of 4 mS cm^{-1}. This was also the optimum EC for leaf protein and leaf calcium yields. The leaf:stem ratio increased with an increase in EC up to 8 mS cm^{-1}. The highest shoot:root ratio was achieved at an EC of 4 mS cm^{-1} at 30DAT, but at 45DAT the best shoot:root ratio was found at both high EC values, 4 mS cm^{-1} and 8 mS cm^{-1}, a further indication that this crop is well-adapted to saline conditions.

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CHAPTER 3

THE EFFECT OF CUTTING HEIGHT ON THE GROWTH OF *Amaranthus tricolor* L., FERTIGATED WITH NUTRIENT SOLUTIONS AT DIFFERENT SALT CONCENTRATIONS

ABSTRACT

Seedlings of *Amaranthus tricolor* were raised in trays in a greenhouse. After four weeks, single seedlings were transplanted into containers with five litres of river sand and placed outdoors, using a "drain to waste" hydroponics system. Plants were fertigated 4 to 6 times per day, using pressure-compensating drippers (2 *l* h^{-1}). Four tanks were used to supply nutrient solutions at electrical conductivity (EC) levels of 1, 2, 4 and 8 mS cm^{-1}. Two cutting heights were used. At 25 days after transplanting (DAT) the first harvest was done by topping the plants at 25% or 50% of their heights. The second harvest was done at 35 DAT and all side-shoots longer than 2 cm were also topped at 25% or 50%. With the third harvest at 45 DAT all of the remaining aboveground material was removed. This 2x4 factorial experiment was replicated seven times using a fully randomised block design. The number of leaves and side-shoots, dry mass of leaves and side-shoots, and leaf calcium and protein contents were monitored as growth parameters. The highest leaf mass, for both cutting heights, was produced with a nutrient solution at an EC of 4 mS cm^{-1}. Only at the highest EC level the 25% topping height produced significantly higher leaf yields than the 50% harvesting method. This harvesting procedure allowed the EC 8 mS cm^{-1} salt-stressed plants to regain growth rates and protein yields, equal to those at EC 2 and 4 mS cm^{-1}.

INTRODUCTION

Previous studies on the physiological response to salt-stress have indicated that *Amaranthus tricolor* L. can tolerate saline conditions (Wang *et al.*, 1999; Shimose *et al.*, 1991). A study on the agronomic response of salt-stress was discussed in the previous chapter, where *A. tricolor* was harvested by uprooting. The best results were found when the plants were grown for 45 days and fertigated with an electrical conductivity of 4 mS cm^{-1} in a hydroponics system. However, *A. tricolor* is commonly harvested by topping, allowing repeated harvesting. Depending on the cutting height, the growth rate may be greatly reduced due to the removal of photosynthetic active young leaves (Auwalu & Tenebe, 1997) and the reduction in carbohydrate reserves in the remaining plant (Mnzava & Masam, 1985). Good results were reported with 20 to 25 cm cutting heights (Grubben, 1976; Mnzava & Masam, 1985; Norman & Shongwe, 1993).

All plant types have specific root-shoot ratios. After removal of the first leaves, the allocation of photosynthates change, associated with a reduction in dry matter accumulation (Poljakoff-Mayber & Lerner, 1994). Klepper (1991) found that after a certain time, the ratio might be restored, probably due to an increased growth of damaged shoots (leaves and stems). The question is: Do plants grown under different salinity conditions have different harvesting needs? In order to answer this question, *A. tricolor* was grown at four different electrical conductivity (EC) levels and were harvested using two cutting heights (CH). Morphological characteristics, yield and calcium and crude protein content of leaves were monitored.

MATERIALS AND METHODS

Plant material

Seeds of *A. tricolor,* obtained from the ARC-Roodeplaat, were sown (1 October 2002) in seedling trays filled with a 1:1:1 mix of vermiculite, composted pine bark and Hygrotech seedling mix. Seedlings were grown in a plastic-covered seedling house at a mean temperature of 20.6°C. The minimum night temperatures ranged from 10.0°C to 18.0°C and maximum day temperatures ranged from 24.0°C to 40.0°C. The Relative Humidity varied from 50% to 90%. Transplanting was done 21 days after more than 50% of the seedlings have emerged (31 October 2002). One seedling was transplanted into 5 litres of washed river sand in plastic bags with 12 mm drainage holes, 25 mm from the base. The sand was sterilised with methyl bromide before the bags were filled. The seedlings had two pairs of open and one pair of closed true leaves at this stage. The plastic bags were placed in double rows spaced at 25 x 35 cm. The experiment was done outdoors, using a "drain to waste" hydroponics system. The plants grew at a mean temperature of 21.1°C. The minimum night and maximum day temperatures ranged from 8.0°C to 23.0°C and 20.0°C to 39.0°C, respectively.

Treatments

Four levels of electrical conductivity; 1, 2, 4 and 8 mS cm^{-1} were combined with two cutting heights. Plants were topped at 25% and 50% of their heights. The first harvest was done at 25 days after transplanting (DAT) by removing 25% or 50% of the main stem height. The second harvesting was done at 35 DAT and all side-shoots longer than 3 cm were also trimmed back with 25% or 50%. The third harvest was done at 45 DAT (15 December 2002), removing all of the remaining aboveground material.

The concentrations of macro-elements in the nutrient solutions were prepared based on the formulation of Steiner

(1984). Sodium chloride (NaCl) was included at the two high EC levels (Table 3.1). The plants were subjected to these treatments from transplanting till the final harvest.

Table 3.1. Macro element concentrations (meq l^{-1}) in nutrient solutions used for the different electrical conductivity (EC) treatments

EC ($mS \cdot cm^{-1}$)	Na^+	K^+	Ca^{++}	Mg^{++}	NO_3^-	$H_2PO_4^-$	$SO_4^{=}$	Cl^-
1.0	0.0	3.5	4.5	2.0	6.0	0.5	3.5	0.0
2.0	0.0	7.0	9.0	4.0	12.0	1.0	7.0	0.0
4.0	10.0	10.5	13.5	6.0	18.0	1.5	10.5	10.0
8.0	35.0	15.8	20.2	9.0	27.0	2.2	15.8	35.0

Fertigation

The plants received nutrient solution through an irrigation system, using pressure-compensating drippers (2 l h^{-1}). The schedule of irrigation from transplanting up to 11 DAT was 4 times per day for 2 minutes each, corresponding to 267 ml $plant^{-1}$ day^{-1}. The duration per dripping was increased to 2.5, 3.0 and 4.5 minutes, corresponding to 333, 400 and 900 ml $plant^{-1}$ day^{-1} at 12, 27 and 35 DAT, respectively. Irrigation frequency was increased to 6 times per day at 35 DAT. The pH of the solutions varied from 5.3 to 5.7.

Experimental design

Two cutting heights and four salt concentrations in nutrient solutions were used as treatments. The experimental design was a randomised complete block with a 2x4 factorial arrangement with seven replicates. An experimental unit consisted of one plant. Duncan´s New Multiple Range Test (DMRT) was used to compare treatment means. The data were analysed using the MSTAT-C Version 1.2 computer program. An ANOVA is presented in the Addendum as Table 2. Figures were used to illustrate significant interactions. Where no interaction was found, main effects were presented in tables.

Measurements

Plant height, length, diameter and numbers

Plant height was measured at transplanting as well as one day before the first harvest (25 DAT) and the difference was used as plant growth in height. With the second harvest (35 DAT), leaves and side-shoots were monitored and side-shoots (>2 cm) were harvested according to treatments (25% or 50%). After the last harvest (45 DAT), the diameter of the remaining main stem and the length and diameter of new side-shoots longer than 2 cm were measured. The number of internodes on these new side-shoots that developed after the second harvest was counted as well. The total number of open leaves and side-shoots were calculated by adding the leaves counted at the 1^{st}, 2^{nd} and 3^{rd} harvests and the side-shoots longer than 2 cm at the 2^{nd} and 3^{rd} harvests.

Leaf moisture content and yield

The fresh weight (FW) of leaves, main stems and side-shoots were measured and the dry mass (DM) determined at each of the different harvests. Using the difference between total FW and DM of leaves, the leaf moisture content (%) was calculated. The leaf dry yield was calculated per plant by adding all the values measured at different harvesting stages. The same procedure was used to calculate the side-shoot dry yield. By dividing the leaf yield by the side-shoot yield on a DM basis, the leaf:side-shoot ratio (LSR) was calculated. For dry mass determinations, the leaves and side-shoot samples were dried at 80°C for 48 hours.

Biomass (aboveground)

Biomass (aboveground) was calculated by adding leaf yields and side-shoot yields on a DM basis. The growth rate (DM basis) was calculated, using the following equation (Greenwood *et al.*, 1977):

$$kt = \ln(w) + w - \ln(w_0) - w_0$$

Where:

k = growth rate coefficient in t ha^{-1} day^{-1};

t = time in days;

w = total dry weight of plant in t ha^{-1};

w_o = weight of seeds sown;

ln = natural logarithm.

Mineral analyses

Leaf calcium (Ca) and nitrogen (N) contents were analysed at the laboratory of the Department of Agriculture, Western Cape at Elsenburg (RSA). The leaf crude protein content was calculated by multiplying nitrogen content with 6.25. By multiplying leaf yield with leaf calcium and crude protein content, the leaf calcium and protein yields were calculated, respectively.

Flowering

Index values were used to evaluate flowering. The first flowers appeared on some of the plants one week before the last harvest. The following index values were used: 1 = no flower; 2 = signs of developing flowers; 3 = flowers. The flower weights were included in side-shoot weights.

RESULTS AND DISCUSSION

General results

The growth and yield of plants fertigated with the EC1 nutrient solution were inferior to all other EC treatments tested. It is therefore clear that an EC of 1 mS cm^{-1} is too low to produce *A. tricolor* in a hydroponics system. Although lettuce is grown at an EC of 1 mS cm^{-1}, most crops grow well in nutrient solutions at concentrations of around 2 mS cm^{-1} (Kreij *et al.*, 1999).

At 20 DAT, the plants fertigated with the EC4 and EC8 treatments started to show stress symptoms on leaves in the

afternoon. This happened at air temperatures higher than 30°C, but the plants recovered during the nights. These symptoms could have been caused by an osmotic effect or by the accumulation of Na^+ and/or Cl^- ions in leaves at high temperature (Shannon & Grieve, 1999; Shannon *et al.*, 1994).

Plant height, main stem diameter and length and diameter of newly formed side-shoots

The increase in plant height during the first 25 DAT (before the first cutting) was not affected by EC (See ANOVA; Table 2 in Addendum). A significant reduction in plant height was reported where pepper (Chartzoulakis & Klapaki, 2000) and tomato plants (Shannon *et al.*, 1993b) were exposed to increases in salinity. This may indicate that the salinity levels used were not high enough to affect main stem elongation in *A. tricolor* during the first three weeks. The short stems observed at 25 DAT (data not shown) are common for this specie (Oomen & Grubben, 1978).

No significant interaction between electrical conductivity and cutting height (EC x CH) was found for main stem diameter, length and diameter of newly formed side-shoots. As shown in Table 3.2, the diameter of the main stem decreased at EC8, but was not affected by cutting height. Poljakoff-Mayber (1975) noted that due to insufficient information it is difficult to describe how salinity affects the structure of the stem. Apart from poor growth at the lowest EC level (EC 1 mS cm^{-1}), neither length nor diameter of the newly formed side-shoots were suppressed by the higher EC levels or by cutting height treatments (Table 3.2).

Table 3.2. Main effects of the electrical conductivity (EC) of nutrient solutions and cutting height (CH) on the diameter of the main stem and the length and diameter of newly formed side-shoots of *Amaranthus tricolor*.

Treatments	Diameter of main stem base (cm)	Newly formed side-shoots	
		Length (cm)	Diameter (cm)
EC ($mS\ cm^{-1}$)			
EC1	1.60 c	6.0 b	0.63 b
EC2	2.41 a	8.2 a	0.78 a
EC4	2.57 a	8.5 a	0.83 a
EC8	2.01 b	7.8 a	0.80 a
LSD (P = 5%)	0.187	1.27	0.084
Cutting Height			
25%	2.19	7.5	0.75
50%	2.11	7.8	0.77
Probability [z]	ns	ns	ns
CV (%)	11.37	21.88	14.57

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ns= P>0.05

Number of leaves, side-shoots and internodes

The main effects of EC and CH were significant for the total number of leaves per plant (Table 3.3). The total number of leaves was the highest at EC4, decreasing at EC8. This decrease is a typical salinity effect on crops (Shannon & Grieve, 1999; Munns, 2002). According to Heuer (1997) the reduction in the number of leaves may be a mechanism used by plants to adapt to salinity. With 50% CH, the number of leaves was lower than with 25% CH, probably due to limited reserves in the remaining shoots (leaves and branches) for leaf regeneration, as was also reported by Mnzava & Masam (1985).

Table 3.3. Main effects of the electrical conductivity (EC) of nutrient solutions and cutting height (CH) on the total number of leaves of *Amaranthus tricolor*.

Treatments	Total number of leaves (plant^{-1})
EC (mS cm^{-1})	
EC1	188.5 d
EC2	250.1 b
EC4	272.1 a
EC8	225.5 c
LSD (P = 5%)	16.88
Cutting Height	
25%	247.8 A
50%	220.3 B
Probability [z]	**
CV (%)	9.46

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ** = P<0.01

The total number of side-shoots as well as the number of internodes on newly formed side-shoots were affected by an EC x CH interaction. At EC2, EC4 and EC8 significant more side-shoots were found with CH 25%, but at EC1, the number of side-shoots was not affected by the CH treatments (Figure 3.1). The number of internodes decreased at EC1, EC2 and EC4 with 25% CH, but cutting height had no effect at EC8 (Figure 3.2).

The fact that a large percentage of available carbohydrates was used to increase the number of internodes on side-shoots (Figure 3.2) especially at EC1, EC2 and EC4, probably limited the reserves needed to stimulate growth in dormant side-shoot buds. In addition, by using CH 50%, more main stem material and buds were removed and lower numbers of side-shoots buds remained. However, at EC1 the balance was restored and at the final harvest, an equal number of side-shoots was found using 25% CH and 50% CH (Figure 3.1). Using EC2, EC4 and EC8, the balance could not be restored, although these plants were

exposed to the same hormonal imbalance caused by the removal of apical dominance (Wareing & Phillips, 1981; Cline, 1991). At EC2 and EC4 more side-shoot internodes developed with CH 50% (Figure 3.2) but this was on less side-shoots (Figure 3.1). At EC8, however, the side-shoot internodes as well as the number of side-shoots were lower with CH 50%, probably due to the production of osmoprotectants (Wang *et al.*, 1999). Andreasen, *et al.* (2002) noted that plant re-growth improves when more nodes with buds and photosynthetic active tissue are left after cutting. This was illustrated where *Amaranthus hybridus* developed more side-shoots with an initial cutting height of 20 cm from the soil surface, compared to drastic cutting heights of 15 and 10 cm (Norman & Shongwe, 1993).

Since newly formed side-shoots that are on the lower part of plants are less exposed to light where only the top 25% is removed compared to 50%, low internode numbers were probably caused by low light intensities, as was also reported by Andreasen, *et al.* (2002). However, Collins & Wein (2000) found more internodes in sprawling annual plants when exposed to low light intensity conditions. By using the 50% CH harvesting method, more meristems or metabolic sinks were removed and this probably lowered carbohydrate competition that could have stimulated growth on the remaining side-shoots. The lack of internode development at EC8 may be ascribed to a salinity effect where these plants needed their reserves to produce osmoprotectants (Wang *et al.*, 1999). In addition, the internode length on these new side-shoots was not affected by the treatments (See ANOVA; Table 2 in Addendum). Several authors associated shorter and fewer internodes with saline conditions (Shannon *et al.*, 1994; Shannon *et al.*, 1993a).

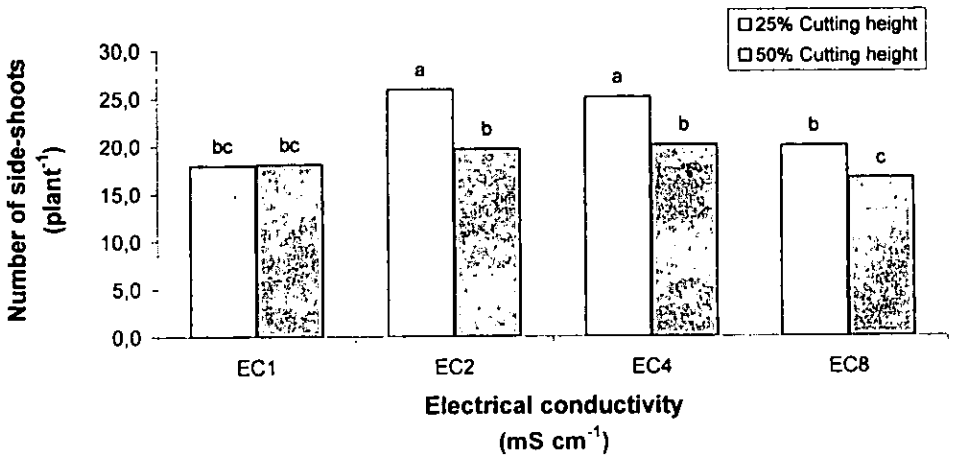

Figure 3.1. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the total number of side-shoots.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 12.16%

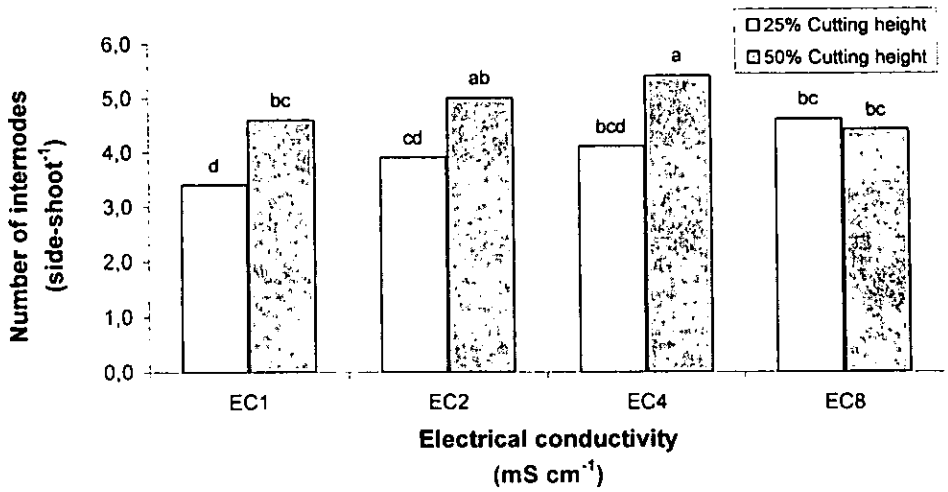

Figure 3.2. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the number of internodes on newly formed side-shoots.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 16.46%

Leaf moisture content, yield of leaves and side-shoots and leaf:side-shoot ratio

The main effects of EC and CH were significant for the leaf moisture content (Table 3.4). The leaf moisture content tended to be high at EC levels of 4 and 8 mS cm^{-1}. This is a typical morphological response to salinity in dicotyledonous species and

it is an adaptation to plants that minimizes the negative effects of high salt concentrations in leaves (Shannon *et al.*, 1994). Cutting at 25% CH, the leaf moisture content was lower than at 50% CH.

Table 3.4. The effects of electrical conductivity (EC) of nutrient solutions and cutting height on leaf moisture content of *Amaranthus tricolor*.

Treatments	Leaf moisture content (%)
EC (mS cm^{-1})	
EC1	84.84 b
EC2	85.11 b
EC4	85.39 ab
EC8	85.74 a
LSD (P = 5%)	0.586
Cutting Height	
25%	84.85 B
50%	85.69 A
Probability [z]	**
CV (%)	0.90

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ** = P<0.01

EC x CH interaction was significant for leaf dry yield. Only at EC8, 25% CH produced higher leaf dry yield than 50% CH (Figures 3.3). This higher leaf yield was due to more side-shoots that were found with 25% CH (Figure 3.1). At EC8, the yield (g $plant^{-1}$) for 25% CH was similar to those at EC2. Since the EC2 treatment produced more leaves than EC8 (Table 3.3), the relatively high leaf yield at EC8 may only be due to an increase in the mass per leaf due to the accumulation of compatible solutes for osmotic adjustment (Yeo, 1998). It is clear that a milder or less destructive cutting height should be used for *A. tricolor* grown under saline conditions to enable the plant to maintain a high level of production. In the previous chapter, it

was shown that the reduction in yield, due to a high EC8 treatment, can be restored by allowing a longer growth period before harvesting.

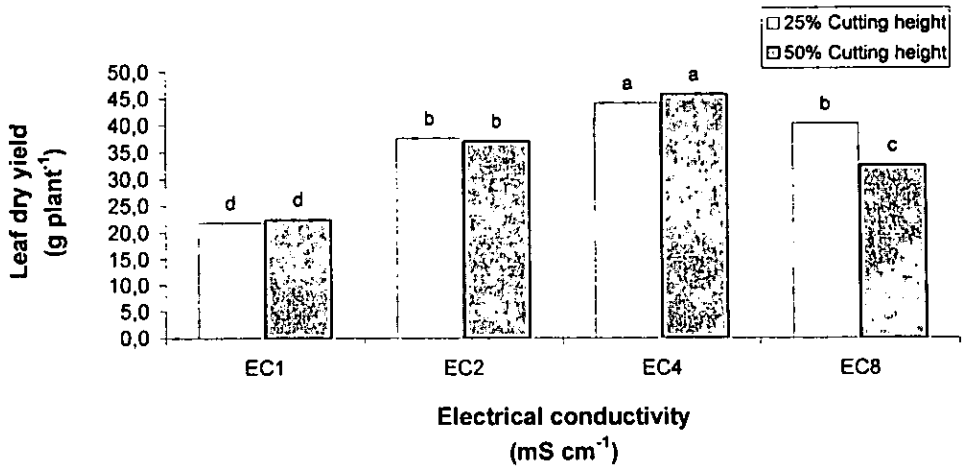

Figure 3.3. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the leaf dry yield of *Amaranthus tricolor*.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 10.20%

No significant EC x CH interaction occurred for dry yield of side-shoots, main stems, or the ratio between leaves and side-shoots. The dry yield of side-shoots increased with an increase in EC from EC1 to EC4, but decreased with further increases to EC8 (Table 3.5). Cutting at 25% CH produced higher side-shoots dry yield than at 50% CH. Norman & Shongwe (1993) found that the initial cutting height had no affect on stem weight of the amaranth plant in the absence of salinity conditions. The leaf:side-shoot ratio (LSR) increased with an increase in EC (Table 3.5). In plants harvested by uprooting at 45 DAT, the LSR also increased with an increase in EC (Chapter 2). The allocation of photosynthates to the leaves, rather than to side-shoots is important for amaranths, as the leaves are the edible part. The LSR was the highest with cutting at 50% CH. In the absence of salinity conditions, Norman & Shongwe (1993) found that an initial cutting at 10 cm above ground resulted in a lower LSR than at 15 and 20 cm cutting heights.

Table 3.5. Effects of the electrical conductivity (EC) of nutrient solutions and cutting heights on stem and side-shoot yield and the leaf:side-shoot ratio (LSR).

Treatments	Main stem and side-shoot yield	Leaf:side-shoot ratio (LSR)
	Dry mass (g plant^{-1})	(Dry mass basis)
EC (mS cm^{-1})		
EC1	7.9 c	2.9 b
EC2	13.8 a	2.8 b
EC4	14.4 a	3.3 ab
EC8	10.6 b	3.7 a
LSD (P = 5%)	2.13	0.49
Cutting Height		
25%	13.5 A	2.8 B
50%	9.9 B	3.5 A
Probability [z]	**	**
CV (%)	23.83	20.47

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ** = P<0.01

Biomass (aboveground) and growth rate

EC x CH interaction was significant for aboveground plant material on DM basis as well as for growth rate. Only at EC8, the DM of aboveground material and the growth rate benefited with cutting at 25% CH (Figures 3.4 and 3.5). These higher aboveground biomass values were due to higher leaf yields (Figures 3.3) and side-shoot numbers (Figure 3.1). Andreasen, *et al.* (2002) also reported an increase in final biomass with higher, less aggressive cutting heights. At EC8, the aboveground production and the growth rate were similar to those found at EC2 with 25% CH (Figures 3.4 and 3.5). This was probably due to more photosynthetic active tissue left after cuttings, resulting in the accumulation of compatible solutes for osmotic adjustment. This is an indication that adaptation to salinity is achieved (Amzallag *et al.*, 1990). However, when harvesting by uprooting, this did not happen even when harvested at 45 DAT

(Chapter 2). It is therefore clear that repeated cuttings at 25% CH were beneficial because it allowed plants to recover under saline conditions.

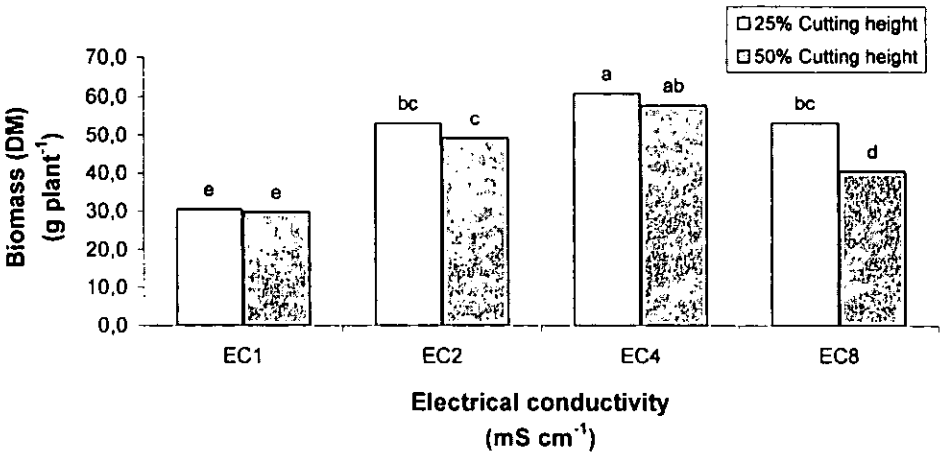

Figure 3.4. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the aboveground biomass on a dry mass basis.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 9.33%

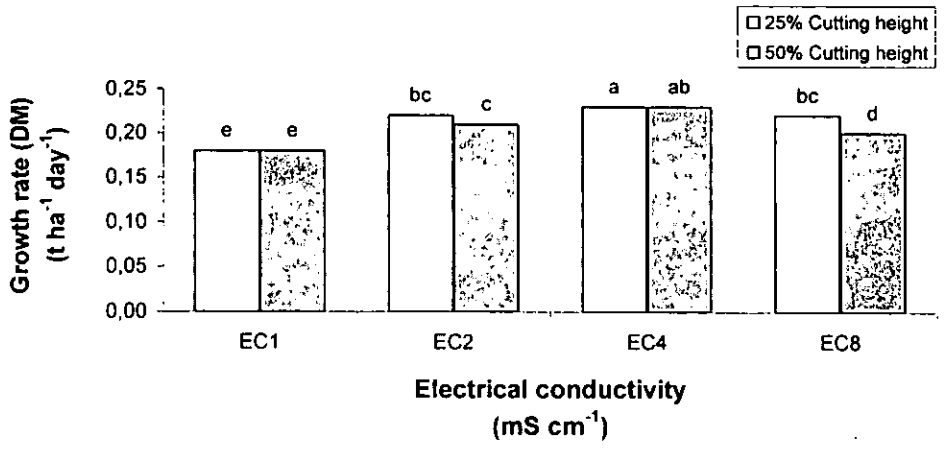

Figure 3.5. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the growth rate of *Amaranthus tricolor*.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 3.69%

Leaf composition and leaf crude protein

Only the main effects of EC and CH were significant with regard to leaf calcium and crude protein concentrations (Table 3.6). The leaf calcium content decreased with EC levels higher than 2 mS cm^{-1}. It was probably caused by the presence of Na^{+} in nutrient solutions at EC4 and EC8 levels (Table 3.1), limiting Ca^{+2} supply to roots (Bernstein & Kafkafi, 2002). Ho & Adams (1994) reported a reduction in Ca uptake in cucumber with an increase in salinity over a range of 3 to 8 mS cm^{-1}. Compare to the 25% CH, the cutting height of 50% resulted in a higher leaf calcium content (Table 3.6).

Leaf crude protein content improved with an increase in EC (Table 3.6), probably due to an increase in the nitrogen concentration in nutrient solutions (Table 3.1), causing an increase in the production of osmoprotectants in leaves (Wang *et al.*, 1999; Wang & Nii, 2000). According to Dubey (1994) some proteins appear to act as osmoprotectants, providing tolerance to salinity or enabling the plant to adapt. Auwalu & Tenebe (1997) found an increase in crude protein content with an increase in nitrogen application in amaranth. Since both the leaf crude protein and leaf calcium contents were highest with the 50% cutting height (Table 3.6), it appears as if vigorous cutting can increase the mineral composition (N and Ca) of amaranth leaves.

Table 3.6. The effects of electrical conductivity (EC) of nutrient solutions and cutting height on leaf calcium and crude protein content of *Amaranthus tricolor*.

Treatments	Leaf composition	
	Calcium (%)	Crude protein (%)
EC (mS cm^{-1})		
EC1	3.59 a	28.13 d
EC2	3.64 a	30.62 c
EC4	3.37 b	32.55 b
EC8	2.84 c	33.33 a
LSD (P = 5%)	0.145	0.761
Cutting Height		
25%	3.23 B	30.43 B
50%	3.49 A	31.89 A
Probability [z]	**	**
CV (%)	5.67	3.20

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ** = P<0.01

An EC x CH interaction affected the leaf calcium and leaf protein yields (Figure 3.6 and 3.7). In both cases, vigorous cutting tended to increase Ca and protein yields, but only at the lower EC levels (1 to 4 mS cm^{-1}). At EC8, the highest leaf calcium and protein yields were obtained with the 25% cutting height treatment. This was associated with a relative high leaf yield (Figure 3.3). Since cutting height did not affect the leaf yield at EC2 and EC4 (Figure 3.3), the decreased leaf calcium yields found at CH 25% was caused by low leaf calcium concentrations (Table 3.6). These results suggest that amaranths growing under saline conditions should be harvested at 25% to increase both leaf calcium and leaf protein yields. When the 50% CH treatment was applied at EC8, both leaf protein (Figure 3.7) and calcium yields (Figure 3.6) were low due to low leaf yield (Figure 3.3).

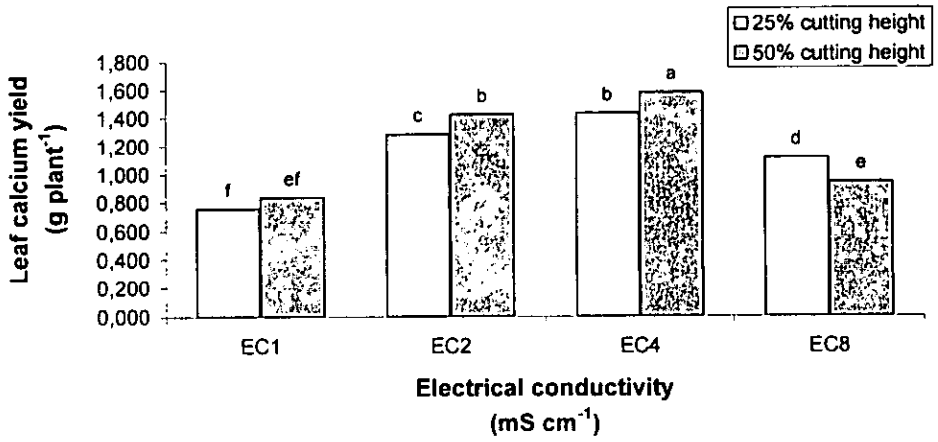

Figure 3.6. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the leaf calcium yield of *Amaranthus tricolor*.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 9.87%

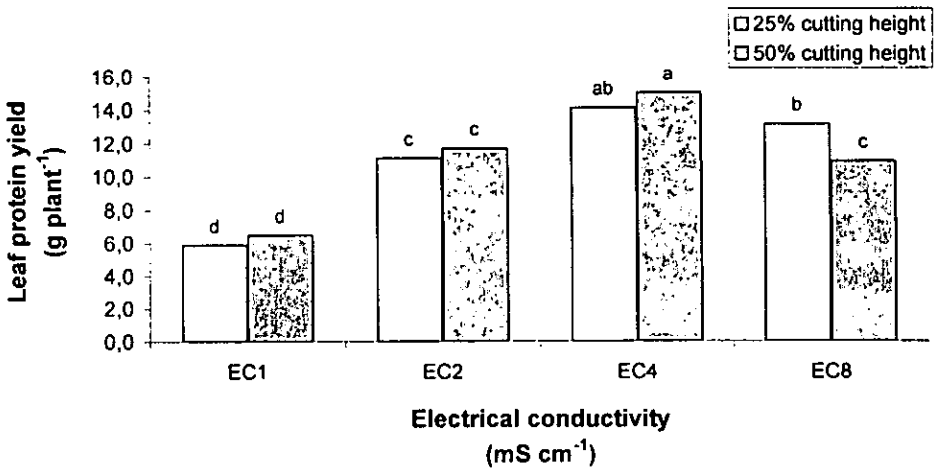

Figure 3.7. An interaction between the electrical conductivity of nutrient solutions and cutting height affecting the leaf protein yield of *Amaranthus tricolor*.
Means followed by the same letters are not significantly different at 5% level probability (DMRT).
EC treatments shown in Table 1.
CV = 10.18%

Flowering

No EC x CH interaction occurred, but the main effects of EC and CH both had significant effects on flowering (Table 3.7). Flowering was delayed with an increase in EC. It could have been caused by the nitrogen concentration in nutrient solutions that increased with each increase in EC level (Table 3.1). According to Shannon *et al.* (1994) the effect of salinity on flowering depends on the species and nutritional level and flowering can be either delayed or promoted. Wareing & Phillips (1981) state that high levels of nitrogen tend to delay flowering. Dumbroff & Cooper (1974) and Pasternak & Twersky (1979) found that salinity delays flowering in tomato while Shannon & Grieve (1999) reported that in onion it promotes flowering. The low nutrient solution (EC1) resulted in early flowering, probably due to the associated low levels of nutrients. Xu *et al.* (2001) found that early flowering of *Capsicum annum* was induced under conditions of low macronutrient levels combined with restricted root volume. Flowering was delayed with cutting at 50% (Table 3.7). Grubben (1976) also found that deep cutting at 12,5 cm above ground resulted in less flowers than cutting at 25 cm. A lower flowering index with 50% CH (Table 3.7) was associated with a lower leaf total (Table 3.3) and lower main stem and side-shoot yields (Table 3.5), this may be due to the reduced production and/or transport of assimilates into floral meristems (Yeh & Chiang, 2001).

Table 3.7. The effect of electrical conductivity (EC) of nutrient solutions and cutting height on the flowering index of *Amarathus tricolor*.

Treatments	Flowering index [y]
EC (mS cm^{-1})	
EC1	2.6 a
EC2	1.9 b
EC4	1.5 c
EC8	1.5 c
LSD (P = 5%)	0.37
Cutting Height	
25%	2.4 A
50%	1.4 B
Probability [z]	**
CV (%)	26.16

Means followed by the same letters are not significantly different at 5% level probability (DMRT).
z ** = P<0.01
y 1 = No flower; 2 = Signs of developing flowers; 3 = Flowers

CONCLUSIONS

With the results from this study, it is clear that plants grown under different salinity conditions have different harvesting needs. With *Amaranthus tricolor* as leafy vegetable, the optimum EC for production appeared to be 4 mS cm^{-1}, using a cutting height of 25%, since these treatments produced the most leaves. Although the highest leaf yield (mass) and leaf protein yield were also produced at an EC of 4 mS cm^{-1}, both of these parameters were not significantly affected by cutting height treatments at this EC level. Under the high EC level (8 mS cm^{-1}), however, the best cutting height was 25% to optimise leaf yield, leaf calcium and protein yields. This harvesting procedure also allowed the salt-stressed plants at EC8 to regain leaf yields, growth rates and protein yields to levels equal and superior to those at EC2.

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CHAPTER 4

SUMMARY

The term 'Glycophyte' used in this study refers to plants that are normally grown under non-saline conditions but with the ability to adapt to saline-induced stress conditions. This term is usually used where salt tolerant crops are studied and it is done to distinguish glycophytes from halophytes, or plants that are grown under saline conditions. The duration of exposure of glycophytes to salinity-induced stress, affects their ability to adapt to saline conditions and different glycophytes may have different responses. Photosynthates are used to produce osmoprotectants, enabling these plants to withstand saline conditions. Since the translocation of photosynthates is affected by cutting height and harvesting stage the effects of these two harvesting procedures were investigated. *Amaranthus tricolor,* is a leafy specie and was selected from other species due to a more stable yield with repeated harvesting procedures.

Soil-less production techniques were used to avoid external factors such as soil-borne diseases, weeds and nutritional imbalances in soil. This was done to minimise variation from factors other than the applied salinity levels and harvesting procedures. Thus, the potential of *A. tricolor* to adapt to saline conditions was studied, using different harvesting procedures. Most hydroponically produced crops grow well in nutrient solutions at concentrations of around 2 mS cm^{-1} under local conditions. Lettuce (moderately salt sensitive) is grown at concentrations of 0.7-1.2 mS cm^{-1}, cucumbers between 1.5 to 2.0 mS cm^{-1}, tomatoes between 2.0 to 2.5 mS cm^{-1} and melons (moderately salt tolerant) can be grown at higher concentrations of 2.5-4.0 mS cm^{-1}. Therefore, EC levels beyond 2.5 mS cm^{-1} may be considered as relatively saline.

The results of this study showed that *A. tricolor* can adapt to saline conditions, up to an EC of 8 mS cm^{-1}. This can be seen

where the shoot:root ratio improved where plants were allowed to grow for 45, rather than 30 days after seedlings were transplanted at an EC of 8 mS cm^{-1}. Under saline conditions the harvesting time and cutting height of amaranth should be adapted in order to optimise leaf yield and quality. With *A. tricolor* grown at an EC of 4 mS cm^{-1}, a growth period of at least 45 days is needed to obtain the best leaf yield as well as leaf calcium and protein yields. Using repeated cuttings at this EC level, 25% to 50% may be topped off without a reduction in leaf yield. However, when plants are fertigated at an EC of 8 mS cm^{-1}, a milder harvesting procedure should be followed, topping at only 25% to optimise leaf yield, leaf calcium and protein yields.

In arid and semi-arid regions, *A. tricolor* is produced in soil at varying moisture and EC levels. The response of salt-stressed plants grown under soil-less conditions may differ from those grown under field conditions. Results from this study with *A. tricolor* as potential saline tolerant crop were promising. However, further studies should be done under field conditions in this regard.

ADDENDUM

Table 1: Analysis of variance (ANOVA) of data used in Chapter 2.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
		Main stem length			
Replication	6	137.565	22.927	2.3184	0.0505
EC	3	390.208	130.069	13.1526	0.0000
Growing period	1	7119.035	7119.035	719.8741	0.0000
Interaction	3	244.345	81.448	8.2360	0.0002
Error	42	415.350	9.889		
Total	55	8306.502			
		Main stem diameter			
Replication	6	0.327	0.055	1.5731	0.1789
EC	3	1.507	0.502	14.4946	0.0000
Growing period	1	7.431	7.431	214.4102	0.0000
Interaction	3	0.476	0.159	4.5751	0.0073
Error	42	1.456	0.035		
Total	55	11.197			
		Leaf number			
Replication	6	1686.964	281.161	2.5946	0.0314
EC	3	9875.339	3291.780	30.3768	0.0000
Growing period	1	112233.018	112233.018	1035.6963	0.0000
Interaction	3	1194.196	398.065	3.6734	0.0194
Error	42	4551.321	108.365		
Total	55	129540.839			
		Side-shoot number			
Replication	6	35.357	5.893	1.8189	0.1186
EC	3	145.339	48.446	14.9535	0.0000
Growing period	1	1197.875	1197.875	369.7378	0.0000
Interaction	3	77.339	25.780	7.9572	0.0003
Error	42	136.071	3.240		
Total	55	1591.982			
		Internodes number			
Replication	6	18.179	3.030	2.7429	0.0243
EC	3	8.071	2.690	2.4357	0.0780
Growing period	1	1420.071	1420.071	1285.6074	0.0000
Interaction	3	17.214	5.738	5.1948	0.0038
Error	42	46.393	1.105		
Total	55	1509.929			
		Internodes length			
Replication	6	0.353	0.059	2.1889	0.0631
EC	3	0.872	0.291	10.8118	0.0000
Growing period	1	1.301	1.301	48.3741	0.0000
Interaction	3	0.286	0.095	3.5452	0.0224
Error	42	1.129	0.027		
Total	55	3.942			
		Leaf moisture content			
Replication	6	17.033	2.839	3.4246	0.0077
EC	3	12.653	4.218	5.0881	0.0043
Growing period	1	100.634	100.634	121.4010	0.0000
Interaction	3	16.372	5.457	6.5835	0.0010
Error	42	34.815	0.829		
Total	55	181.507			

Table 1 continue on next page

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
Leaf dry yield					
Replication	6	211.004	35.167	19.2340	0.0000
EC	3	1269.613	423.204	231.4615	0.0000
Growing period	1	4697.614	4697.614	2569.2471	0.0000
Interaction	3	259.211	86.404	47.2563	0.0000
Error	42	76.793	1.828		
Total	55	6514.236			
Stem dry yield					
Replication	6	24.685	4.114	1.5463	0.1870
EC	3	156.464	52.155	19.6024	0.0000
Growing period	1	2142.731	2142.731	805.3451	0.0000
Interaction	3	98.210	32.737	12.3041	0.0000
Error	42	111.747	2.661		
Total	55	2533.837			
Leaf:stem ratio (Dry mass)					
Replication	6	0.970	0.162	1.2167	0.3170
EC	3	11.284	3.761	28.3118	0.0000
Growing period	1	73.029	73.029	549.6902	0.0000
Interaction	3	1.274	0.425	3.1961	0.0330
Error	42	5.580	0.133		
Total	55	92.137			
Shoot dry weight					
Replication	6	365.632	60.939	9.9443	0.0000
EC	3	1921.855	640.618	104.5392	0.0000
Growing period	1	13185.652	13185.652	2151.6992	0.0000
Interaction	3	421.671	140.557	22.9368	0.0000
Error	42	257.377	6.128		
Total	55	16152.186			
Root dry weight					
Replication	6	23.069	3.845	4.3431	0.0017
EC	3	67.391	22.464	25.3742	0.0000
Growing period	1	806.362	806.362	910.8456	0.0000
Interaction	3	28.522	9.507	10.7392	0.0000
Error	42	37.182	0.885		
Total	55	962.526			
Shoot:root ratio (Dry mass)					
Replication	6	0.901	0.150	6.3294	0.0001
EC	3	22.943	7.648	322.1865	0.0000
Growing period	1	16.056	16.056	676.4398	0.0000
Interaction	3	1.728	0.576	24.2735	0.0000
Error	42	0.997	0.024		
Total	55	42.626			
Growth rate (Dry mass)					
Replication	6	0.001	0.000	2.8947	0.0188
EC	3	0.007	0.002	51.7237	0.0000
Growing period	1	0.015	0.015	341.4080	0.0000
Interaction	3	0.001	0.000	6.2500	0.0013
Error	42	0.002	0.000		
Total	55	0.026			

Table 1 continue on next page

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
		Leaf calcium content			
Replication	6	0.971	0.162	1.9440	0.0959
EC	3	4.113	1.371	16.4637	0.0000
Growing period	1	3.970	3.970	47.6750	0.0000
Interaction	3	0.179	0.060	0.7184	
Error	42	3.497	0.083		
Total	55	12.730			
		Crude protein content			
Replication	6	25.368	4.228	2.0746	0.0767
EC	3	465.763	155.254	76.1817	0.0000
Growing period	1	49.632	49.632	24.3540	0.0000
Interaction	3	1.395	0.465	0.2282	
Error	42	85.594	2.038		
Total	55	627.752			
		Leaf calcium yield			
Replication	6	0.172	0.029	10.2971	0.0000
EC	3	1.218	0.406	145.8000	0.0000
Growing period	1	5.523	5.523	1982.7430	0.0000
Interaction	3	0.247	0.082	29.5530	0.0000
Error	42	0.117	0.003		
Total	55	7.278			
		Leaf protein yield			
Replication	6	18.337	3.056	16.9608	0.0000
EC	3	174.279	58.093	322.4028	0.0000
Growing period	1	369.816	369.816	2052.4009	0.0000
Interaction	3	34.518	11.506	63.8550	0.0000
Error	42	7.568	0.180		
Total	55	604.517			

Table 2: Analysis of variance (ANOVA) of data used in Chapter 3.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
Increase in height					
Replication	13	52.712	4.055	0.7514	
EC	3	1.089	0.363	0.0673	
Error	39	210.461	5.396		
Total	55	264.262			
Diameter of main stem base					
Replication	6	1.024	0.171	2.8570	0.0200
EC	3	7.905	2.635	44.1283	0.0000
Cutting height	1	0.079	0.079	1.3189	0.2573
Interaction	3	0.045	0.015	0.2502	
Error	42	2.508	0.060		
Total	55	11.560			
Length of newly formed side-shoots					
Replication	6	52.615	8.769	3.1447	0.0123
EC	3	53.826	17.942	6.4342	0.0011
Cutting height	1	1.750	1.750	0.6276	
Interaction	3	6.688	2.229	0.7994	
Error	42	117.120	2.789		
Total	55	231.998			
Diameter of newly formed side-shoots					
Replication	6	0.347	0.058	4.7300	0.0009
EC	3	0.335	0.112	9.1336	0.0001
Cutting height	1	0.004	0.004	0.3653	
Interaction	3	0.096	0.032	2.6256	0.0628
Error	42	0.513	0.012		
Total	55	1.296			
Total leaves number					
Replication	6	9169.929	1528.321	3.1196	0.0128
EC	3	53903.071	17967.690	36.6758	0.0000
Cutting height	1	10587.500	10587.500	21.6113	0.0000
Interaction	3	2753.357	917.786	1.8734	0.1488
Error	42	20576.071	489.906		
Total	55	96989.929			
Number of internodes on newly formed side-shoots					
Replication	6	5.964	0.994	1.8704	0.1087
EC	3	4.429	1.476	2.7776	0.0529
Cutting height	1	10.286	10.286	19.3536	0.0001
Interaction	3	4.714	1.571	2.9568	0.0432
Error	42	22.321	0.531		
Total	55	47.714			
Internodes length on newly formed side-shoots					
Replication	6	0.984	0.164	0.7105	
EC	3	1.438	0.479	2.0758	0.1179
Cutting height	1	0.568	0.568	2.4617	0.1242
Interaction	3	1.151	0.384	1.6624	0.1896
Error	42	9.697	0.231		
Total	55	13.839			
Total number of side-shoots					
Replication	6	74.964	12.494	2.0213	0.0840
EC	3	288.482	96.161	15.5572	0.0000
Cutting height	1	182.161	182.161	29.4705	0.0000
Interaction	3	78.625	26.208	4.2401	0.0105
Error	42	259.607	6.181		
Total	55	883.839			

Table 2 continue on next page

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
Leaf moisture content					
Replication	6	32.179	5.363	9.0747	0.0000
EC	3	6.317	2.106	3.5631	0.0220
Cutting height	1	9.795	9.795	16.5731	0.0002
Interaction	3	1.677	0.559	0.9460	
Error	42	24.822	0.591		
Total	55	74.790			
Leaf dry yield					
Replication	6	176.724	29.454	2.3099	0.0512
EC	3	3722.190	1240.730	97.3046	0.0000
Cutting height	1	32.406	32.406	2.5415	0.1184
Interaction	3	190.652	63.551	4.9840	0.0048
Error	42	535.541	12.751		
Total	55	4657.514			
Main stem and side-shoot yield (Dry mass)					
Replication	6	72.917	12.153	1.5641	0.1816
EC	3	380.872	126.957	16.3393	0.0000
Cutting height	1	181.235	181.235	23.3248	0.0000
Interaction	3	27.697	9.232	1.1882	0.3258
Error	42	326.342	7.770		
Total	55	989.063			
Leaf:side-shoot ratio (Dry mass)					
Replication	6	0.793	0.132	0.3152	
EC	3	7.266	2.422	5.7737	0.0021
Cutting height	1	8.094	8.094	19.2966	0.0001
Interaction	3	0.397	0.132	0.3153	
Error	42	17.618	0.419		
Total	55	34.168			
Biomass (Dry mass)					
Replication	6	415.027	69.171	3.6366	0.0054
EC	3	6292.149	2097.383	110.2688	0.0000
Cutting height	1	366.915	366.915	19.2903	0.0001
Interaction	3	280.962	93.654	4.9238	0.0051
Error	42	798.867	19.021		
Total	55	8153.918			
Growth rate (Dry mass)					
Replication	6	0.001	0.000	1.4558	0.2168
EC	3	0.019	0.006	105.4587	0.0000
Cutting height	1	0.001	0.001	17.2308	0.0002
Interaction	3	0.001	0.000	5.7436	0.0022
Error	42	0.003	0.000		
Total	55	0.024			
Leaf calcium content					
Replication	6	1.403	0.234	6.4506	0.0001
EC	3	5.606	1.869	51.5433	0.0000
Cutting height	1	0.978	0.978	26.9737	0.0000
Interaction	3	0.159	0.053	1.4576	0.2398
Error	42	1.523	0.036		
Total	55	9.668			

Table 2 continue on next page

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
		Crude protein content			
Replication	6	48.647	8.108	8.1454	0.0000
EC	3	225.998	75.333	75.6813	0.0000
Cutting height	1	30.047	30.047	30.1862	0.0000
Interaction	3	5.030	1.677	1.6843	0.1849
Error	42	41.807	0.995		
Total	55	351.529			
		Leaf calcium yield			
Replication	6	0.240	0.040	3.0169	0.0152
EC	3	4.251	1.417	106.9167	0.0000
Cutting height	1	0.033	0.033	2.4715	0.1234
Interaction	3	0.243	0.081	6.0994	0.0015
Error	42	0.557	0.013		
Total	55	5.323			
		Leaf protein yield			
Replication	6	13.515	2.252	1.7885	0.1248
EC	3	511.208	170.403	135.3023	0.0000
Cutting height	1	0.008	0.008	0.0061	
Interaction	3	23.098	7.699	6.1134	0.0015
Error	42	52.896	1.259		
Total	55	600.725			
		Flowering			
Replication	6	2.750	0.458	1.9046	0.1025
EC	3	10.768	3.589	14.9152	0.0000
Cutting height	1	15.018	15.018	62.4064	0.0000
Interaction	3	1.482	0.494	2.0530	0.1210
Error	42	10.107	0.241		
Total	55	40.125			