Resilience of cassava (*Manihot esculenta* Crantz) to salinity: implications for food security in low-lying regions

Ros Gleadow*, Amelia Pegg and Cecilia K. Blomstedt

School of Biological Sciences, Monash University, Clayton, Melbourne, Victoria 3800, Australia

* Correspondence: ros.gleadow@monash.edu

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Abstract

Rising sea levels are threatening agricultural production in coastal regions due to inundation and contamination of groundwater. The development of more salt-tolerant crops is essential. Cassava is an important staple, particularly among poor subsistence farmers. Its tolerance to drought and elevated temperatures make it highly suitable for meeting global food demands in the face of climate change, but its ability to tolerate salt is unknown. Cassava stores nitrogen in the form of cyanogenic glucosides and can cause cyanide poisoning unless correctly processed. Previous research demonstrated that cyanide levels are higher in droughted plants, possibly as a mechanism for increasing resilience to oxidative stress. We determined the tolerance of cassava to salt at two different stages of development, and tested the hypothesis that cyanide toxicity would be higher in salt-stressed plants. Cassava was grown at a range of concentrations of sodium chloride (NaCl) at two growth stages: tuber initiation and tuber expansion. Established plants were able to tolerate 100 mM NaCl but in younger plants 40 mM was sufficient to retard plant growth severely. Nutrient analysis showed that plants were only able to exclude sodium at low concentrations. The foliar cyanogenic glucoside concentration in young plants increased under moderate salinity stress but was lower in plants grown at high salt. Importantly, there was no significant change in the cyanogenic glucoside concentration in the tubers. We propose that the mechanisms for salinity tolerance are age dependent, and that this can be traced to the relative cost of leaves in young and old plants.

Key words: Cassava, cyanogenic glucosides, food security, Konzo, linamarin, salinity, sea level.

Introduction

Rising sea levels and salinization of groundwater is becoming an increasing problem in many parts of the world, reducing the area of land available for agricultural crops (Church et al., 2013; Nunn, 2013). Globally, one-fifth of irrigated land is salt affected (450 000 km$^2$), resulting in huge losses in productivity (Munns and Gillman, 2015). The ability to tolerate salt varies within and between species. Barley (*Hordeum vulgare*), for example, is one of the more salt-tolerant crops, while lupins (*Lupinus albus*) is one of the most sensitive (Munns, 2002). Little is known about the impact of salinity on some of the world’s major staples.

Cassava (*Manihot esculenta* Crantz) is the most widely grown root crop in the world and the tuberous roots are the principle source of calories for many of the world’s poorest people (Nweke et al., 2002; FAO, 2014). Cassava has the ability to cope with a wide range of environmental stresses and continues to produce tubers under poor growing conditions, such as low nutrients and drought (El-Sharkawy, 1993;...
Jørgensen et al., 2005; Burns et al., 2010), yet little is known about how it responds to salt stress. Its classification as ‘moderately sensitive’ by the FAO is based on three early studies (Anon, 1976; Hawker and Smith, 1982; Indira, 1978). More recently, Carretero et al. (2008), in a study of pre-tuberous plants, found large effects on growth at 68 mM sodium chloride (NaCl) and only 30% survival at the highest concentration (136.8 mM NaCl).

Even less is known about the impact of salinity on the nutritional value of cassava. Unlike all other staple foods, cassava can be lethal if not correctly processed due to the high levels of the cyanogenic glucosides, linamarin and lotaustralin, that break down to release cyanide when mixed with specific β-glucosidases (McMahon et al., 1995; McKey et al., 2010). Consumption of cassava that is high in cyanide may cause diseases in humans, such as Konzo (an irreversible paralysis of the lower limbs), tropical ataxia, and even death (Cliff et al., 1997; Burns et al., 2010; Bradbury et al., 2011). There is a strong link between Konzo epidemics and the increase in cyanide concentrations during droughts (Ernesto et al., 2002), but there are no published studies on whether salinity-induced physiological droughts also result in higher levels of cyanide, and possible threats to health and food security. Ballhorn and Elias (2014), in the only published study on the effect of salinity on the production of cyanogenic glucosides, found for Trifolium repens that the cyanogenic glucoside concentration did increase in proportion to increasing salinity. Salinity is also known to affect the micronutrient concentration in plants. Given that children consuming cassava as a staple food are at risk for malnutrition (136.8 mM NaCl), it is important to assess the possible impact of salinity on their availability in cassava food products.

Cyanogenic glucosides are an effective defence against herbivores (Gleadow and Woodrow, 2002), but they represent a significant drain on resources that might otherwise be used for growth (e.g. Simon et al., 2010). This is balanced by the alternative roles they may play in primary metabolism, such as the transport and storage of nitrogen and mitigation of oxidative stress (Gleadow and Woodrow, 2002; Kongswadworakul et al., 2009; Selmar and Kleinwächter, 2013; Gleadow and Moller, 2014). Thus the partitioning of resources is a balance between defence, particularly when growth is limited, and, for example, stress tolerance and nitrogen turnover.

The aim of this study was, therefore, to determine the effect that salinity had on biomass and nutritional composition at two different life stages of cassava. We tested the tolerance of established plants, with well-developed tubers, to a wide range of NaCl solutions. The second experiment involved a detailed study on young clonally propagated plantlets through to tuber initiation. Photosynthetic parameters, growth indices, mineral nutrient composition, and cyanogenic glucoside concentration were determined and used to estimate the impact of salinity on plant production and nutritional value.

Materials and methods

Plant material and growing conditions

Cassava (Manihot esculenta Cranz) cv. MAus7 was grown in a greenhouse at the School of Biological Sciences complex Monash University, Clayton. Average temperature was 25 °C/23.5 °C day/night with a relative humidity of 75%. Glasshouses received natural light. The photoperiod was extended to 14 h from May to September 2014 using sodium lamps (MK-1 Just-a-shade, Abite Australia, Sunnfield Enterprises, Allambie Heights, NSW, Australia). Plants were rotated weekly to reduce microenvironment effects.

Experiment 1: effect of a range of salt concentrations on established cassava plants

The effect of salt on cassava plants with established tuberous roots (‘tubers’, hereafter) was tested. Cassava (one plant per pot) was grown in 8 l pots containing commercial potting mix with slow-release fertilizer (Osmocote®) for 8 months (June 2013–January 2014) and then treated with four different concentrations of NaCl for 4 weeks. Eight-month-old cassava plants were matched according to height and leaf number to form five sets with seven plants in individual pots in each group. One group was harvested for initial biomass determination (n=7 plants). The other groups were assigned to one of four different salt treatments (0, 50, 100, or 150 mM NaCl) and the subsequent harvests were randomized matched-pair design. Plants were watered with 1.4 litres of solution two times per week, such that the solution ran through the pots. Pots were flushed weekly with 2 litres of water to prevent the build-up of salt in the soil. All plants were destructively harvested after 28 d for biomass determination and chemical analysis.

Experiment 2: long-term effects of salinity on growth and tuber initiation

In the second experiment, longer term effects of salt on plants prior to tuber initiation were tested. In this experiment, young plants were established from cuttings (January 2014) and transplanted after 2 months (~15 cm tall, three leaves) into 2 l pots containing potting mix (as above) and mixed 5:1 (v/v) with soil containing mycorrhizal fungi, from the Jock Marshall Reserve, Monash University following Vandegheer et al. (2013). During this time, they were watered twice a week, once with a commercial full nutrient solution (Aquadol®, 10 ml l−1 H2O), and once with plain water. Before treatments were imposed, a set of 10 plants were harvested for dry mass determination. Plants were then watered with three different concentrations of salt [0, 40, and 80 mM NaCl (n=10)] based on the results of Experiment 1. In order to avoid a shock response, the salt concentration was increased gradually, starting at 20 mM and increasing by 20 mM every 3 d until the levels of 40 mM and 80 mM were reached. These levels were then maintained for the remainder of the experiment, a total of 70 d. The plants were watered with 300 ml of their appropriate solution twice a week. This volume was enough to saturate the soil. Pots were flushed weekly with 500 ml of water to prevent accumulation of salt in the soil. To ensure plants did not become nutrient limited, they were watered with AquaSol® on days 49, 52, and 56 of the study at a rate of 10 ml l−1.

The stem height and leaf number of each plant were recorded each week. Stem height was measured from the soil surface to the point where the newest leaf was expanding. The number of leaves of the third fully expanded leaf and the length of the middle lobe of that leaf was also recorded weekly. The lobe length was the distance from the point where the leaf blade was attached to the petiole to the tip of the lobe. All plants (n=30) were destructively harvested after 70 d (see below).

Harvesting protocol

The biomass of plants was separated into above-ground (stem, petioles, leaves) and below-ground (cutting, roots, tubers). The above-ground biomass of plants was removed by cutting the stem
at the soil surface. Leaves were removed from the plant and separated into three classes: fully expanded, expanded, or senescent. Expanded leaves were defined as fully developed leaves, expanded leaves were young, soft leaves, and senescent leaves were defined as leaves that were yellow or brown in colour on >50% of the leaf surface. Leaf fresh weight was recorded for all classes and leaf area was taken for expanded and expanded leaves using a leaf area meter (LI-3000 Portable Area Meter, Li-Cor, Lincoln, NE, USA). Three leaf discs (diameter=5 mm) were taken from the third fully expanded leaf and one disc from tubers and frozen at −20 °C for later analysis for cyanogenic glcosides. Height, and stem and petiole weight were recorded. Roots and tubers were washed and all soil removed where possible. For Experiment 1, tubers and roots were weighed separately, oven-dried at 60 °C for 14 d, and dry weights determined. For Experiment 2, roots were separated into fine roots and thick roots (tuberous roots). Thick roots were defined as roots that were white in appearance and >2 mm in diameter. All leaf and root tissue was frozen in liquid nitrogen and freeze-dried for 5 d, and weighed. The remaining biomass (stems and petioles) was oven-dried at 60 °C for 14 d and dry weights determined.

Growth indices

The root:shoot ratio was calculated by dividing the total below-ground biomass (DW) by the total above-ground biomass (DW). The relative growth rate (RGR) was calculated as follows: RGR=log(Wf)/log(Wi)/(t2−t1), where W1 and W2 are the initial and final dry mass at time 1 (t1) and time 2 (t2). The specific leaf area (SLA) of fully expanded leaves was calculated as leaf area per g dry leaf mass.

Photosynthetic parameters: assimilation rates, Fv/Fm, greenness, and chlorophyll

Light-saturated measurements of the photosynthetic rate and light-adapted chlorophyll fluorescence, Fv/Fm, were made using a Li-Cor 6400 gas exchange system on all living plants in Experiment 2 in the week before harvesting (20 August 2014). Three concordant measurements were made on the third fully expanded leaf from each living plant at 700 μmol m−2 s−1 PAR, 400 ppm CO2, 25 °C (growth temperature), and a relative humidity of 50%, following Gleadle et al. (2009). Total chlorophyll concentration was measured following Gleadle et al. (1983) as modified by Burns et al. (2002). Freeze-dried leaf tissue (100 mg) was extracted twice in 2 ml of 80% acetone and the absorbance measured at 647, 665, and 750 nm using a FLUOstar Galaxy plate reader (BMG, Australia). Greenness measurement readings were made of the third fully expanded leaf using the GreenIndex+ Ap (Spectrum Technologies Inc.) by taking a photograph of the middle lobe against a background of known colour and using in-built algorithms. To our knowledge, this is the first time GreenIndex+ measurements have been made on cassava.

Plant composition: cyanogenic glucosides, total nitrogen, and micro- and macronutrients

The cyanogenic glucoside concentration was measured by the evolved cyanide method. Freeze-dried tissue (three leaf discs diameter=1 cm2 or one tuber disc, 2 mm thick) was placed into sealed vials with 270 μl of 0.1 M pH 6.4 phosphate buffer, and a separate 0.2 ml inner tube containing 200 μl of 1 M NaOH. Vials were then frozen and thawed at room temperature twice to disrupt the cells, and incubated for ~19 h at 37 °C. The cyanide evolved from the cyanogenic glucosides trapped in the NaOH was assayed colorimetrically following Blomstedt et al. (2012). To ensure complete conversion of the cyanogenic glucosides to cyanide, latex [30 μl, in 0.1 M phosphate buffer pH 6.4, 1:100 (v/v)] was added to the reaction vial, as latex contains the degradative β-glucosidases and α-hydroxynitrile lyases necessary to degrade both linamarin and lotaustralin (Jørgensen et al., 2005). Tissue in vials was rinsed, dried, and weighed for biomass determination to allow the calculation of the hydrogen cyanide potential (HCNp) of the tissue analysed.

Total elemental nitrogen and carbon were measured on finely ground, freeze-dried leaf and tuber samples using a LECO CNS2000 analyser. The remaining nutrient analyses were performed using inductively coupled plasma (ICP)-MS on microwave-digested samples (Environmental Analysis Laboratory, Southern Cross University, NSW, Australia).

Statistical analysis

Data were analysed with Prism Graphpad 6th using ANOVA and t-tests. Matched-pair ANOVAs were used for analysis of Experiment 1 as plants were grouped according to size. Means were compared using post-hoc Tukey’s tests (P<0.05) where a statistical significance was detected between groups. Data were log transformed if required in order to satisfy the assumptions of normality.

Results

Experiment 1: effect of a range of salt concentrations on established cassava plants

Plants were grown at four concentrations of salt for 28 d. Above-ground biomass decreased with increasing salinity and was significantly lower in plants grown at 100 mM and 150 mM NaCl (Fig. 1A) compared with controls. Mean above-ground dry weight in control plants was 169 ± 14.5 g, but was only 67.9 ± 8.9 g in plants grown at 150 mM NaCl (F3,24=20.35, P<0.0001; Fig. 1A). The root:shoot ratio was higher as a consequence of increased salinity, driven by the difference in above-ground biomass, as there was no significant difference in tuber biomass between treatments (F6,16=3.7, P=0.85; Fig. 1B, C). Leaf area of plants decreased as the salinity level increased, primarily through plants shedding leaves, with significant differences detected between control and 100 mM, control and 150 mM, and 50 mM and 150 mM NaCl treatment groups (F5,24=16.64, P=0.021, 0.003, and 0.005, respectively; Fig. 1D).

The HCNp in the leaves decreased with increasing salt concentration (Fig. 1E). In the tubers, HCNp initially increased and was significantly higher at 50 mM and 100 mM NaCl (F3,22=6.46, P=0.006 and 0.015, respectively). At the highest salt treatment, the HCNp decreased in the tubers, but there was no significant difference compared with any other treatment.

Experiment 2: long-term effects of salinity on young cassava plants and tuber initiation

Plant growth, biomass, and phenology

The number of leaves present on each plant was recorded once a week for the duration of the study. After 5 weeks, plants in the 80 mM NaCl group began to lose leaves at a steady rate, whereas plants in the control and 40 mM NaCl groups had steady increases in their number of leaves over the course of the experiment (Fig. 2A). Six of the 10 plants...
grown at 80 mM NaCl died (i.e. all leaves abscised and there was no subsequent sign of recovery), four at day 56 and the other two at day 60 of the study. Surviving plants were shorter (Fig. 2B), had fewer fully expanded leaves, and had more senescent leaves than plants grown at 40 mM NaCl (Fig. 2; Supplementary Table S1 at JXB online). There was a significant decrease in leaf area of plants grown at both 40 mM and 80 mM NaCl (Fig. 2C). Total biomass was highest in plants grown without salt, with an average mass of 7.68 ± 0.42 g compared with the plants grown at 40 mM (5.73 ± 0.32 g) and 80 mM NaCl 2.09 ± 0.43 g, respectively (F<sub>2,27</sub>=52.24, P<0.0001; Fig. 2D). Control plants had greater above-ground biomass than plants from either of the salt treatments (F<sub>2,27</sub>=72.40, P<0.0001, Fig. 2D), but the below-ground biomass was not significantly different between the control and 40 mM NaCl groups. Thus, the higher root:shoot ratio in the 80 mM plants of 2.98 ± 0.64 compared with 1.33 ± 0.08 in the 40 mM-grown plants (Supplementary Table S1; F<sub>2,27</sub>=7.58, P=0.0038) was driven primarily by the lower above-ground biomass, as the fine root mass was small and only one plant produced a tuber (Fig. 2D; Supplementary Table S1). There was no significant difference in the RGR between plants grown at 0 mM and 40 mM NaCl, but there was a significant decrease in plants at 80 mM salt (Fig. 2E).

**Photosynthetic parameters:** assimilation rate, stomatal conductance, F<sub>v</sub>/F<sub>m</sub>′, and chlorophyll

Assimilation, stomatal conductance, and F<sub>v</sub>/F<sub>m</sub>′ were measured on the third fully expanded leaf. The assimilation rate of control plants was 4.57 ± 0.82 mmol m<sup>-2</sup> s<sup>-1</sup>, 2-fold greater than that of plants grown at 40 mM (2.24 ± 0.27 mmol m<sup>-2</sup> s<sup>-1</sup>) and nearly 5-fold higher than plants from the highest 80 mM (1.24 ± 0.51 mmol m<sup>-2</sup> s<sup>-1</sup>) salt treatment (F<sub>2,21</sub>=7.53, P=0.0182 and 0.0055, respectively; Fig. 3A). Stomatal conductance followed a similar pattern to that of the photosynthetic rate; however, there was only a significant difference between control (0.04 ± 0.01 mmol m<sup>-2</sup> s<sup>-1</sup>) and 80 mM- (0.01 ± 0.005 mmol m<sup>-2</sup> s<sup>-1</sup>) treated plants (F<sub>2,21</sub>=3.54, P=0.0420; Fig. 3B). Internal CO<sub>2</sub> concentrations were similar in all treatments (Fig. 3D). There was no significant difference in F<sub>v</sub>/F<sub>m</sub>′ between control and 40 mM plants, with an overall mean of 0.59 ± 0.01. F<sub>v</sub>/F<sub>m</sub>′ was much lower in plants grown under 80 mM NaCl, with a mean of 0.33 ± 0.12 (F<sub>2,21</sub>=8.77, P=0.0017), indicating severe stress (Fig. 3E).

Total chlorophyll concentration also decreased with increasing salt concentration. Plants grown under 40 mM NaCl had a lower chlorophyll concentration than control plants but the difference was not significant (F<sub>2,21</sub>=411.10, P=0.0036; Fig. 3C) and the concentration of chlorophyll in leaves from 80 mM-grown plants was significantly lower than
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that of both the control plants and plants at 40 mM NaCl ($F_{2,22}=411.10$, $P<0.0001$). The Chl $a/b$ ratio was significantly higher for 40 mM and 80 mM plants compared with control plants ($F_{2,22}=7.23$, $P=0.0095$ and 0.0110, respectively; Fig. 3F). Measurement of leaf greenness was to approximate total chlorophyll concentration, with a highly significant Pearson’s correlation coefficient of 0.67 ($P<0.001$) (Supplementary Table S1).

Cyanogenic glucoside and nutrient analysis

Cyanide assays were performed on freeze-dried leaf and tuber tissue. HCNp was highest in the leaves of plants grown at 40 mM NaCl ($F_{2,22}=9.32$, $P=0.0049$ and 0.0008, respectively, Fig. 4A). There was no difference between control and 40 mM groups in the amount of HCNp found in tubers (Fig. 4A). Statistical tests on HCNp were unable to be performed for tubers grown at 80 mM NaCl as only one plant grown at this salt level contained tuberous roots. Overall, there was no significant difference in the HCNp:total biomass ratio between treatments ($F_{2,22}=0.43$, $P=0.6536$), indicating that observed changes in HCNp were not driven by changes in biomass.

Total nitrogen concentration was higher in the leaves of control plants compared with 40 mM plants ($F_{2,22}=8.14$, $P=0.0019$; Table 1; Fig. 4B), although no difference between control and 80 mM plants or 40 mM and 80 mM plants was found. Carbon was lower in leaves of 80 mM-grown plants so there was no overall difference in the C:N ratio between control and 80 mM-grown plants. However, the leaves of plants grown at 40 mM NaCl had a higher C:N ratio compared with control and 80 mM plants ($F_{2,22}=14.86$, $P=0.0014$ and 0.0002, respectively; Table 1).

Analysis of leaves (all treatments) and tubers (0 and 40 mM NaCl treatment only due to lack of tuber initiation at 80 mM NaCl) showed that there were significant differences in concentrations of key nutrients and trace elements between treatments (Table 1; Fig. 4C, D). The most striking difference is seen in the concentration of sodium. Plants at 80 mM NaCl had higher amounts of sodium in their leaves (1.80%) compared with control (0.01%) and 40 mM (0.02%) plants ($F_{2,22}=50.58$, $P<0.0001$ and 0.0073, respectively; Table 1). The sodium concentration in the tubers in plants grown at 40 mM NaCl was nearly 20 times higher than in control plants ($P=0.0022$). The amount of potassium was also
significantly higher in the tubers of plants grown at 40 mM salt, but the magnitude of the increase was much less, with a 1.4-fold increase (Table 1).

Iron and zinc concentrations were significantly higher in leaves of plants grown at 80 mM NaCl (Fig. 4C, D). Concentrations of these two nutrients were somewhat lower in the tubers of salt-stressed plants, but the differences were not significant. Of the remaining macronutrients, the concentration of manganese in leaves also increased with rises in salinity levels and ranged from 43 mg kg\(^{-1}\) in control plants to 107 mg kg\(^{-1}\) in 80 mM plants (\(F_{2,22}=47.13, P<0.0001, <0.0001, \) and 0.0146, respectively; Table 1). The amount of magnesium increased with increasing NaCl concentration in leaf tissue, and was significantly different between all treatments. Leaf phosphorus was marginally higher in the leaf tissue of plants grown at 80 mM NaCl and also 10-fold higher in the tubers of 40 mM plants (Table 1). Leaf sulphur levels in 40 mM plants were lower than in control and 80 mM-grown plants. The levels of trace elements in addition to iron and zinc, such as copper and cobalt, increased in leaf tissue with increasing salt (Table 1). In general, salt stress results in an increase in the nutrient levels in leaves and a decrease in the tubers (Table 1).

**Discussion**

The impact of salinity on growth and nutritional value (i.e. the cyanogenic glucoside and micronutrient concentrations) depended on the age of the plant. Older plants that had already developed tubers were more salt tolerant than younger, pre-tuberous plants in terms of survival and growth. The key effect of salinity on cassava was a reduction in biomass, leaf area, and photosynthetic rate. There was an increase in HCNp in the leaves of young cassava plants under moderate stress (Fig. 4), but in the leaves of mature cassava plants, HCNp decreased step-wise with increases in salinity (Fig. 1). The age-affected differences may be related to: (i) the propensity for cassava to shed leaves in response to
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Abiotic stress; (ii) the relatively high costs involved in excluding sodium; and (iii) relatively higher investment by younger plants in leaves compared with older plants.

Growth and photosynthesis

We found that tuberous plants were able to tolerate fairly high concentrations of salt, up to 150 mM NaCl (Fig. 1). In contrast, growth and survival of pre-tuberous plants was severely retarded at 80 mM NaCl, with only one plant developing a tuber (Fig. 2). In both experiments, there was a decrease in mass of ~1% for every 1 mM NaCl increase, relative to the initial biomass ($y=-1.0197x+456.6$; $R^2=0.20$; Experiment 1; Supplementary Fig. S1) and ($y=-0.0699x+7.966$; $R^2=0.77$; Experiment 2; Supplementary Fig. S2). These results are broadly consistent with earlier studies that report severe

![Graphs showing concentration of cyanogenic glucosides, N, C, Fe, and Zn in leaves and tubers of young cassava plants after 9 weeks treatment with nutrient solution containing three different concentrations of salt. No tubers were present in the 80 mM NaCl treatment. Values are means ± SE. n = 10, except at 80 mM NaCl where final n = 4 for analysis. Significant differences are indicated on the line over each group of means. ***P < 0.001; **P < 0.01; n.s. = not significant. A full list of macro- and micronutrients is given in Table 1.](https://academic.oup.com/jxb/article-abstract/67/18/5403/2485531?search_type=ref&search_type=abstract&search_query=fig_4_concentration_of_cyanogenic_glucosides.png)
stunting and death of cassava plants between 50 mM and 135 mM, depending on variety, length of treatment, and soil environment, with older plants and those with mycorrhizae generally being more tolerant (Indira, 1978; Hawker and Smith, 1982; Carretero et al., 2008).

The large reduction in biomass in both pre- and post-tuberous plants can be attributed to the loss of photosynthetic area and reduced rates of carbon assimilation. Weekly phenological measurements allowed us to track individual plants over the entire treatment period. In young plants, there was no significant reduction in leaf number until nearly 8 weeks in plants grown at 40 mM NaCl and even in the 80 mM treatment leaves were retained until 4 weeks after treatments commenced. This is consistent with the characteristic shedding of older leaves when plants are under stress, for example when droughted (Setter and Fregene, 2007; Vandegeer et al., 2013). Final harvest data showed that leaf area was even more sensitive to salt than leaf number, with a significant reduction in pre- and post-tuberous plants at 40 mM and 100 mM NaCl, respectively, (Figs 2 and 1, respectively).

The concentration of chlorophyll, greenness, and Fv/Fm (measured on the third fully expanded leaf) were not significantly lower in plants grown at 40 mM, indicating that these leaves were not under severe stress. Plants from the 80 mM treatment, on the other hand, did show signs of stress with a lower Fv/Fm, and decreased chlorophyll concentration. Vandegeer et al. (2013) showed that the health of these highly productive, expanded leaves is maintained well after drought has affected other parts of the plant metabolism, and leaves are only discarded after prolonged drought. The significant leaf loss after 4 weeks growth and the reduction in photosynthetic capacity suggest that at 80 mM salt cassava is severely stressed.

Nutrient analysis and evidence for ionic exclusion at low salinity

Evidence that cassava is able to tolerate low to moderate concentrations of salt comes from the ionic composition of the tissues. Foliar sodium concentration was the same at 40 mM NaCl as in plants grown under control conditions, indicating that survival is from ionic exclusion, rather than tissue tolerance. This ability to exclude sodium breaks down at the higher concentrations, with a 100-fold increase in foliar sodium in plants grown at 80 mM. This type of response is typical of plants that are sensitive to salt. Salt-tolerant species, in contrast, are able to tolerate quite high concentrations of tissue salt (Munns and Gillman, 2015). Some plants cope with excess available Na by accumulating K as a balancing cation and this may influence K⁺ uptake (Mattius, 2014; Munns and Gillman, 2015). We found no evidence for a change in ionic balance in salt-stressed cassava in the leaves. However, in the tubers, there was a significant increase in both Na and K with salt stress, though the relative increase was much greater for Na. This agrees with the results of Carretero et al. (2008) for roots/tubers but not leaves where it was unchanged (Carretero et al., 2008), and there are also reports of potassium being lower (Hawker and Smith, 1982) than controls due to salt stress. It is not clear why there is such a difference in the effect of salinity on potassium concentration reported in these experiments, but it may be the result of plant age or that particular leaves were tested, rather than pooling material, as we did here.

While tubers are an excellent source of carbohydrates, they are very poor in nutrients (Burns et al., 2010). On the other hand, leaves are an important source of protein and micronutrients for many subsistence farmers in Africa (Latif and Müller, 2015). The concentration of foliar and tuber micro- and macro-nutrients measured here were in the same range of concentrations as plants grown in the field in Africa (Burns et al., 2012). Any change in foliar nutrients, therefore, could have a major impact on human health. In this study, there was a significant reduction in above-ground biomass in both young and old plants, and nutrient analysis of the young plants shows a significant change in important nutrients, such as iron and zinc. The concentration of these increase in leaf tissue but in terms of total nutrients available for food there is a major reduction due to salt stress. In tubers grown under moderate salt stress, there is a reduction in the concentration of macro- and micronutrients, indicating that cassava under even moderate salt stress is not a nutritious food source. There was also a small but significant decrease in foliar nitrogen concentration.

Impact of salinity on cyanogenic glucosides and nutritional status

Changes in the concentration of cyanogenic glucosides in our study were very different in both magnitude and direction in old and young plants. In established plants, foliar cyanogenic glucoside concentration decreased with each increment of salt from 0 to 150 mM. In contrast, in pre-tuberous plants, the cyanogenic glucoside concentration was somewhat higher in the leaves of plants grown at 40 mM than in those of the control plants, but much lower in plants grown at 80 mM NaCl (Fig. 4A). One explanation for this difference is to consider the relative cost of the investment in defence and leaf infrastructure in the two age groups. The primary mechanism for coping with salinity stress in older plants is to discard leaves and rely on the tubers for storage, thus any investment in foliar defence is unnecessary. The significant increase in cyanogenic glucosides in the tubers of these plants is consistent with this hypothesis and also the fact that in cassava cyanogenic glucosides are synthesized in the leaves and transported to the tubers (Jorgensen et al., 2005). On the other hand, in younger plants, the leaves represent a relatively large proportion of total plant biomass. Moreover, as there are no tubers, there is no sink for the storage of cyanogenic glucosides and little prospect of recovery should the shoots die. In younger plants, therefore, it is to the plant’s advantage to protect and maintain the leaves that it has already produced. Further detailed analysis of plants over time based on the hypotheses illustrated in the schematic in Fig. 5 are required.

The higher concentration of cyanogenic glucosides in the young plants from the 40 mM treatment and the significant decrease in HCNp in severely salt stressed (80 mM NaCl) plants may also be directly linked to the plant’s ability to
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Mattius, 2014; Roy et al., 2014). One way to deal with stress is to have a ROS-scavenging system, for example via the up-regulation of antioxidant enzymes such as superoxide dismutase. Such enzymes are induced within minutes of salt being applied—mainly in the form of H$_2$O$_2$ (Cabello et al., 2012; Mattius, 2014). It has been proposed that the synthesis of cyanogenic glucosides may also act to mitigate oxidative stress: NADPH$^+$ is essential for the conversion of precursor amino acids to the intermediate oxime, plus the putative in vivo cyanogenic glucoside turnover pathway consumes H$_2$O$_2$ (Møller, 2010; Gleadow and Møller, 2014; Pičmanová et al., 2015). The nitrogen levels in the leaves of plants at both moderate and severe salt stress do not differ significantly but the levels of HCNp do. We hypothesize that at moderate stress cyanogenic glucosides are acting in defence whilst at the higher salinity levels the cyanogenic glucosides are turned over and protect the plant from oxidative stress and ROS scavenging (Fig. 5B). However, details of the putative pathway need to be elucidated before this hypothesis can be confirmed.

**Conclusions and implications for food security**

The study of climatic variables and the consequences for cassava growth is key to evaluating the effect of global change on food security. Cassava is able to tolerate a wide range of conditions and so it is often assumed that the impact of climate change on cassava will be minimal (e.g. Lobell et al., 2008; Burns et al., 2010). Rising sea levels and reliance on saline irrigation water in increasingly hot, dry climates poses risks that have not been previously explored for many crops.

Changes in cassava tissue chemistry in response to salinity reflect a combination of factors, including differences in the balance in trade-offs in resource allocation between growth and defence in plants at different stages of development and the possible reduction in oxygen radicals. This is summarized in the model presented in Fig. 5. This model presents a number of testable hypotheses. It is noteworthy that all the measurements of cyanogenic glucosides are above the FAO/WHO Codex (1989) standard for edible cassava flour of 10 ppm cyanide, underscoring again the importance of processing before consumption.

We conclude cassava to be sensitive to low to moderate concentrations of salt, particularly at early stages of development and, therefore, that cassava is not suitable for planting in regions contaminated with even relatively low levels of salt. In coastal areas, impacts may be minimized by irrigating with less saline water, or during periods of high rainfall to allow time for plants to become established before they are exposed to higher concentrations of salt. Given that alternative tuberous crops such as sweet potatoes are even more salt sensitive.
than cassava (Shannon and Grieve, 1999), breeding for more salt-tolerant varieties is necessary if cassava is to continue to expand its role as a staple in a future, more saline world.

Supplementary data

Supplementary data are available at JXB online.

Table S1. Height, leaf area, biomass, growth indices, and chlorophyll measurements of cassava plants grown at three levels of salinity (0, 40, and 80 mM NaCl) for 70 d.

Figure S1. Total biomass of 6-month-old, tuberous cassava plants after 4 weeks of treatment with different concentrations of salt (Experiment 1).

Figure S2. Total biomass of young, pre-tuberous cassava plants plotted versus salinity (Experiment 2).

Figure S3. Correlation between total chlorophyll and greenness measured using the GreenIndex Ap.

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