Assessment of genetic and nutritional diversity, and salinity tolerance of Kenyan guava (*Psidium guajava* L.): an underutilized naturalized fruit species

Dissertation

to obtain the Ph. D. degree

in the International Ph. D. Program for Agricultural Sciences in Goettingen (IPAG)

at the Faculty of Agricultural Sciences,

Georg-August-University Göttingen, Germany

presented by

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born in Kenya

Göttingen, May, 2018

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Date of dissertation: 18.07.2018

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Chapter one

1. General introduction

1.1. Overview of global tropical fruit production

Tropical fruits are important to developing countries from both nutritional and economic perspective. They are cultivated widely in the tropics and subtropics at commercial and subsistence levels. About 90% of these fruits are consumed in producing countries themselves, while 10 percent are traded internationally as fresh fruits and processed products (Available: http://www.fao.org/docrep/meeting/028/ma937e.pdf – Accessed 08.05.2018). The dominant fruits produced and marketed are mango, pineapples, papaya and avocado – hence referred to as 'major tropical fruits.' The major tropical fruits account for approximately 75% of global fresh tropical fruit production. Other fruits such as lychees, passion fruit and guavas are referred to as 'minor tropical fruits' – usually traded in smaller volumes. Asia and the Pacific region were projected to be the major producing regions in the year 2010, accounting for about 56% of global tropical fruit production, followed by Latin America and the Caribbean (32%), and Africa (11%) (Available: http://www.fao.org/faostat/en/#data/QC – Accessed 08.05.2018).

Notwithstanding significant growth in the major tropical fruits, the opportunities to grow, consume and export more fruit from tropical regions remain under-exploited compared to temperate regions (Griesbach 2007). The minor tropical fruits are traded in smaller volumes, however, their market shares have been expanding rapidly in recent years. Therefore, more efforts geared towards improvement of the minor tropical fruits could enhance their production and economic capacities.

1.2. Distribution and botanical description of guava

Guava (*Psidium guajava* L.) is a fruit crop cultivated in the tropics and some sub-tropical regions (Gautam et al. 2010; Rodríguez et al. 2010). Leading countries in guava production

include India, Pakistan, Mexico, Brazil, Egypt, Thailand, Columbia, and Indonesia (Pommer and Murakami 2009). Guava belongs to the Myrtaceae family with about 150 genera and more than 5,000 species (Govaerts et al. 2008). The common guava has several secondary centres of diversity in the wet tropics, mainly in South America, Australia, and tropical Asia, and also occurs in Africa and even South Europe (Grattapaglia et al. 2012). The fruit tree has been cultivated for a long time, and its distribution has been promoted by man, birds and other animals (Pommer and Murakami 2009). The tree has a good potential to grow on wastelands, including soils with high pH levels (Gautam et al. 2010), explaining its wide distribution.

The guava is a small (2-10 m tall) monoecious tree, with broad, spreading tops branching freely close to the ground (Crane and Balerdi 2005) (Figure 1). The fruit is a fleshy, pyriform or ovoid berry that can weigh up to 500 g (Orwa et al. 2009) and varies greatly depending on the genotype and the environment (Babu et al. 2007; Patel et al. 2011). The fruit requires about 120 days to mature after flowering (Crane and Balerdi 2005). The skin colour of ripe fruits varies from light green to yellow, while the pulp may be red, white, yellow or pink (Ecocrop 2015; Orwa et al. 2009). The fruit varies from having a thin pericarp, with many seeds in the pulp, to a thick pericarp, with only a few seeds (Mehmood et al. 2014). The flavour ranges from sweet to highly acidic, while the aroma may be strong and penetrating, or even mild and pleasant (Mehmood et al. 2014).

Guava can be propagated by seeds and vegetative means (Kakon et al. 2008). Plants propagated through seed generally take many years to come into bearing, and normally do not produce true-to-type and often bear fruits of inferior quality (Kakon et al. 2008). Guava is also propagated by air layering (Nyambo et al. 2005). Other methods include use of cuttings, stooling, budding and micropropagation or recently by wedge grafting (Mishra et al. 2005). Properly propagated and cultured trees may start to bear within two to four years.

A mature tree will produce from 54 to 100 kg of fruit per year, with two seasons of production – one in which there is a major crop, and another with a minor second crop (Nyambo et al. 2005). Self-pollination is possible in guava; however, cross-pollination by insects results in higher yields (Crane and Balerdi 2005). Guava is an allogamous fruit crop which is highly heterozygous (Chandra and Mishra 2007).

Figure 1. Photos of guava trees growing in farmers' field (a) young guava tree, and (b) mature guava tree with fruits. Source: photos by J.C. Chiveu.

There are probably more than 400 guava cultivars around the world, but only a few are under common cultivation (Pommer and Murakami 2009). The cultivated cultivars are widely diverse regarding tree size, bearing habit, and yield, as well as fruit size, shape, ripening season and quality in terms of nutrient composition (Pommer and Murakami 2009; Sharma et al. 2010). Irrespective of the morphological and nutritional diversities observed in these cultivars, several reports indicate that selection of the accessions was based on a few morphological traits that were considered important (e.g. Mehmood et al. 2013; Galli et al. 2015; Mehmood et al. 2015; Valera-Montero et al. 2016), and, therefore, much of the variation is left untapped. This is likely to lead to genetic vulnerability of the crop (Nogueira et al. 2014), especially with respect to climate change.

1.3. Nutritional properties and uses

Guava fruit is mostly consumed fresh and has been reported to be rich in several important nutrients. Recent studies have reported appreciable amount of vitamin C and other antioxidants, calcium, potassium and phosphorus, as well as dietary fibres (Youssef and Ibrahim 2016; Singh 2005; Prakash et al. 2002; Jiménez-Escrig et al. 2001). Lyophilized extract of raw fruit peel has been experimentally shown to reduce low-density lipoprotein (LDL) cholesterol and triglycerides (Rai et al. 2010; Setiawan et al. 2001). Furthermore, the fruits, leaves, flowers, roots, bark, and stems are traditionally used for their medicinal properties (Gutiérrez et al. 2008). Guava wood is useful for tool manufacturing, fencing and use as firewood in the form of charcoal (Orwa et al. 2009). Guava processing yields 25% by-products that can be used in animal feeding (Azevêdo et al. 2011). In recent years, the guava fruit has gained popularity in the international trade due to its nutritional value and the diversity of processed products including jam, jelly, and juice (Available: *http://pdf.usaid.gov/pdf_docs/PA00KP1Z.pdf.* Accessed 30.11.2017).

Fruit chemical and mineral composition is affected by the climatic and soil factors where the fruit tree grows (Wall 2006). Moreover, other factors such as climatic conditions, cultivar, fruit size and maturity stage of the fruit also impact on its chemical and mineral composition (Burlingame et al. 2009). There is however limited knowledge on the effect of each of these factors on the chemical and mineral composition of guava.

1.4. Salinity effect in plants

Salinity is the concentration of dissolved mineral salts present in soils (soil solution) and waters (Hu and Schmidhalter 2004). Salinity decreases the agricultural production of most crops and also affects the physicochemical properties of the soil (Hu and Schmidhalter 2004). Soil salinity affects about 7% of the world's total land area and 23% of arable land (Flowers et al. 1997). In semiarid and arid areas, low precipitation, high level of evaporation

and existence of saline parent rock are the major causes of salinity. However, salinity also results from poor techniques of irrigation, irrigation with salinized water and salt accumulation from high doses of mineral fertilization (Bresler et al. 2012).

Mechanisms of salt stress and tolerance by plants are very complex (Kozlowski, 1997) and have been a topic in many baseline studies (Kozlowski 1997; Grattan and Grieve 1998; Hu and Schmidhalter 2004; Munns and Tester 2008). Salinity generally impairs plant growth in a quick osmotic phase, during which development of young leaves is inhibited and a tardy ionic phase in which senescence of older leaves is accelerated (Munns 2002). Plants have developed different types of tolerance mechanisms, which include osmotic stress tolerance, ion exclusion, and tissue tolerance to ion accumulation (Munns and Tester 2008).

Crop salt tolerance is the ability of plants to survive and produce economic yields under adverse conditions of salinity (Hu and Schmidhalter 2004). It is usually determined by the percentage of biomass production in saline versus control conditions over a period of time (Martin et al. 1994). Tolerance can also be determined by uptake and accumulation of sodium ions (Na^+) in the above-ground biomass as genetic differences in Na^+ exclusion from the transpiration stream have been reported (Munns and James 2003). In addition, the relationship between salinity tolerance and potassium/sodium ratio (K^+/Na^+) discrimination by the plant is usually considered – with a higher K^+/Na^+ regarded as a means to reduce Na^+ toxicity in the plant (Munns 2005).

1.5. Effect of salinity in guava

Fruit trees are generally regarded as very sensitive to soil salinity (Ebert 1999). However, a diversity in salt tolerance between plant species and between cultivars within a crop species exist (Kozlowski 1997). Guava production faces salinity challenges in many guava-producing countries, such as Brazil (Cavalcante et al. 2007), Australia (Noble and West 1988), India (Singh et al. 2016), Kenya (Mugai 2004), and Sudan (Ali-Dinar et al.

1999). The tolerance threshold for most cultivated guava varieties is generally reported to vary between 30 mM sodium chloride (NaCl) and 50 mM NaCl in the rhizosphere (Ali-Dinar et al. 1999; Desai and Singh 1983). In fact, guava has been found to be more sensitive to salinity during the seedling phase (Cavalcante et al. 2007), as shoot growth is impaired by salinity in this stage. Some guava types have been reported to perform better under salinity stress than others (Singh et al. 2016). Most studies on salinity tolerance of guava have mainly focused on mechanisms to alleviate the effect of salt stress – consequently, their salinity experiments comprised of treatments that could help plants tolerate salinity. For instance, by application of nitrate fertilizers (Ali-Dinar et al. 1998), calcium nitrate (Ebert et al. 2002) and organic manure (da Silva et al. 2008). There is therefore a need to provide uniform growing conditions for salinity experiments in guava. This will enable selection of guava genotypes to saline environments as the genotypes would only vary mainly due to the salt treatments.

1.6. Guava diversity and production status in Kenya

Fruit and nut production offers tremendous opportunities for enhancing the incomes of small-scale farming families in Kenya and elsewhere in Africa. It also helps in improving the nutrition of the poor people who have been reported to suffer from deficiencies of vitamins, minerals and other micronutrients as a consequence of their low consumption (FAO 2003). For instance, it is estimated that about 50 million African children are at risk of vitamin A deficiency as a result of its low intake, hence considered Africa's third greatest public health problem after HIV/AIDS and malaria (WHO 2002). In Kenya for instance, 35% of children under the age of five years are stunted, 16% are underweight and 7% are wasted as a result of malnutrition (M.O.H.S 2012). To exacerbate the situation, reports also indicate that East Africa has the least fruit consumption anywhere in the world. For example, fruit consumption is generally low in Kenya and is with 36 g fruits per person and day far

below the World Health Organization (WHO) recommended minimum of 400 g fruits and vegetables per person and day (WHO 2003, 2002).

Kenya has diverse agro-ecological zones that contribute to production of a wide diversity of both exotic and indigenous fruits (Simitu et al. 2008). However, some of the naturalized fruit species such as guava and many indigenous ones, are underutilized (Chikamai et al. 2004). This implies that their potential in terms of health benefits, contribution to food security and income generation is under-exploited. Fruit growing in Kenya is mainly carried out by farmers with a low resource base and their level of fruit species diversification is also low (Mbora et al. 2008). Moreover, little information concerning the under-utilized fruit species limits their promotion along the fruits' product value chain with regard to utilization, production potential, processing, and effective and efficient marketing (Mbora et al. 2008). Guava is found in all the agroecological regions in Kenya apart from very arid areas and highlands. It is however not clear when it was introduced in Kenya, but has been naturalized and occurs in the wild and on farmer's fields. Most guava fruits are collected for home consumption and the domestic market. According to the Horticultural Crops Directorate, HCD (2014), the trees are mainly unattended, growing from seeds dispersed unintentionally. In spite of this, the HCD (2014) reported increases in the acreage, production, productivity and value of Kenyan guava fruits over the years (Figure 2).

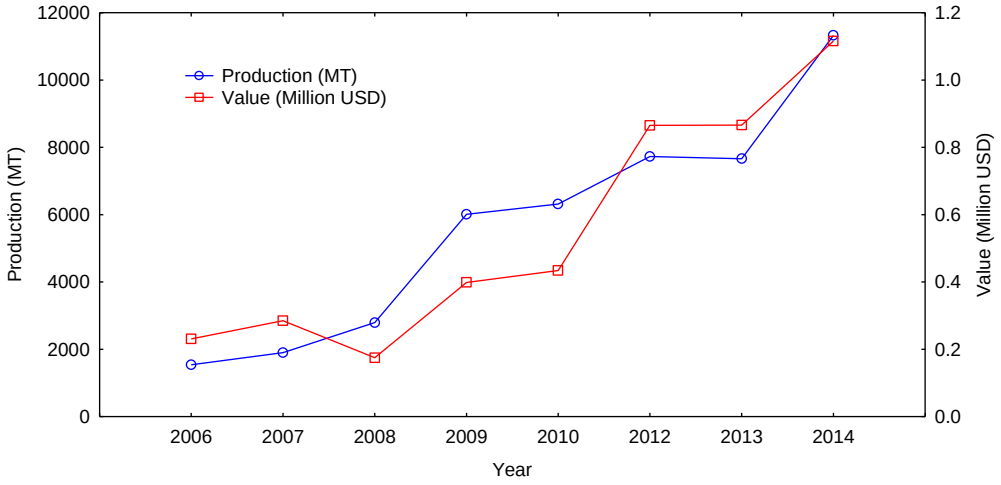

Figure 2. Production trend and value of Kenyan guava fruit from the year 2006-2014. The graph was plotted from data of HCD (2014) and HCDA (2010) reports, and data for 2011 was not included.

In the year 2014, the area under guava production was given as 1,260 ha and about 11,000 tons of fruits worth 112 million Kenya shillings (approximately USD 1.1 million) were produced. The HCD report cited the lack of suitable superior varieties, limited knowledge of agronomic and postharvest practices, and limited value addition as constraints in guava production in Kenya. The starting point for guava improvement in Kenya is, therefore, to collect germplasm for characterization and conservation, and synthesize knowledge of the existing genetic and nutritional diversity and production situation.

Genetic diversity studies can be performed using both phenotypic and molecular data that provide complementary information regarding each genotype (Nogueira et al. 2014). Unlike the morphological characters, molecular marker analysis is more expensive, but independent from environmental influences; thus, it is suitable for the identification of landraces in field gene banks and for breeding purposes (Sennhenn et al. 2013). In guava research, several molecular marker techniques have been employed. Random amplified polymorphic DNA (RAPD) markers have been used to estimate the molecular diversity of guava genotypes in India (Chandra and Mishra 2007). Inter-simple sequence repeats (ISSRs)

have been used to assess their association with the Vitamin C content in the Egyptian guava genotypes (Youssef and Ibrahim 2016). Additionally, the ISSR markers have been used to assess the genetic stability of micro-propagated guava (Liu and Yang 2012). Co-dominant markers, such as simple sequence repeats (SSRs), have been used to study the genetic diversity of guava in Cuba (Rodríguez et al. 2007), Mexico (Quiroz-Moreno et al. 2009) and the United States (Sitther et al. 2014). SSRs have also been used for the mapping of quantitative trait loci (QTLs) for vegetative and reproductive characters in guava (Rodríguez et al. 2007).

The increasing prospect for the utilization of guava through commercialization, particularly for processing and export, requires the use of a wide genetic base for selection and breeding of most suitable varieties for different environments and purposes. Therefore, to tap the genetic resource of this species, there is need to obtain knowledge on the existing genotypes, their exact distribution within the countries agro-climatic zones, their level of genetic diversity, nutritional value of their fruits and salinity tolerance of the genotypes.

This dissertation presents the findings of the three aims of our study. Chapter one presents the genetic diversity of guava in Kenya based on SSR markers. Chapter two presents the results of the nutritional and chemical diversity of guava fruit in relation to climatic, fruit morphological traits and soil properties. In chapter three, genetically diverse accessions of guava were tested for their level of salinity tolerance, and thus the implication for their potential area of cultivation.

Chapter two

2. Genetic diversity of common guava in Kenya: an underutilized naturalized fruit species

Abstract

Common guava (*Psidium guajava* L.) fruit has a significant nutritional and medicinal potential besides its economic importance. Currently, the world guava fruit production is based only on a few cultivars. It is not clear when guava was introduced in Kenya - but the species is currently naturalized. There is no detailed study on guava diversity in Kenya to enable a comparison with other guava producing countries for purposes of characterization and improvement. Genetic diversity of 177 guava accessions collected in four regions of Kenya (Coast, Eastern, Rift Valley and Western) was assessed using 13 simple sequence repeat (SSR) markers. The neighbour-joining (NJ) phylogenetic tree revealed most accessions generally clustering into multiple weakly supported groups. Only 46 out of 177 accessions were supported by bootstrap values above 50% and clustered in twenty two groups, each comprising two or three individual accessions only. The principle coordinates analysis (PCoA) did not reveal clear-cut clusters along geographic origins or fruit flesh colour of the samples. The fixation index (F_{IS}) was very high (F_{IS} = 0.511) that could be due to a high level of either inbreeding and/ or differentiation. The white-fleshed accessions were clustered together with the red-fleshed types, indicative of some degree of genetic similarity, but also pointing to a possibility of shared ancestry between them. For guava conservation, selection and improvement in Kenya, we recommend sampling many individual accessions covering the geographical range of the species.

2.1. Introduction

Common (also known as yellow or lemon) guava (*Psidium guajava* L.) is one of the most important fruit crops domesticated in Mesoamerica and widely cultivated in the tropics and some sub-tropical regions (Gautam et al. 2010; Rodríguez et al. 2010). The fruit is consumed fresh and processed and is rich in several important nutrients. The fresh fruit pulp is high in vitamins, particularly vitamin C, phosphorus, and potassium, as well as many antioxidants and dietary fibres (Jiménez-Escrig et al. 2001; Lukmanji et al. 2008; Flores et al. 2015). Furthermore, not only fruits, but leaves, flowers, roots and bark are traditionally used also in medicine (Gutiérrez et al. 2008). In recent years, the guava fruit has gained popularity in the international trade due to its nutritional value and the diversity of processed products including jam, jelly, and juice (*http://pdf.usaid.gov/pdf_docs/PA00KP1Z.pdf.* Accessed 30.11.2017).

There are probably more than 400 guava cultivars around the world, but only a few are under common cultivation (Pommer and Murakami 2009). The cultivated cultivars are widely diverse regarding tree size, bearing habit, and yield, as well as fruit size, shape, ripening season and quality in terms of nutrient composition (Pommer and Murakami 2009; Sharma et al. 2010). Irrespective of the morphological and chemical diversities observed in these cultivars, several reports indicate that selection of the accessions was based on a few traits considered important (Mehmood et al. 2013; Galli et al. 2015; Mehmood et al. 2015; Valera-Montero et al. 2016), and, therefore, much of the variation is left untapped. This is likely to lead to genetic vulnerability of the crop (Nogueira et al. 2014), especially with respect to climate change.

With regard to Kenya, guava is found in all the agroecological regions apart from very arid areas and highlands. It is however not clear when it was introduced in Kenya, but has been naturalized and occurs in the wild and on farmer's fields. Most guava fruits are

collected for home consumption, although lately fresh fruits could be found being marketed in major Kenyan towns. According to the Horticultural Crops Directorate, HCD (2014), the trees are mainly unattended, growing from seeds dispersed unintentionally. The HCD report cited the lack of suitable superior varieties, limited knowledge of agronomic and postharvest practices, and limited value addition as constraints in guava production in Kenya. The starting point for guava improvement in Kenya is, therefore, to collect germplasm for characterization and conservation, and synthesize knowledge of the existing genetic diversity and production situation.

The objective was to analyse the genetic diversity and differentiation of guava accessions collected in four different regions of Kenya. We hypothesized that due to diverse range of agroecological conditions of guava, the accessions are highly differentiated. In addition, white and red-fleshed accessions would cluster separately. Accurate knowledge of the genetic diversity and the origin of the accessions would assist in the selection of parental materials in breeding programmes. Such information will consequently eliminate the possibility of redundant collection of identical individuals for conservation and improvement, thereby enhancing cost effective use of land, space and time regarding field gene bank establishment and breeding activities.

2.2. Materials and methods

2.2.1. Study site selection and sampling procedure

Four known major guava-producing regions in Kenya were selected for guava sampling based on the horticulture-validated report data (HCD 2014). The sites for sampling within these regions included Meru (Eastern region), Uasin-Gishu and Elgeyo-Marakwet (Rift Valley region), Homabay, Siaya, Kakamega and Vihiga (Western region), and Kwale, Kilifi, and Mombasa (Coastal region) (Figure 1). Most sampled trees were found on individual farmer's fields, but also one prison fruit farm and one commercial fruit farm were

included in the sampling. A majority of the sampled trees (27%) growing on farmers' fields were found either growing in fruit orchards together with other fruit trees or in crop fields. About 25% of the sampled trees were found in the farmers' homestead fence and 11% were growing in uncultivated farm parts together with other wild trees and shrubs. The remainder of the trees were found growing as shade trees in farmers' compounds, along rivers, and in fallow fields. The trees were sampled randomly in cases where more than 10 trees occurred on the same farm, though in most cases all guava trees within the farm were sampled.

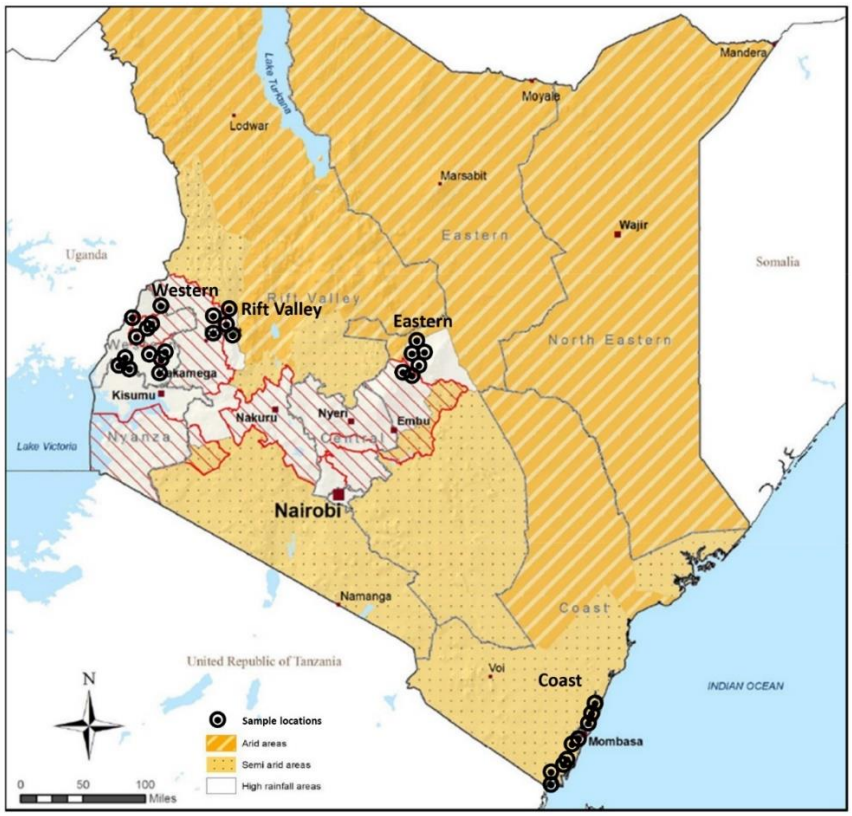

Figure 1. Sample collection locations for the guava accessions (circles) in four regions of Kenya (Coastal, n = 38; Eastern, n = 19; Rift Valley, n =48 and Western, n = 72). The map was adapted from the International Fund for Agricultural Development (IFAD) report (available: https://www.ifad.org/documents/10180/42d2d8ea-644f-4bc3-a977-c3edb103b148. Accessed 03.12.2017) (i.e. see also Supplemental Information – Table S1).

2.2.2. Leaf material sampling

Leaves from a total of 177 guava trees (here also referred to as accessions or sample) were sampled (Supplemental Information - Table S1). At least five young fully developed healthy leaves were picked at random from each of the 177 accessions (72 in Western, 48 in Rift Valley, 38 in Coastal, and 19 in Eastern regions). The leaves were then briefly dried under a shade in the field and placed in the sealable polythene bags containing silica gel for complete drying and preservation. Afterwards, the leaf samples were taken to the laboratory for DNA isolation and subsequent fragment analysis.

2.2.3. DNA isolation and quantification

Nuclear DNA from silica gel dried-leaf samples (about 300—500 mg) was extracted using the DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany). DNA quality and quantity were checked on a 3% (w/v) agarose gel by comparing it with a known λ DNA concentration. The stock DNA preps were diluted accordingly with molecular-grade water and then stored at -20°C for eventual analyses.

2.2.4. Primer selection for polymerase chain reaction (PCR) amplification

We used the PCR primers that were previously designed for guava by Risterucci et al. (2005) and had been proved successful in assessing guava diversity (Valdés-Infante et al. 2010; Sitther et al. 2014). We tested 20 primer pairs used by Risterucci et al. (2005) and selected the best 13, which were also good for multiplexing. The primers were labelled with fluorescent dyes; and those primers that amplified alleles with non-overlapping fragment lengths were pooled to save on the PCR cost and time (Supplemental Information - Table S2). Table S2 also shows the allele size ranges in base pairs (bp) observed for each primer pair in our guava accessions.

The PCR amplification was conducted in a 14 µL volume containing 1 µL of genomic DNA (20 ng/µL), 1.5 µL PCR buffer (0.8 M Tris–HCl pH 9.0, 0.2 M $[NH_4]_2SO_4$,

0.2% w/v Tween-20; Solis BioDyne, Tartu, Estonia), 1.5 µL $MgCl_2$, 1 µL dNTPs, 2 µL fluorescent dye-labelled forward and reverse primers, 0.2 µL *Taq* DNA polymerase (HOT FIREPol DNA Polymerase, Solis BioDyne, Tartu, Estonia), and 6.8 µL distilled water. The amplification procedure included an initial denaturation step of 95°C for 15 min followed by 35 cycles of 94°C for 1 min (denaturation), 50°C for 1 min (annealing), 72°C for 1 min (extension), and a final extension step of 72°C for 20 min. The PCR reactions were conducted in a T-Professional thermocycler (Biometra, Analytik Jena, Germany).

In preparation for fragment analysis, the PCR products were diluted with water in a ratio of 1:100. Next, 2 µL of the diluted PCR product comprising of 12 µL of Hi-Di formamide and 0.6 µL of internal size standard Genescan 500 Rox (Applied Biosystems Inc.) was denatured at 95°C for 3 min in a thermocycler. The fragments were then analysed in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Waltham, MA USA). The sizes of the microsatellite fragments were determined, and the microsatellite loci were genotyped using the GeneMapper software v. 4.0 (Applied Biosystems Inc.).

2.3. Data analysis

The number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosities were computed using the GenAlEx 6.5 software (Peakall and Smouse 2012). The duplicates were checked by multi-locus matching. The fixation index (F_{IS}) in the entire sample was computed following the definition of Wright (1965) using Genepop software v 4.0 (Rousset 2008). Nei's chord distance (1983) matrix between accessions was generated using microsatellite analyser (Dieringer and Schlötterer 2003) with 10,000 bootstrappings. The distances were then used to generate a phylogenetic tree using the neighbour-joining (NJ) method of clustering (Saitou and Nei 1987) available in PHYLIP (Felsenstein 1993), which was visualized using the Geneious software v. 10.1.3. (*www.geneious.com*; Kearse et al. (2012).

A PCoA with covariance standardization available in GenAlEx 6.5 was used to determine the spatial distribution of the samples based on their genetic distances. Moreover, AMOVA was performed from a triangular distance matrix with 1000 permutations to quantify genetic variation within and among regions. The SSR data was also subjected to a Bayesian cluster analysis using the STRUCTURE software v. 2.3.2 (Pritchard et al. 2000) in order to infer the most likely number of subpopulations or groups (clusters) in the sample. The admixture model was applied without assigning individual trees to particular groups or geographic regions *a priori*; and the samples were tested for number of potential clusters (*K*) ranging from 1 to 10. Ten runs per each *K* were performed, each consisting of a burn-in of 100,000, followed by 1,000,000 Monte Carlo Markov Chain iterations. The ΔK value approach (Evanno et al. 2005) was used to determine the most probable number of clusters using the STRUCTURE Harvester program (Earl and von Holdt 2012).

2.4. Results

All the PCR primers were able to generate fragments in all our samples, and all the amplified alleles were polymorphic. No samples with genotypes identical for all markers (supposedly duplicates) were found in the collected 177 guava accessions. The 13 primer pairs amplified 84 alleles in the studied guava accessions in total. The highest number of alleles (13) was found in locus mPgCIR10, while the least number of alleles (four) – in loci mPgCIR08, mPgCIR11, mPgCIR13 and mPgCIR21. The expected heterozygosity (H_e) values ranged from 0.507 to 0.843 with an average of 0.630, while the observed heterozygosity (H_o) values ranged from 0.192 to 0.497 with an average of 0.312. The fixation index (F_{IS}) among the accessions for the entire sample ranged from 0.410 to 0.621 for different markers with an average of 0.511 (Table 1).

Table 1. Summary genetic variation statistics for 177 guava accessions collected from four regions of Kenya assessed with 13 simple sequence repeats.

Locus	N_a	N_e	H_o	H_e	F_{IS}
mPgCIR07	6	2.2	0.220	0.545	0.596
mPgCIR08	4	2.1	0.203	0.518	0.608
mPgCIR09	6	3.6	0.356	0.721	0.506
mPgCIR10	13	6.4	0.497	0.843	0.410
mPgCIR11	4	2.7	0.282	0.634	0.554
mPgCIR13	4	2.0	0.192	0.507	0.621
mPgCIR15	8	5.2	0.418	0.809	0.483
mPgCIR17	5	2.3	0.232	0.565	0.590
mPgCIR19	7	2.4	0.322	0.590	0.454
mPgCIR20	9	2.4	0.311	0.583	0.467
mPgCIR21	4	2.2	0.305	0.540	0.435
mPgCIR22	6	2.4	0.328	0.592	0.446
mPgCIR25	8	3.9	0.390	0.740	0.474
mean	6.5	3.1	0.312	0.630	0.511

N_a- number of alleles, N_e- number of effective alleles, H_o- observed heterozygosity, H_e- expected heterozygosity, F_{IS}- fixation index (i.e. see also Supplemental Information -Table S2).

Most samples in the cluster analysis were not supported by bootstrap values above 50% (Supplemental Information - Figure S1). Only 46 samples had bootstrap values above 50% and clustered mainly into groups of two or three accessions in the observed 22 clusters (Figure 2). In general, samples from all regions were found in the well sustained 22 clusters. It was interesting to observe that the white-fleshed accessions were clustered together with red-fleshed accessions in two of the clusters. It was also noted that the accessions within the well supported 22 clusters grouped mainly according to their geographical origins, except in two of the groups.

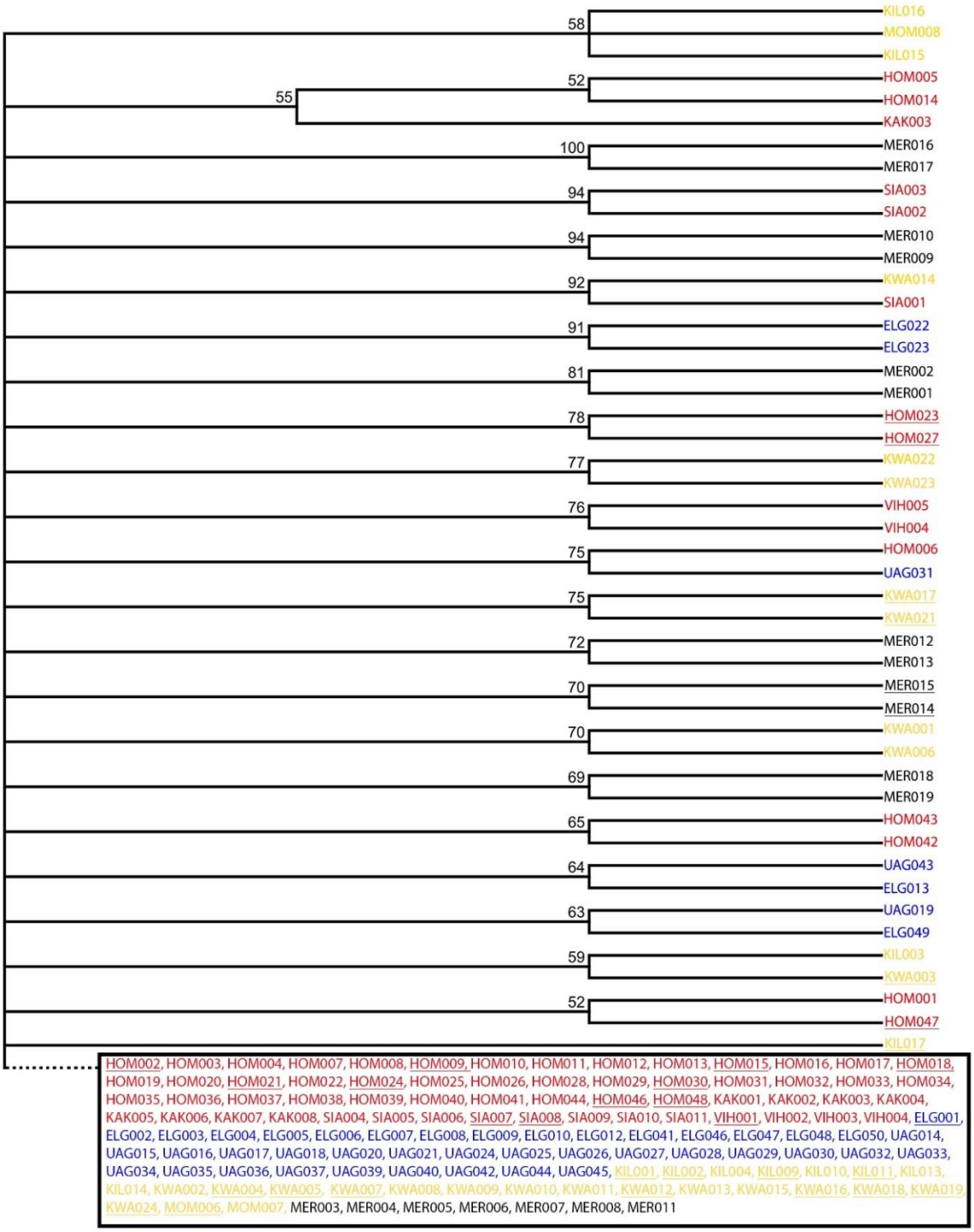

Figure 2. The neighbour-joining phylogenetic tree of 177 guava accessions collected from four regions of Kenya. The accession colour codes depict the region of collection (Red = Western, Blue = Rift Valley, Gold = Coast, Black = Eastern). The white-fleshed accessions are underlined. Only bootstrap values of 50% and more are indicated for tree nodes after 10,000 bootstrapping. Accessions within the text box were supported by bootstrap values below 50% (i.e. see also Supplemental Information – Figure S1).

The STRUCTURE analysis, however, did not reveal any genetic clusters based on both the *LnP(D)* and Δ*K* value (Evanno et al. 2005) analyses (Figure 3 and Supplemental Information - Figure S2). Consequently, the accessions were significantly admixed with any number of clusters, thereby pointing at the possibility of existence of only one genetic cluster. There was no preferential grouping of the accessions based on fruit flesh colour.

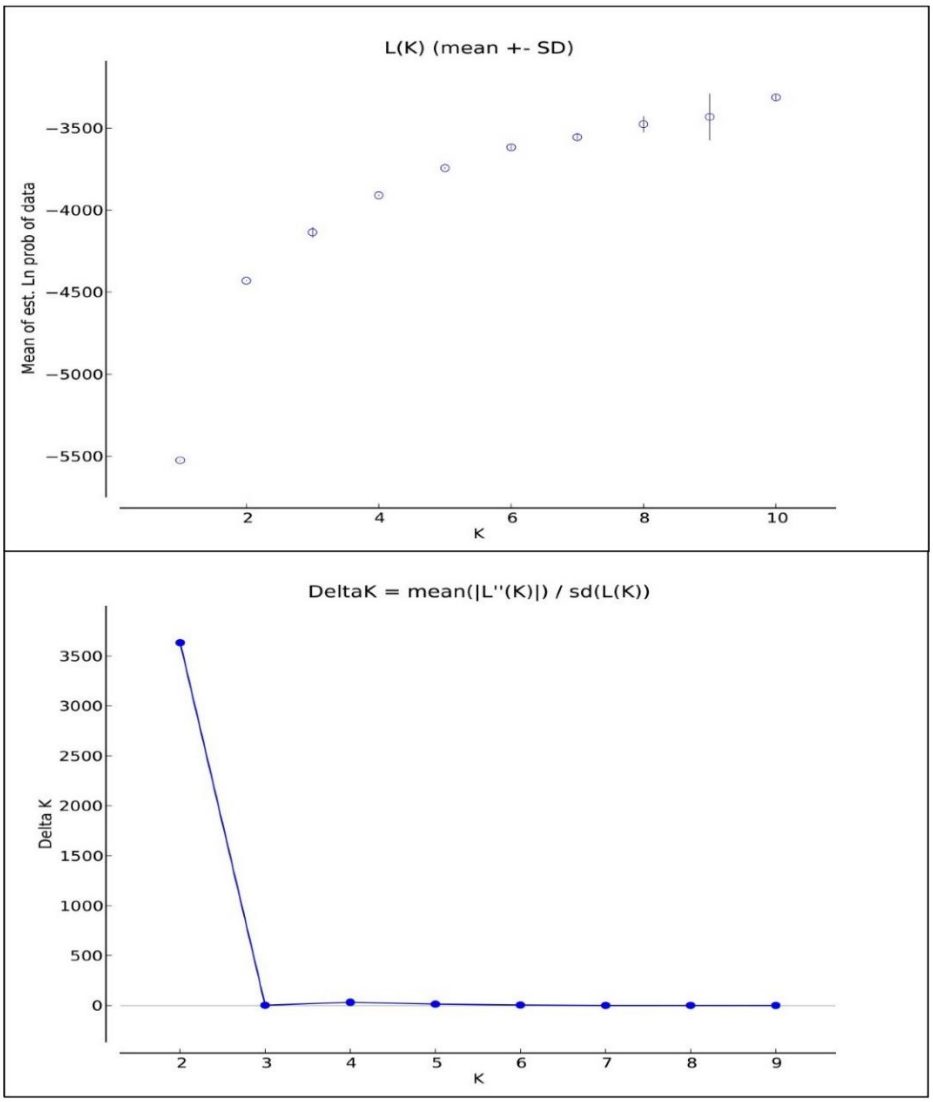

Figure 3. Estimate of probability of the data for a given *K, (LnP(D)* (Pritchard et al. 2000) and plots for detecting the most probable number of K groups (Δ*K)* (Evanno et al. 2005) based on 13 SSR loci genotyped in 177 Kenyan guava accessions (i.e. see also Supplemental Information – Figure S2).

The PCoA confirmed the lack of strongly differentiated groups or clusters among accessions (Figure 4)—this is similar to the results of the NJ clustering and Bayesian cluster analysis. Similarly, AMOVA performed in accordance with the region of collection of the accessions revealed that much of the genetic variation (87%) resided within accessions in a region (Table 2).

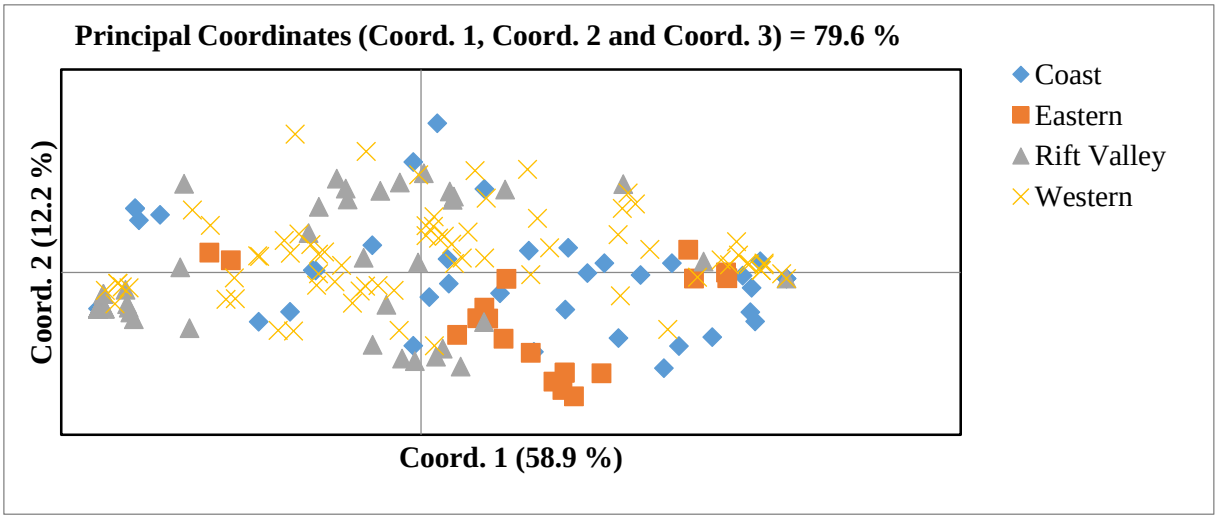

Figure 4. Principal coordinate analysis of 177 guava accessions collected from four regions of Kenya. The first three axes explained 79.6% of the total variation, with the first axis explaining 58.9%, second - 12.2%, and third - 8.5%.

Table 2. AMOVA based on the region of collection of 177 guava accessions from four regions of Kenya.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Estimated Variance	Variation %	Amova Statistic	*P**
Among regions	3	243.844	81.281	1.689	13	0.131	0.001
Within regions	173	1936.557	11.194	11.194	87		
Total	176	2180.401		12.883	100		

*After 1000 random permutations.

2.5. Discussion

The comparison of the Kenyan guava germplasm multilocus SSR genotypes revealed no identical or duplicate accessions; therefore, each accession was genetically distinct from the others. The low levels of observed heterozygosity (mean = 0.312) with respect to expected heterozygosity (mean = 0.630) likely indicates a high level of genetic differentiation between accessions within identified groups, including those that existed within the same geographical locations. Similar results were also reported using SSR markers by Sitther et al. (2014), where the observed and expected heterozygosities were 0.2 and 0.7 on the average, respectively, in the guava germplasm found in the United States. The expected heterozygosity was even much higher and varied between 0.392 and 0.961 with an average of 0.824 in the Indian guava based on the SSR genotypes (Kanupriya et al. 2011), while a much lower expected heterozygosity ranging from 0.027 to 0.172 with an average of 0.085, was found in the Pakistan guava germplasm also based on the SSR markers (Mehmood et al. 2015). Similarly, low to moderate levels of expected heterozygosity (0.057 to 0.568) were detected in the Cuban guava germplasm using microsatellites (Rodríguez et al. 2007). The differences in the heterozygosity indices in the aforementioned studies were attributed to the high inbreeding and a possibility of cross incompatibility occurring in guava. The difference in the diversity among the mentioned studies, however, could be also due to the different microsatellite loci used (Pommer and Murakami 2009), but it is more likely that they were accession or sample specific (Belaj et al. 2003).

The average fixation index in our study was 0.511 (Table 1), implying a high genetic substructure within our guava accessions or a high inbreeding rate. The Myrtaceae flower has been reported to be hermaphrodite, which increases the possibility of selfing (Grattapaglia et al. 2012). Nakasone and Paull (1998) estimated the outcrossing rate as only 35-40% in *Psidium guajava*, which is in agreement with our results. In contrast, very high

inbreeding coefficients of 0.8 and 0.85 have been reported in the SSR studies by Sitther et al. (2014) and Mehmood et al. (2015), respectively. These very high values of an average inbreeding coefficient point at the possibility of cross-incompatibility, which may hinder the effectiveness of creating true hybrids and recombining favourable alleles from parental clones in guava as reported by Mehmood et al. (2015).

Based on the NJ phylogenetic tree, some of the Kenyan guava accessions mainly from one region were well supported by bootstrap values above 50% and grouped together in clusters of two or three individuals (Fig. 2); although accessions from all the regions could be found together in different small genetic clusters when lower than 50% bootstrap values were considered (Supplemental Information - Figure S1). The PCoA, however, depicted an overlap between these clusters and groups that was also supported by the observed genetically admixed individuals based on the Bayesian clustering implemented in the STRUCTURE software. This implies that some accessions are very similar and can form genetic groups, while others are genetically distinct and admixed irrespective of their existence within the same geographical environment. This high intra-regional genetic heterogeneity was also supported by results of the AMOVA (Table 2). Population structure is as a result of geographic adaptation and natural selection (Lehermeier et al. 2015). Thus we expected that many individual guava accessions from one region rather than just two or three would cluster together and form distinct groups based on their geographic origin, which was not the case in our study. The lack of a robust sub-structuring in the Kenyan guava accessions could therefore be attributed to the high inbreeding as reported in other studies (Nakasone and Paull 1998) and plant material (seeds and seedlings) transfer across different regions.

The white-fleshed guava accessions were found in groups together with the red-fleshed types in the NJ phylogenetic tree. Therefore, the expectation based on previous

studies (Chen et al. 2007; Kanupriya et al. 2011) that all the white-fleshed accessions would be clustered in the same one group was not confirmed in our study. Moreover, grouping white- together with the red-fleshed types could be due to a shared ancestry of these accessions at some point in time, as supported by the Bayesian clustering in our study. However, the possibility of sympatric speciation cannot be ruled out in the Kenyan guava germplasm, especially when few accessions with similar flesh colour cluster together with higher bootstrap values. In a related study, Chen et al. (2007) identified two genetic groups based on RAPD markers. The commercial and wild genotypes of guava were clustered separately in their study, possibly depicting selection pressure on the traits of interest for the commercial group. The latter group included two subgroups, which roughly clustered white- and red-fleshed guavas separately. In Bajpai et al. (2008), 22 guava genotypes were clustered according to their regions of origin based on the RAPD and directed amplification of minisatellite DNA (DAMD) markers. Additionally, molecular data allowed Coser et al. (2012) and Nogueira et al. (2014) to cluster most genotypes in accordance with their origins in Brazil. Notably, although the genotypes were registered as cultivars having been highly selected based on production quality traits, but they still clustered according to the initial parental material origin with minimal segregation (Coser et al. 2012). In the Indian guava germplasm, the pink flesh cultivars were reported to group separately from those with white flesh (Kanupriya et al. 2011), probably pointing at their distinct evolutionary pathways.

Initial efforts to improve guava production in Kenya have only concentrated on the conservation of the available germplasm through collection based on morphological attributes such as leaf shape or fruit flesh colour, among others. A few of these genotypes collected from various regions of the country have been conserved at the Kenya Agricultural and Livestock Research Organization (KALRO). Therefore, guava farmers in Kenya rely on the genetic variation existing in the wild populations on their farms and probably on limited

exchange of some genotypes they consider superior for production and quality traits between individual farmers.

2.6. Conclusion

In conclusion, the SSRs markers were able to distinguish among the Kenyan guava accessions. Much of the genetic variation resided within individual accessions found in different geographical locations of the country, and therefore we reject the hypothesis that the accessions would cluster according to their agroecological environments. In addition, the white-fleshed guava accessions clustered together with the red-fleshed guava accessions, hence suggesting a shared ancestry. Therefore, we recommend that sampling for conservation and improvement should aim at including trees from different regions as well as covering the full ecological range of the species. This data also forms a basis for comparison of guava genetic diversity studies with other guava producing countries, and therefore joint research aimed at guava improvement could be initiated.

2.7. Supplementary information

Table S1. Accession codes, region of collection, fruit flesh colour, altitude and geographic coordinates of the locations of the sampled Kenyan guava accessions, Related to Figure 1.

Sample number	Accession code	Region	Fruit flesh colour	Latitude [N°/S°]	Longitude [E°]	Altitude (m)
1	KIL001	Coast	White	03.69568 °S	039.72340 °E	208
2	KIL002	Coast	White	03.69580 °S	039.72343 °E	199
3	KIL003	Coast	Red	03.69679 °S	039.72604 °E	202
4	KIL004	Coast	Red	03.69518 °S	039.72219 °E	200
5	KIL009	Coast	White	03.92239 °S	039.74352 °E	23
6	KIL010	Coast	Red	03.92240 °S	039.74314 °E	25
7	KIL011	Coast	White	03.92226 °S	039.74282 °E	22
8	KIL013	Coast	Red	03.91339 °S	039.74015 °E	18
9	KIL014	Coast	Red	03.91348 °S	039.74015 °E	17
10	KIL015	Coast	Red	03.91338 °S	039.73997 °E	18
11	KIL016	Coast	Red	03.91332 °S	039.73999 °E	21
12	KIL017	Coast	White	03.91347 °S	039.73988 °E	20
13	KWA001	Coast	Red	04.16923 °S	039.59783 °E	23
14	KWA002	Coast	Red	04.16853 °S	039.59749 °E	19
15	KWA003	Coast	White	04.16856 °S	039.59748 °E	19
16	KWA004	Coast	White	04.16854 °S	039.59750 °E	19
17	KWA005	Coast	White	04.16494 °S	039.57737 °E	104
18	KWA006	Coast	Red	04.16495 °S	039.57743 °E	97
19	KWA007	Coast	White	04.16496 °S	039.57764 °E	119
20	KWA008	Coast	Red	04.16782 °S	039.56780 °E	108
21	KWA009	Coast	Red	04.16837 °S	039.56796 °E	92
22	KWA010	Coast	Red	04.16860 °S	039.56822 °E	94
23	KWA011	Coast	Red	04.34928 °S	039.53458 °E	22
24	KWA012	Coast	White	04.34926 °S	039.53447 °E	23
25	KWA013	Coast	Red	04.34938 °S	039.53400 °E	26
26	KWA014	Coast	Red	04.34318 °S	039.51459 °E	35

27	KWA015	Coast	Red	04.33752 °S	039.44971 °E	117
28	KWA016	Coast	White	04.33753 °S	039.44975 °E	118
29	KWA017	Coast	White	04.49746 °S	039.25124 °E	39
30	KWA018	Coast	White	04.49765 °S	039.25125 °E	45
31	KWA019	Coast	White	04.49763 °S	039.25131 °E	41
32	KWA021	Coast	White	04.49715 °S	039.25139 °E	45
33	KWA022	Coast	Red	04.60348 °S	039.18504 °E	25
34	KWA023	Coast	Red	04.60352 °S	039.18509 °E	20
35	KWA024	Coast	White	04.60323 °S	039.18452 °E	21
36	MOM006	Coast	White	03.96482 °S	039.73122 °E	15
37	MOM007	Coast	Red	03.96493 °S	039.73089 °E	14
38	MOM008	Coast	Red	03.96229 °S	039.73233 °E	16
39	MER001	Eastern	Red	00.17234 °S	037.64283 °E	1564
40	MER002	Eastern	Red	00.17239 °S	037.64275 °E	1545
41	MER003	Eastern	Red	00.16647 °S	037.65030 °E	1457
42	MER004	Eastern	Red	00.16708 °S	037.65543 °E	1449
43	MER005	Eastern	Red	00.17249 °S	037.65120 °E	1479
44	MER006	Eastern	Red	00.17247 °S	037.65128 °E	1481
45	MER007	Eastern	Red	00.17251 °S	037.63130 °E	1481
46	MER008	Eastern	Red	00.19338 °S	037.66548 °E	1429
47	MER009	Eastern	Red	00.08721 °S	037.66675 °E	1455
48	MER010	Eastern	Red	00.08726 °S	037.66695 °E	1452
49	MER011	Eastern	Red	00.08583 °S	037.66500 °E	1474
50	MER012	Eastern	Red	00.08564 °S	037.66451 °E	1478
51	MER013	Eastern	Red	00.08536 °S	037.66438 °E	1481
52	MER014	Eastern	White	00.11461 °S	037.69637 °E	1384
53	MER015	Eastern	White	00.11443 °S	037.69638 °E	1380
54	MER016	Eastern	Red	00.18701 °S	037.69572 °E	1290
55	MER017	Eastern	Red	00.18693 °S	037.69600 °E	1288
56	MER018	Eastern	Red	00.12048 °S	037.72087 °E	1393
57	MER019	Eastern	Red	00.12024 °S	037.72074 °E	1385

58	ELG001	Rift Valley	White	00.64776 °N	035.51977 °E	2089
59	ELG002	Rift Valley	Red	00.64203 °N	035.52221 °E	2064
60	ELG003	Rift Valley	Red	00.64265 °N	035.52145 °E	2077
61	ELG004	Rift Valley	Red	00.64264 °N	035.52150 °E	2071
62	ELG005	Rift Valley	Red	00.67029 °N	035.51809 °E	2214
63	ELG006	Rift Valley	Red	00.67030 °N	035.51812 °E	2209
64	ELG007	Rift Valley	Red	00.64350 °N	035.51839 °E	2104
65	ELG008	Rift Valley	Red	00.64349 °N	035.51843 °E	2104
66	ELG009	Rift Valley	Red	00.64338 °N	035.51852 °E	2102
67	ELG010	Rift Valley	Red	00.64505 °N	035.51627 °E	2132
68	ELG012	Rift Valley	Red	00.63469 °N	035.52243 °E	2031
69	ELG013	Rift Valley	Red	00.63185 °N	035.52095 °E	2024
70	ELG018	Rift Valley	Red	00.58769 °N	035.46060 °E	2325
71	ELG022	Rift Valley	Red	00.63766 °N	035.51977 °E	2079
72	ELG023	Rift Valley	Red	00.64214 °N	035.52221 °E	2056
73	ELG041	Rift Valley	Red	00.63469 °N	035.52043 °E	2021
74	ELG046	Rift Valley	Red	00.63187 °N	035.52195 °E	2024
75	ELG047	Rift Valley	Red	00.66551 °N	035.53129 °E	1972
76	ELG048	Rift Valley	Red	00.66582 °N	035.53104 °E	1985
77	ELG049	Rift Valley	Red	00.56152 °N	035.30367 °E	2142
78	ELG050	Rift Valley	Red	00.58151 °N	035.30357 °E	2150
79	UAG014	Rift Valley	Red	00.57152 °N	035.30377 °E	2142
80	UAG015	Rift Valley	Red	00.57151 °N	035.30377 °E	2150
81	UAG016	Rift Valley	Red	00.58574 °N	035.46054 °E	2317
82	UAG017	Rift Valley	Red	00.57162 °N	035.30367 °E	2142
83	UAG019	Rift Valley	Red	00.58788 °N	035.46055 °E	2322
84	UAG020	Rift Valley	Red	00.66651 °N	035.53149 °E	1972
85	UAG021	Rift Valley	Red	00.66682 °N	035.53004 °E	1985
86	UAG024	Rift Valley	*	00.64256 °N	035.52145 °E	2067
87	UAG025	Rift Valley	*	00.64264 °N	035.52150 °E	2076
88	UAG026	Rift Valley	*	00.67019 °N	035.51809 °E	2267

89	UAG027	Rift Valley	*	00.67028 °N	035.51812 °E	2210
90	UAG028	Rift Valley	*	00.64352 °N	035.51839 °E	2114
91	UAG029	Rift Valley	*	00.64356 °N	035.51843 °E	2106
92	UAG030	Rift Valley	*	00.64109 °N	035.51783 °E	2119
93	UAG031	Rift Valley	*	00.64348 °N	035.51852 °E	2112
94	UAG032	Rift Valley	*	00.64509 °N	035.51627 °E	2125
95	UAG033	Rift Valley	*	00.64109 °N	035.51783 °E	2120
96	UAG034	Rift Valley	*	00.63469 °N	035.52243 °E	2041
97	UAG035	Rift Valley	*	00.63185 °N	035.52095 °E	2021
98	UAG036	Rift Valley	*	00.66651 °N	035.53149 °E	1972
99	UAG037	Rift Valley	*	00.66682 °N	035.53004 °E	1985
100	UAG039	Rift Valley	*	00.57152 °N	035.30377 °E	2142
101	UAG040	Rift Valley	*	00.64348 °N	035.51852 °E	2102
102	UAG042	Rift Valley	*	00.64438 °N	035.51752 °E	2102
103	UAG043	Rift Valley	*	00.64507 °N	035.51632 °E	2142
104	UAG044	Rift Valley	*	00.64505 °N	035.51627 °E	2142
105	UAG045	Rift Valley	*	00.64129 °N	035.51783 °E	2112
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107	HOM002	Western	White	00.59580 °N	034.57707 °E	1302
108	HOM003	Western	Red	00.59585 °N	034.57596 °E	1307
109	HOM004	Western	Red	00.59594 °N	034.57690 °E	1306
110	HOM005	Western	Red	00.59596 °N	034.57690 °E	1306
111	HOM006	Western	Red	00.59593 °N	034.57688 °E	1303
112	HOM007	Western	Red	00.59593 °N	034.57692 °E	1307
113	HOM008	Western	Red	00.59596 °N	034.57689 °E	1307
114	HOM009	Western	White	00.59600 °N	034.57698 °E	1305
115	HOM010	Western	Red	00.59596 °N	034.57703 °E	1307
116	HOM011	Western	Red	00.59603 °N	034.57717 °E	1302
117	HOM012	Western	Red	00.60963 °N	034.58897 °E	1329
118	HOM013	Western	Red	00.60974 °N	034.58366 °E	1335
119	HOM014	Western	Red	00.60961 °N	034.58369 °E	1339

120	HOM015	Western	White	00.60961 °N	034.58374 °E	1337
121	HOM016	Western	Red	00.60984 °N	034.58377 °E	1336
122	HOM017	Western	Red	00.60610 °N	034.63214 °E	1463
123	HOM018	Western	White	00.60611 °N	034.63223 °E	1456
124	HOM019	Western	Red	00.61762 °N	034.64497 °E	1498
125	HOM020	Western	Red	00.61760 °N	034.64495 °E	1502
126	HOM021	Western	White	00.61766 °N	034.64488 °E	1800
127	HOM022	Western	Red	00.53904 °N	034.50943 °E	1242
128	HOM023	Western	White	00.53907 °N	034.50946 °E	1238
129	HOM024	Western	White	00.53907 °N	034.50945 °E	1240
130	HOM025	Western	Red	00.53907 °N	034.50941 °E	1237
131	HOM026	Western	Red	00.53908 °N	034.50942 °E	1238
132	HOM027	Western	White	00.53906 °N	034.50946 °E	1242
133	HOM028	Western	Red	00.53905 °N	034.50951 °E	1239
134	HOM029	Western	Red	00.53893 °N	034.50956 °E	1240
135	HOM030	Western	White	00.53880 °N	034.50989 °E	1239
136	HOM031	Western	Red	00.53887 °N	034.51012 °E	1238
137	HOM032	Western	Red	00.53987 °N	034.50855 °E	1246
138	HOM033	Western	Red	00.72493 °N	034.45583 °E	1289
139	HOM034	Western	Red	00.72484 °N	034.45608 °E	1292
140	HOM035	Western	Red	00.72481 °N	034.45610 °E	1289
141	HOM036	Western	Red	00.72479 °N	034.45597 °E	1290
142	HOM037	Western	Red	00.72493 °N	034.45608 °E	1293
143	HOM038	Western	Red	00.72485 °N	034.45566 °E	1285
144	HOM039	Western	Red	00.72471 °N	034.45581 °E	1292
145	HOM040	Western	Red	00.72468 °N	034.45585 °E	1289
146	HOM041	Western	Red	00.72472 °N	034.45564 °E	1287
147	HOM042	Western	Red	00.72455 °N	034.45533 °E	1283
148	HOM043	Western	Red	00.72442 °N	034.45531 °E	1283
149	HOM044	Western	Red	00.72436 °N	034.45530 °E	1285
150	HOM046	Western	White	00.72439 °N	034.45518 °E	1283

151	HOM047	Western	White	00.72412 °N	034.45534 °E	1265
152	HOM048	Western	White	00.72412 °N	034.45539 °E	1275
153	KAK001	Western	Red	00.27951 °N	034.67358 °E	1419
154	KAK002	Western	Red	00.27863 °N	034.67363 °E	1409
155	KAK003	Western	Red	00.27861 °N	034.67367 °E	1420
156	KAK004	Western	Red	00.27791 °N	034.69564 °E	1447
157	KAK005	Western	Red	00.27700 °N	034.69589 °E	1441
158	KAK006	Western	Red	00.27777 °N	034.69579 °E	1443
159	KAK007	Western	Red	00.24446 °N	034.82470 °E	1571
160	KAK008	Western	Red	00.24442 °N	034.82479 °E	1572
161	SIA001	Western	Red	00.19481 °N	034.34081 °E	1297
162	SIA002	Western	Red	00.19376 °N	034.33390 °E	1286
163	SIA003	Western	Red	00.19423 °N	034.33385 °E	1280
164	SIA004	Western	Red	00.13007 °N	034.42597 °E	1358
165	SIA005	Western	Red	00.13003 °N	034.42687 °E	1357
166	SIA006	Western	Red	00.12687 °N	034.42089 °E	1340
167	SIA007	Western	White	00.12680 °N	034.42102 °E	1342
168	SIA008	Western	White	00.12804 °N	034.42337 °E	1347
169	SIA009	Western	Red	00.12810 °N	034.42309 °E	1347
170	SIA010	Western	Red	00.13046 °N	034.42354 °E	1348
171	SIA011	Western	Red	00.13008 °N	034.42255 °E	1349
172	VIH001	Western	White	00.08540 °N	034.79936 °E	1680
173	VIH002	Western	Red	00.08539 °N	034.79936 °E	1679
174	VIH003	Western	Red	00.08532 °N	034.79938 °E	1682
175	VIH004	Western	Red	00.84470 °N	034.79931 °E	1683
176	VIH005	Western	Red	00.84360 °N	034.79930 °E	1684
177	VIH006	Western	Red	00.08413 °N	034.79875 °E	1688

*There were no fruits on the trees at the time of sampling, hence fruit flesh colour was not determined

Table S2. PCR primer sequences and pools used for the PCR multiplexing in guava (*Psidium guajava* L.) DNA fragment analysis and size ranges of alleles amplified, Related to Table 1.

	Primer				
Multiplex pool	Name	Forward	Reverse	Fluorescent dye	Allele size range, bp
1	mPgCIR11	TGAAAGACAACAAACGAG	TTACACCCACCTAAATAAGA	HEX	301—316
	mPgCIR15	TCTAATCCCCTGAGTTTC	CCGATCATCTCTTTCTTT	HEX	146—166
	mPgCIR17	CCTTTCGTCATATTCACTT	CATTGGATGGTTGACAT	HEX	225—243
	mPgCIR19	AAAATCCTGAAGACGAAC	TATCAGAGGCTTGCATTA	HEX	255—280
2	mPgCIR07	ATGGAGGTAGGTTGATG	CGTAGTAATCGAAGAAATG	HEX	143—158
	mPgCIR09	GCGTGTCGTATTGTTTC	ATTTTCTTCTGCCTTGTC	FAM	155—175
	mPgCIR10	GTTGGCTCTTATTTTGGT	GCCCCATATCTAGGAAG	FAM	260—326
	mPgCIR13	CCTTTTTCCCGACCATTACA	TCGCACTGAGATTTTGTGCT	FAM	246—258
3	mPgCIR08	ACTTTCGGTCTCAACAAG	AGGCTTCCTACAAAAGTG	HEX	214—224
	mPgCIR20	TATACCACACGCTGAAAC	TTCCCCATAAACATCTCT	FAM	265—296
	mPgCIR21	TGCCCTTCTAAGTATAACAG	AGCTACAAACCTTCCTAAA	HEX	147—162
	mPgCIR22	CATAAGGACATTTGAGGAA	AATAAGAAAGCGAGCAGA	HEX	237—253
	mPgCIR25	GACAATCCAATCTCACTTT	TGTGTCAAGCATACCTTC	FAM	99—131

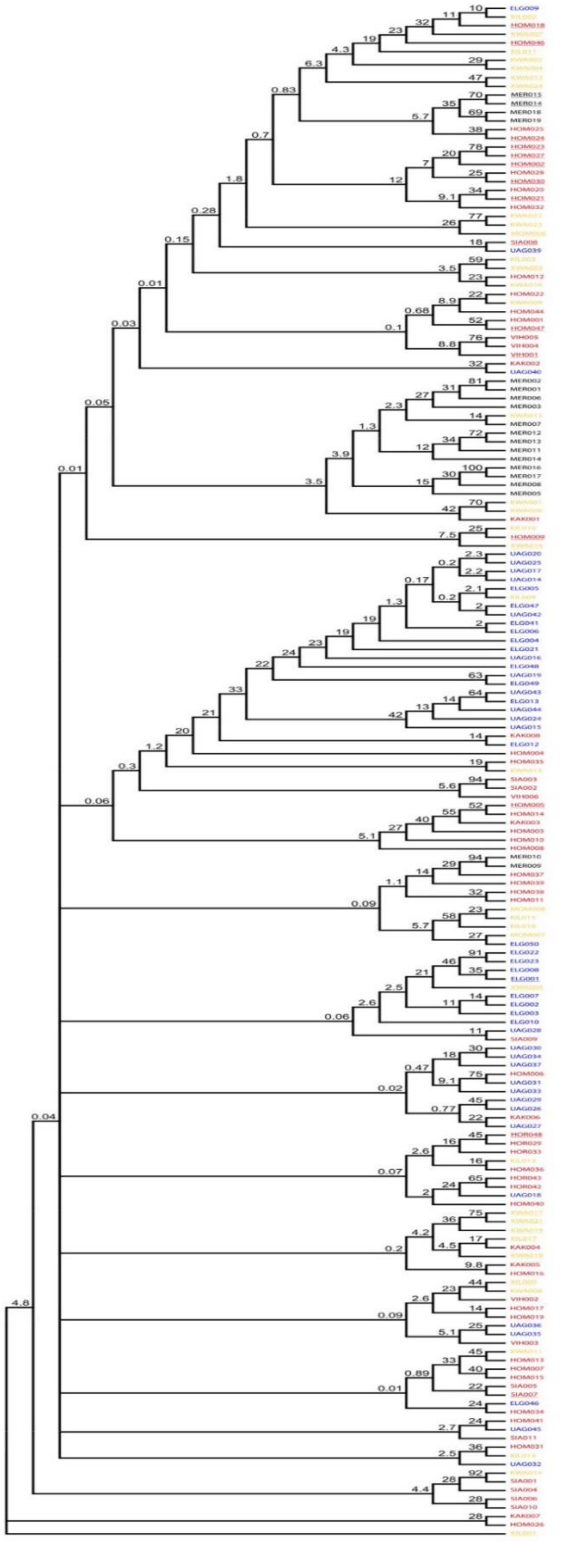

Figure S1. The neighbour-joining phylogenetic tree of 177 guava accessions collected from four regions of Kenya. The accession colour codes depict the region of collection (Red = Western, Blue = Rift Valley, Gold = Coast, Black = Eastern). The white-fleshed accessions are underlined. The bootstrap values are indicated at the tree nodes after 10,000 bootstrapping, Related to Figure 2.

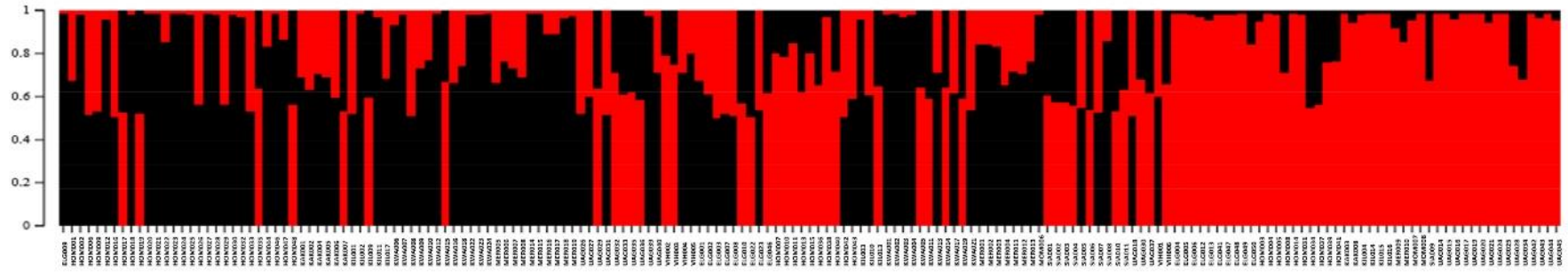

Figure S2. Bayesian analysis cluster plot of 177 guava accessions from four regions of Kenya. The most probable number of clusters (K = 2) is represented by colours, which however, depict the accessions as having admixed genotypes, Related to Figure 3.

Chapter three

3. Variation in fruit chemical and mineral composition of guava (*Psidium guajava* L.): Inferences from climatic conditions, soil nutrients, and fruit morphological traits

Abstract

There is limited knowledge about the impact of climatic conditions, soil nutrients, and fruit morphological traits on the nutritional composition of guava fruits. Fruits were gathered from 128 guava trees across four geographically diverse regions of Kenya and soils collected under 50 trees of the 128 trees. The fruits were morphologically characterized, and analysed for their chemical and mineral composition, and the soil nutrient content was also determined. The ascorbic acid content correlated positively only with total annual precipitation while the total soluble solids (TSS) correlated positively with mean annual temperature. TSS also correlated positively with soil nutrients (P, Mg, and Zn) but negatively with pulp weight, and was higher in white-fleshed fruits than in the red-fleshed types. The mineral content of the fruits mainly correlated negatively with most of the fruit weight- and size-based morphological traits and also with the total annual precipitation. This information could act as a guide in the selection of specific regions for upscaling guava production, the selection of accessions for improvement programmes, and the design of appropriate fertilizer regimes that enhance guava fruit nutritional composition.

3.1. Introduction

Tropical fruits are important to developing countries from both nutritional and economic perspective, with about 90% of these fruits being consumed in producing countries themselves, while 10 percent are traded internationally as fresh fruits and processed products (Available: http://www.fao.org/docrep/meeting/028/ma937e.pdf – Accessed 18.05.2018). Besides some efforts seen in the production of tropical fruits such as mangoes and avocados, the opportunities to grow, consume, and export more fruits from tropical regions remain under-exploited compared to those in temperate regions (Griesbach 2007). For instance, the supply of fruits and vegetables in lower income countries fall on average 58% short based on nutritional recommendations (Siegel et al. 2014). Consequently, low-quality monotonous diets are common in these regions, leading to high risks of nutrient deficiencies (Arimond et al. 2010). Research to improve fruit production, therefore, offers tremendous opportunities for raising the incomes of small-scale farming families in these regions, while also improving their nutritional status as observed by Keding et al. (2017).

Guava (*Psidium guajava* L.) is an important tropical fruit tree that is grown mainly for its edible fruits which are eaten raw or made into purée (pulp), jam, jelly, paste, juice, syrup, chutney, and so on (Leite et al. 2006). The guava tree is cultivated in orchards and in home gardens in many tropical countries (CABI 2013). In Kenya, for example, the guava tree exists in all regions of the country (HCD 2014) and mainly grows unattended. Despite the lack of attention devoted to guava tree husbandry, guava fruit production in Kenya has recently shown an increase (HCD 2014). However, most of these guava fruits are collected from the wild, and not much effort has been put to improve tree husbandry and the production potential (Mbuvi and Boon 2009).

Most recent studies have reported an appreciable amount of ascorbic acid and other antioxidants in guava fruit (Araújo et al. 2015; Flores et al. 2015; Gull et al. 2012), which

are essential dietary components (Flores et al. 2013). Guava fruit consumption has also been observed to reduce low-density lipoprotein (LDL) cholesterol and triglyceride levels (Rai et al. 2010; Setiawan et al. 2001). Besides, guava fruit has also been reported to contain appreciable amounts of minerals such as K, P, and Ca (Ogoloma et al. 2013; Natale et al. 2007), which could significantly contribute towards meeting a person's daily dietary requirements.

The nutritional composition of a fruit is largely a reflection of the geographic region where the fruits grows and the mineral composition of the soil there (Wall 2006; Forster et al. 2002). The traits also vary with climate (Rodriguez-Amaya et al. 2008), fruit maturity (Gull et al. 2012) and cultivar (Burlingame et al. 2009; Toledo and Burlingame 2006a). The soil quality determines the sustainability and productivity of any agro-ecosystem (Forster et al. 2002); hence, the growth and development of a plant is a function of soil–plant interaction and the prevalent weather conditions (Haque et al. 2009). The nutritional composition of fruits may therefore vary from one continent to another, from one country to another in the same continent and in the same country, as well as from region to region due to changes in climatic conditions (Haque et al. 2009) and soil quality parameters. However, there is limited data on the nutrient content of tropical fruits in relation to these variables (Natale et al. 2007).

The objectives of this study were: (1) to characterize and correlate the variation in the fruit chemical and mineral composition of guava with the climatic variables (temperature and precipitation), (2) to determine the extent of correlation between soil nutrient content and guava fruit chemical and mineral composition, and (3) to determine if the fruit morphological traits (flesh colour and size- and weight-based traits) influence the chemical and mineral composition of guava fruit and if they could be correlated. The assumption is that variations in fruit chemical and mineral composition are correlated to each of the climatic, soil nutrient content and fruit morphological traits which lead to their differences

in guava fruits. The information would help in establishing the species' actual and potential contributions to nutritional security, especially in relation to these factors.

3.2. Materials and methods

3.2.1. Sampling

The regions for sampling in Kenya were chosen based on their high guava fruit production trends (HCD 2014). Fruit sampling was carried out between September and November 2015. This coincided with the time when the fruits were available and ready for harvesting in the specific regions. With the help of key informants and field guides, the main guava-producing locations within the regions were identified. Households and institutions were randomly selected within these locations and trees with ripe fruits targeted for fruit collection. The geographical locations of the trees were recorded with a hand-held Global Positioning System (GPS) (Table S1). The latitudes and longitudes also enabled the retrieval of the mean annual temperature and annual precipitation data from WorldClim—Global Climate Data: http://www.worldclim.org/bioclim; (Fick and Hijmans 2017) for individual accessions (Table S1). The monthly meteorological data (temperature, relative humidity [RH], and precipitation) based on the nearest meteorological station within the regions is shown in Figure S1. Healthy and clean fruits and leaves from 128 trees were collected from the Coast (36 trees), Eastern (12 trees), Rift Valley (19 trees), and Western (61 trees) regions (Figure 1).

From the 128 trees selected for fruit nutrient and chemical characterization, 50 trees were randomly selected (Coast = 19, Eastern = 7, Rift Valley =13, and Western = 11) for soil sample collection under their crowns from two spots that were equidistant from the main trunk. These spots were also in opposite directions to each other. The top five cm-layer comprising the organic litter was first removed before collecting the soil. The soil was collected using a soil auger and by drilling vertically, first 0–15 cm and then 15–30 cm. The

two collected soil samples from top- and subsoil from each tree were then mixed thoroughly and air-dried before sieving through a two-mm-mesh.

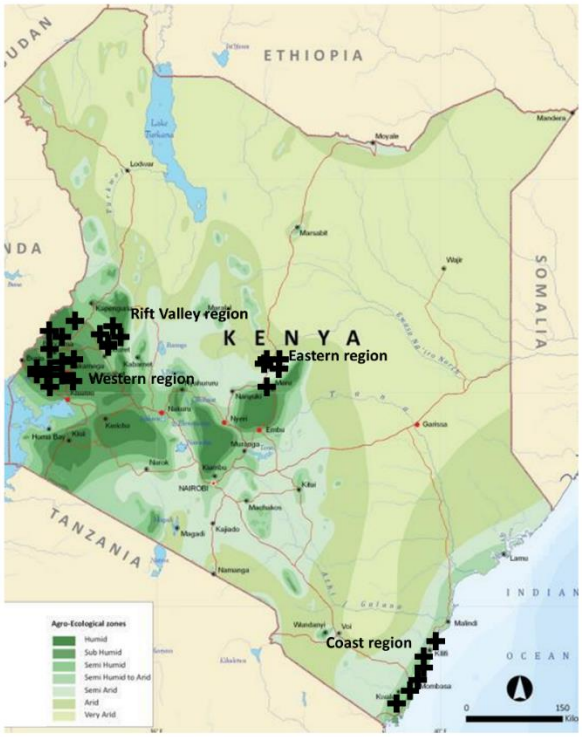

Figure 1. Sample collection locations for the guava accessions (crosses) from four regions of Kenya with Coast = 36, Eastern = 12, Rift Valley = 19, and Western = 61.

3.2.2. Fruit morphological characterization

A descriptor list for mango (IPGRI 2006) was modified (e.g. for tree shape, leaf size and seed traits) to accommodate the guava tree, leaf, and fruit traits for characterization; the modification also considered the results of other guava characterization studies (e.g. Singh et al. 2015; Mehmood et al. 2014; Nasution and Hadiati 2014; Sharma et al. 2010) and the authors' own observations. A total of 64 characteristics comprising 23 quantitative and 41 qualitative traits were evaluated (Table S2). Tree characteristics such as tree height, crown diameter, number of branches, and some leaf traits such as leaf colour were measured in the field, while 10 leaves and 20 fruits per tree were randomly collected for further measurements in the laboratory at the World Agroforestry Centre (ICRAF), Nairobi. During

morphological fruit characterization, fruits which were found to be infested by maggots and could only be discovered after longitudinal dissection were not characterized. This therefore reduced the number of accessions for morphological characterization. As the minimum number of fruits for size- and weight-based fruit morphological characterization was set to be at least 20, the characterization was eventually carried out for fruits from 105 trees (Coast = 23, Eastern = 12, Rift Valley = 17, and Western = 53), except for characterization of fruit flesh colour of which at least one fruit per tree was used. Therefore, all the 128 trees were used for determination of flesh colour. The fruit and the various fruit parts which were measured are depicted in Figure 2.

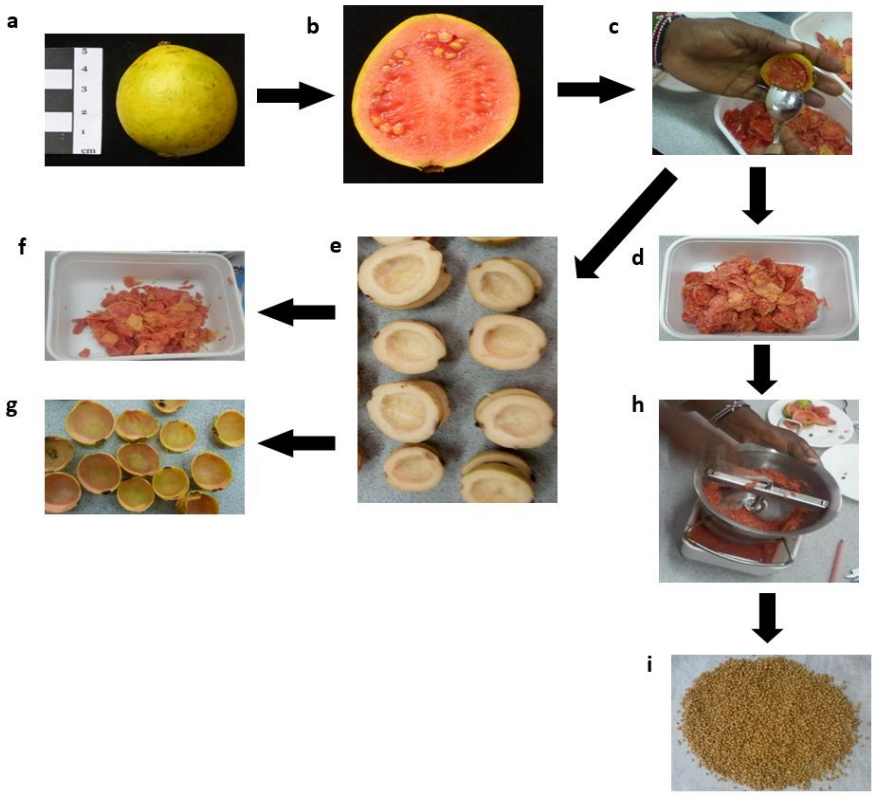

Figure 2. Guava fruit and fruit parts used in morphological characterization: **(a)** entire guava fruit, **(b)** fruit longitudinally cut into two parts, **(c)** pulp and seed removed with a spoon, **(e)** pericarp, hence pericarp thickness and weight measured, **(f)** mesocarp removed from pericarp with a spoon and weight measured, **(g)** fruit exocarp/skin after removing the mesocarp, thickness and weight measured, **(h)** seed and pulp separated by a fruit mill and pulp weight measured, **(i)** guava seeds washed and dried for weighing.

3.2.3. Determination of fruit chemical and mineral composition

Since chemical and mineral characterization of the fruits considered between five to 20 healthy and undamaged ripe fruits, fruits from all the 128 trees were characterized for their chemical and mineral content. The ripeness of the fruits was determined as the yellow colour of the skin based on the colour chart of The Royal Horticultural Society (RHS 2015) in addition to the softness of the fruits to touch (Araújo et al. 2015; Gull et al. 2012). The fruits were cleaned and separated into skin, pulp, and seeds, and the edible portion (pulp plus skin) was divided into two sub-samples. One fresh sample was used for the analysis of ascorbic acid content, TSS, and titratable acidity (TA) immediately after processing. The other sub-sample was weighed and then freeze-dried. The freeze-dried sample was weighed again to determine the water loss, which was expressed as %. The sample was later used to analyse the protein, sugar, total phenolic compounds, and mineral contents. All the results were expressed per fruit fresh weight (FW).

The ***ascorbic acid*** content was determined in fresh samples by reduction with 2,6-dichloroindophenol solution to a colourless dye using the titration method according to the procedure developed by Puwastien et al. (2011). To increase precision, the samples were measured titrimetrically.

The ***total phenolic compounds*** were extracted from 0.25 g of freeze-dried sample by adding 5 ml of 80% ethanol in a falcon tube. The tube was thoroughly vortexed and then centrifuged at 5,000 g for 10 minutes. The supernatant was transferred to a 10 ml flask. The extraction was repeated and the supernatants combined. The flask was then filled up to the 10 ml mark with 80% ethanol. Estimation of the total phenolic compounds was carried out in triplicate photometrically at 735.8 nm, immediately after extraction with the Folin–Ciocalteu reagent and expressed as mg per gallic acid equivalent (mg/GAE) according to the protocol developed by Singleton and Rossi (1965).

TSS was measured by placing a few drops of the squeezed guava juice from fresh fruits on a hand-held refractometer. The values were read directly as % brix.

TA was determined on extracted guava juice from fresh fruits by titrating to a pH of 8.1 by adding 0.1N NaOH according to the method by LMBG (1983). The result was expressed as mg of citric acid per 100 g of sample.

Sugars (glucose and fructose) were extracted from 200 mg of freeze-dried and milled guava fruit samples by adding 8 ml of pure water, vortexing, and then shaking the samples for one hour. Then 0.5 ml of 0.25 M Carrez I (containing potassium hexacyanoferrate (II) trihydrate, $K_4[Fe(CN)_6].3H_2O$) and 0.5 ml of 0.09 M Carrez II (containing zinc sulphate heptahydrate, $ZnSO_4.7H_2O$) were added to each sample and mixed by vortexing. The tubes were then centrifuged at 5,000 rpm for 20 minutes and the supernatant transferred into 25 ml volumetric flasks. The extraction was repeated by adding 7 ml of pure water alone. The volumetric flasks were then filled up to the 25 ml level and the extract filtered into scintillation vessels. Soluble carbohydrates were separated according to the procedure used by Keutgen and Pawelzik (2008), and the sugar content (glucose and fructose) detected by High-Performance Liquid Chromatography (HPLC) (Jasco, 26600 Mary's Court Easton, MD 21601).

Proteins were extracted using the phenol protocol established by Faurobert et al. (2007) on 200 mg of milled freeze-dried samples. The proteins were measured photometrically, each in three replications, according to Bradford (1976).

Fruit mineral contents—that is, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P), sulphur (S), iron (Fe), boron (B), zinc (Zn), and copper (Cu)—were extracted from 100 mg of each milled freeze-dried sample according to the procedure by Wheal et al. (2011) and determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Vista-RL ICP-OES, Varian Inc., USA).

3.2.4. Determination of soil pH and soil nutrients

The ***soil pH*** was determined according to the procedure by Jackson (1967). 25 ml of 0.01 M $CaCl_2$ solution was added to 10 g of air-dried soil. The mixture was stirred thoroughly using a glass rod and the pH was measured after 30 minutes using a pH metre.

Soil nutrients (P as P_2O_5, K as K_2O, and Mg) were extracted using calcium acetate lactate ($C_5H_8CaO_5$) (commonly known as the CAL method) according to the procedure by Schüller (1969). Na, Cu, Zn, B, Fe, and manganese (Mn) were extracted using calcium chloride dehydrate ($CaCl_2.2H_2O$) and diethylenetriaminepentaacetic acid ($C_{14}H_{23}N_3O_{10}$), which is commonly referred to as the CAT method (Schachtschabel 1954). The nutrient composition of the soil was determined using ICP-AES (Vista-RL ICP-OES, Varian Inc., USA). Owing to the high concentrations of Mn in the samples, Mn was determined separately by atomic absorption spectrophotometry. Soil Nitrogen (N) and Carbon (C) were determined in sample weights of 35–40 mg of soil using the N analyzer (Verardo et al. 1990) (Model NA 1500-R/AS 200, Thermo Quest, Fisons).

3.3. Data analysis

Analysis of variance, mean separation and correlation analyses for fruit traits and soil nutrients were conducted using SPSS version 20.0 (IBM 2011). A Shapiro–Wilk test ($p < 0.05$) and the resulting histograms, normal Q-Q plots, and box plots showed that the data for fruit chemical composition and soil properties was not normally distributed. The Welch test of homogeneity of variance also depicted variance inhomogeneity. Therefore, these data sets were analysed non-parametrically using the Kruskal–Wallis test for one-way analysis of variance, Mann-Whitney U-test for comparison of two sample means, and Spearman's rank for correlations. Only correlations with $r \geq 0.3$ at $p \leq 0.01$ were considered as significant, and hence presented in the results. The morphological data, however, was normally distributed and analysed by analysis of variance (ANOVA) followed by a post hoc Tukey

test, and independent sample t-test for mean separation. Regarding the fruit traits (both chemical and mineral, and morphological), analysis was first done per region to check if regional variations existed. Next, mean annual temperature and annual precipitation data obtained from WorldClim–Global Climate Data: http://www.worldclim.org/bioclim, (Fick and Hijmans 2017) for individual accessions was correlated to the fruit chemical and mineral composition data. Furthermore, the fruit chemical and mineral composition data was again tested for variation based on the colour of the fruits' pulp.

Due to the observed existence of variation in morphological characteristics within the regions and even within tree sampling locations, the morphological data was processed further to capture this variation more precisely. Field tree traits easily influenced by horticultural practices, such as number of branches, crown diameter, and tree height, were not included in the analysis. A combined cluster analysis of z-standardized qualitative and quantitative morphological variables using Ward's clustering method and Squared Euclidean Distances was performed. Discriminant analysis was then performed on the identified clusters to identify the most important variables responsible for the formation of the observed clusters. These variables had higher loadings based on the Standardized Canonical Discriminant Function coefficients. In addition, Principal Component Analysis (PCA) was conducted using Statistica software (Statsoft.com 2016), considering several clustering possibilities and to identify the variables responsible for their formation. Eventually, the two methods—Discriminant analysis and PCA—depicted two clusters that could be inferred by some variables which grouped consistently in the two methods. ANOVA was then performed on these variables to check whether a significant difference existed between the two clusters. Finally, a cluster analysis was repeated based only on the identified key descriptors to check if sample distribution in the final clusters was similar to the one when all the quantitative and qualitative descriptors were used. Lastly, ANOVA

followed by independent sample t-test was performed to test the existence of significant differences between the two clusters based only on the key selected descriptors. Fruit chemical and mineral data was therefore analysed again for significant differences based on the two identified clusters and correlations performed with the key morphological descriptors. A PCA scatter plot considering temperature, precipitation, soil, and fruit traits was finally performed using Statistica software to check the correlations of all the variables together.

3.4. Results

3.4.1. Fruit chemical and mineral composition based on region and climate

The chemical and mineral composition of guava fruits from the four regions of Kenya are provided in Table 1. The edible fruit portion (pulp and skin) of the 128 sampled trees had a mean ascorbic acid content of 83.8 mg 100 g^{-1} FW, although with an extremely high variability ranging from 9.8 to 377.1 mg 100 g^{-1} FW. The highest mean value was recorded in fruits from the Eastern region (147.4 mg 100 g^{-1} FW), followed by the Western region (91.0 mg 100 g^{-1} FW). The samples from Coast and Rift Valley regions recorded the lowest values (66.8 mg 100 g^{-1} and 52.9 mg 100 g^{-1} FW, respectively).

The contents of total phenolic compounds were statistically similar across the regions, with a total mean value of 150.9 mg 100 g^{-1} FW. However, there were wide variations of these compounds within the regions with the total mean minimum and maximum values ranging between 108.6 mg 100 g^{-1} FW and 285.8 mg 100 g^{-1} FW.

Lower values of TSS (% brix; 9.13%) were registered in samples from the Rift Valley region, especially in comparison to those from the Coast (13.2%) region, which recorded higher values. The brix value ranged from 5.9% to 20% with an overall mean of 11.3%. Consequently, higher fructose and glucose values were also recorded in samples from the Coast region as 3.76 g 100 g^{-1} FW and 1.55 g 100 g^{-1} FW, respectively. On the other hand,

TA was highest in samples from the Eastern region (1.22 mg 100 g^{-1} FW) and lowest in the Rift Valley region (0.78 mg 100 g^{-1} FW). TA ranged from 0.59 100 g^{-1} FW to 2.73 100 g^{-1} FW with an overall mean of 0.96 g 100 g^{-1} FW. The protein content was highest in fruits from the Coast region (0.69 mg 100 g^{-1} FW) and lowest in the Eastern region (0.38 mg 100 g^{-1} FW).

The lowest Ca levels were found in fruits from the Eastern region (9.77 mg 100 g^{-1} FW), and the highest values were recorded in the Coast, Rift Valley and Western regions (16.0 mg 100 g^{-1} FW, 15.3 mg 100 g^{-1} FW and 13.4 mg 100 g^{-1} FW, respectively) with a total mean of 14.1 mg 100 g^{-1} FW for all the regions together. Fruits from the Coast region had the highest K, Mg, and S contents (406 mg 100 g^{-1} FW, 13.1 mg 100 g^{-1} FW, and 17.1 mg 100 g^{-1} FW, respectively); the other regions recorded lower values that were similar to each other. The fruit Na content was significantly higher in fruits from the Coast (5.63 mg 100 g^{-1} FW) and the Rift Valley (3.63 mg 100 g^{-1} FW) than those from the Western and Eastern regions (1.49 mg 100 g^{-1} FW and 0.69 mg 100 g^{-1} FW, respectively). A similar trend was observed with respect to fruit P content. The Fe content was significantly higher in fruits from the Rift Valley (0.47 mg 100 g^{-1} FW) and Coast (0.38 mg 100 g^{-1} FW), compared to those from the Western region (0.27 mg 100 g^{-1} FW). B was highest in samples from the Coast region (0.27 mg 100 g^{-1} FW) and lowest in those from the Rift Valley region (0.15 mg 100 g^{-1} FW). Fruits collected in the Eastern region had highest Zn content (0.13 mg 100 g^{-1} FW) while those from the Western region recorded the lowest Zn values (0.04 mg 100 g^{-1} FW). However, fruits from the Eastern region recorded significantly lower Cu values (0.07 mg 100 g^{-1} FW), as compared to those from the Rift Valley region. Fruits sampled in the Eastern, Western and Rift Valley regions had significantly higher water content than those from the Coast region (Table 1).

Table 1. Fruit chemical and mineral composition of 128 guava accessions sampled from four regions of Kenya.

Fruit chemical and mineral composition	Region of collection				Mean (n= 128)	*p*-value
	Rift Valley (n= 19)	Western (n= 61)	Coast (n= 36)	Eastern (n= 12)		
Ascorbic acid (mg 100 g^{-1} FW)	52.9 ^{b} (11.1-04.5)	91.0 ^{a} (11.5-206.5)	66.8 ^{b} (9.77-279.1)	147.4 ^{a} (58.6-377.1)	83.8 (9.77-377.1)	0.000
Phenolics (mg 100 g^{-1} FW)	141.8 ^{a} (110.6-167.5)	151.8 ^{a} (108.6-285.8)	151.3 ^{a} (113.7-248.7)	159.5 ^{a} (121.0-201.2)	150.9 (108.6-285.8)	0.283
TSS (% brix)	9.13 ^{c} (5.90-11.2)	11.1 ^{b} (6.9-14.9)	13.2 ^{a} (8.1-20.0)	9.53 ^{bc} (7.7-10.7)	11.3 (5.90-20.0)	0.000
TA (mg 100 g^{-1} FW)	0.78 ^{b} (0.62-0.98)	0.95 ^{a} (0.61-2.25)	1.00 ^{a} (0.60-1.58)	1.22 ^{a} (0.59-2.73)	0.96 (0.59-2.73)	0.000
Fructose (g 100 g^{-1} FW)	2.51 ^{b} (1.28-3.78)	2.77 ^{b} (0.80-6.05)	3.76 ^{a} (1.55-5.96)	2.30 ^{b} (0.88-2.95)	2.97 (0.80-6.05)	0.000
Glucose (g 100 g^{-1} FW)	1.09 ^{ab} (0.52-1.94)	1.08 ^{b} (0.10-2.80)	1.55 ^{a} (0.14-2.51)	0.88 ^{b} (0.36-1.24)	1.19 (0.10-2.80)	0.002
Protein (mg 100 g^{-1} FW)	0.43 ^{b} (0.26-0.58)	0.49 ^{b} (0.24-0.91)	0.69 ^{a} (0.38-1.16)	0.38 ^{b} (0.31-0.57)	0.53 (0.24-1.16)	0.000
Ca (mg 100 g^{-1} FW)	15.3 ^{a} (7.64-27.7)	13.4 ^{ab} (5.84-28.4)	16.0 ^{a} (7.09-36.5)	9.77 ^{c} (4.98-14.1)	14.1 (4.98-36.5)	0.002
K (mg 100 g^{-1} FW)	270.8 ^{b} (106.3-391.5)	287.6 ^{b} (126.2-680.5)	406.0 ^{a} (208.3-704.8)	243.9 ^{b} (187.7-376.4)	314.3 (106.3-704.8)	0.000
Mg (mg 100 g^{-1} FW)	8.15 ^{b} (3.43-16.9)	9.59 ^{b} (4.96-18.8)	13.1 ^{a} (7.91-19.6)	8.57 ^{b} (6.05-12.9)	10.3 (3.43-19.6)	0.000
Na (mg 100 g^{-1} FW)	3.63 ^{a} (0.61-6.02)	1.49 ^{b} (0.23-7.47)	5.63 ^{a} (2.54-16.0)	0.69 ^{b} (0.32-1.54)	2.90 (0.23-16.0)	0.000
P (mg 100 g^{-1} FW)	18.4 ^{a} (11.4-32.0)	12.3 ^{b} (4.18-32.4)	18.8 ^{a} (10.0-33.5)	11.1 ^{b} (6.94-16.1)	14.9 (4.18-33.5)	0.000
S (mg 100 g^{-1} FW)	10.7 ^{b} (3.98-15.9)	10.8 ^{b} (3.21-24.7)	17.1 ^{a} (7.95-28.4)	10.1 ^{b} (7.75-15.5)	12.5 (3.21-28.4)	0.000
Fe (mg 100 g^{-1} FW)	0.47 ^{a} (0.18-1.17)	0.27 ^{b} (0.10-0.67)	0.38 ^{a} (0.18-0.94)	0.37 ^{ab} (0.16-0.82)	0.34 (0.10-1.17)	0.000
B (mg 100 g^{-1} FW)	0.15 ^{c} (0.08-0.21)	0.21 ^{b} (0.08-0.42)	0.27 ^{a} (0.11-0.43)	0.18 ^{bc} (0.12-0.25)	0.22 (0.08-0.43)	0.000
Zn (mg 100 g^{-1} FW)	0.09 ^{ab} (0.00-0.19)	0.04 ^{b} (0.00-0.40)	0.11 ^{ab} (0.00-0.84)	0.13 ^{a} (0.00-0.28)	0.08 (0.00-0.84)	0.001
Cu (mg 100 g^{-1} FW)	0.13 ^{a} (0.06-0.52)	0.11 ^{ab} (0.02-0.46)	0.10 ^{ab} (0.05-0.17)	0.07 ^{b} (0.05-0.13)	0.11 (0.02-0.52)	0.017
Water content (%)	86.3 ^{a} (82.1-91.6)	85.2 ^{a} (75.1-92.6)	78.5 ^{b} (65.6-88.2)	88.4 ^{a} (83.7-90.6)	83.8 (65.6-92.6)	0.000

Values within the same row followed by the same letter are not significantly different at $p < 0.05$ according to Kruskall-Wallis test followed by pairwise comparisons. Values within parenthesis depict the range.

A correlation between fruit chemical and mineral composition of individual accessions and mean annual temperature and annual precipitation at their growth location is shown in Table 2. The ascorbic acid content correlated positively with annual precipitation but not with the mean annual temperature. Similarly, the fruit water content correlated positively with annual precipitation, but negatively with the mean annual temperature. However, TSS, protein, and most of the fruit minerals (e.g. K, Mg, Na, P, and S) correlated positively with the mean annual temperature and negatively with annual precipitation.

Table 2. Spearman correlation coefficients between fruit chemical and mineral composition traits with annual mean temperature and annual precipitation based on the individual accession climatic data (Table S1) of 128 guava accessions

Fruit chemical and mineral composition	**Climatic data**	
	Mean annual	Annual
Ascorbic acid	ns	0.37***
Phenolics	ns	ns
TSS	0.37***	-0.31***
TA	ns	ns
Fructose	ns	-0.33***
Glucose	ns	ns
Protein	0.43***	-0.45***
Ca	ns	ns
K	0.41***	-0.46***
Mg	0.34***	-0.42***
Na	0.49***	-0.66***
P	0.33***	-0.54***
S	0.38***	-0.50***
Fe	ns	-0.41***
B	0.32***	ns
Zn	ns	ns
Cu	ns	ns
Water content	-0.45***	0.49***

***Correlation is significant at $p \leq 0.001$ level. ns = correlation was not significant at $p \leq 0.01$ level. Only $r \geq 0.3$ at $p \leq 0.01$ values were considered as significant.

3.4.2. Correlation of fruit chemical and mineral composition with soil nutrients

In general, the regional variation in the fruit chemical and mineral traits of the 50 trees was fairly similar to those of the 128 trees analysed (Table S3), thus enabling a correlation analysis with soil nutrients that could be generalized for the entire fruit sample.

The soil pH and nutrients in the four regions were fairly similar, with the soil textures being sand, sandy loam, loamy sand, and loam. The results of the soil analyses are presented in Table S4. Mean soil pH was similar under trees in all the regions with an overall mean value of 5.97, and ranged between 4.33 and 7.98. The highest soil N was recorded under trees from the Rift Valley region (0.23%), and this was statistically similar to those of the Eastern (0.20%) and the Western (0.15%) regions. The soil under the trees from the Coast region had the least N content (0.07%), and the lowest levels of C, while C was highest in the Rift Valley region. The C/N ratio was similar in samples from the Rift Valley (13.0) and the Coast (12.4) regions, while statistically similar values were also recorded for Coast (12.4), Western (10.6) and Eastern (10.5) regions. The P_2O_5 content was highest in soil samples from the Coast (101.1 mg kg^{-1}) but least in those from the Western region (19.9 mg kg^{-1}), and K_2O content was also highest in soil samples from the Coast region (656.8 mg kg^{-1}), but least in samples from the Rift Valley region (138.3 mg kg^{-1}). Soil Mg content was also highest in samples from the Coast and lowest in those from the Rift Valley region (325.9 mg kg^{-1} versus 134.2 mg kg^{-1}, respectively). With regard to micronutrients, samples from the Coast region were highest in B content (4.06 mg kg^{-1}) that was least in those from the Rift Valley region (1.59 mg kg^{-1}). The other soil micronutrients (Cu, Mn, Fe, Zn, and Na) did not vary among the studied regions.

Table 3 presents the results of the correlation between the soil nutrients and fruit chemical and mineral composition. Soil P_2O_5 was observed to correlate positively with TSS.

A positive correlation between soil Mg with fructose and glucose contents was also observed. Soil Zn positively correlated with TSS and fructose.

Table 3. Spearman correlation coefficients between fruit chemical and mineral composition and soil nutrients based on 50 fruit and soil samples for all the regions

	Fruit chemical and mineral composition			
Soil nutrients	TSS	Fructose	Glucose	Cu
P_2O_5	0.39^{**}	ns	ns	ns
Mg	ns	0.37^{**}	0.42^{**}	ns
Zn	0.58^{**}	0.56^{**}	ns	ns
Cu	ns	ns	ns	0.40^{**}

***Correlation is significant at $p \leq 0.001$ level. **Correlation is significant at $p \leq 0.01$ level. ns = correlation was not significant at $p \leq 0.01$ level. Only $r \geq 0.3$ at $p \leq 0.01$ values were considered as significant.

3.4.3. Fruit chemical and mineral composition based on pulp colour

The chemical and mineral composition of the fruits based on fruit pulp colour is depicted in Table 4. There was no variation in the ascorbic acid content of the fruits. However, the total phenolic compound content of the red-fleshed fruits (154.0 mg 100 g^{-1} FW) was significantly higher than that of the white-fleshed fruits (138.9 mg 100 g^{-1} FW).

The TA did not vary with the fruit pulp colour. However, the TSS of the rather few fruits with white pulp was significantly higher than that of fruits from the red-fleshed group (12.8% versus 10.9%). Similar to TSS, the fructose content was also higher in the white-fleshed fruits than in the red-fleshed fruits. There was no variation in the glucose content with regard to fruit pulp colour.

There was no variation in the fruit mineral contents of P, Ca, Fe, Zn, and Cu with respect to the fruit flesh colour. However, interestingly, the white-fleshed fruits were superior to the red-fleshed group with regard to the protein content and the content of minerals K, Mg, Na, S, and B. In contrast, the water content was higher in the red-fleshed group (84.4%) as opposed to the white-fleshed group (81.0%).

Table 4. Chemical and mineral composition of fruits from 128 guava accessions based on the fruit pulp colour irrespective of region of collection.

Fruit chemical and mineral composition	Fruit pulp colour		Mean (n = 128)	*p*-value
	White (n = 26)	Red (n = 102)		
Ascorbic acid (mg 100 g^{-1}	83.4 [a] (11.9-235.4)	84.0 [a] (9.77-377.1)	83.8 (9.77-377.1)	0.852
Phenolics (mg 100 g^{-1} FW)	138.9 [b] (113.7-190.5)	154.0 [a] (108.6-285.8)	150.9 (108.6-285.8)	0.011
TSS (% brix)	12.8 [a] (9.15-20.0)	10.9 [b] (5.90-15.9)	11.3 (5.90-20.0)	0.000
TA (mg 100 g^{-1} FW)	0.94 [a] (0.60-1.54)	0.97 [a] (0.59-2.73)	0.96 (0.59-2.73)	0.861
Fructose (g 100 g^{-1} FW)	3.46 [a] (1.05-5.96)	2.85 [b] (0.80-6.05)	2.97 (0.80-6.05)	0.019
Glucose (g 100 g^{-1} FW)	1.38 [a] (0.29-2.50)	1.15 [a] (0.10-2.80)	1.19 (0.10-2.80)	0.183
Protein (g 100 g^{-1} FW)	0.61[a] (0.26-1.16)	0.51 [b] (0.24-1.03)	0.53 (0.24-1.16)	0.009
Ca (mg 100 g^{-1} FW)	14.2 [a] (5.84-28.0)	14.1 [a] (4.98-36.6)	14.1 (4.98-36.6)	0.972
K (mg 100 g^{-1} FW)	370.9 [a] (162.0-704.8)	302.4 [b] (106.2-680.5)	314.3 (106.3-704.8)	0.014
Mg (mg 100 g^{-1} FW)	12.4 [a] (6.22-19.6)	9.79 [b] (3.43-18.8)	10.3 (3.43-19.6)	0.003
Na (mg 100 g^{-1} FW)	4.44 [a] (0.23-16.0)	2.55 [b] (0.32-12.9)	2.90 (0.23-16.0)	0.030
P (mg 100 g^{-1} FW)	17.6 [a] (7.27-33.5)	14.4 [a] (4.18-32.4)	14.9 (4.18-33.5)	0.050
S (mg 100 g^{-1} FW)	15.2 [a] (6.34-27.0)	11.9 [b] (3.21-28.4)	12.5 (3.21-28.4)	0.006
Fe (mg 100 g^{-1} FW)	0.31 [a] (0.10-0.59)	0.35 [a] (0.10-1.17)	0.34 (0.10-1.17)	0.738
B (mg 100 g^{-1} FW)	0.25 [a] (0.13-0.43)	0.21 [b] (0.08-0.42)	0.22 (0.08-0.43)	0.027
Zn (mg 100 g^{-1} FW)	0.07 [a] (0.00-0.27)	0.08 [a] (0.00-0.84)	0.08 (0.00-0.84)	0.863
Cu (mg 100 g^{-1} FW)	0.09 [a] (0.04-0.17)	0.11 [a] (0.02-0.52)	0.11(0.02-0.52)	0.503
Water content (%)	81.0 [b] (65.6-91.5)	84.4 [a] (68.1-92.6)	83.8 (65.6-92.6)	0.005

Values within the same row followed by the same letter are not significantly different at $p < 0.05$ according to the Mann-Whitney U-test. Values within parenthesis depict the range.

3.4.4. Guava fruit morphological traits and effect on chemical and mineral composition

The analysis of the morphological traits identified two clusters (Figure S2). The clusters were distinct from each other based on seven key descriptors that were only fruit-based. The tree, leaf, and seed traits were not found to be important in discriminating among the guava samples. Table S5 shows variations of the key descriptors between the two cluster groups of our samples. Cluster 2, which consisted of only 15 samples, had the highest of both fruit size- and weight-based characteristics; meanwhile, Cluster 1, comprising 90 samples, had the least of these traits. However, a comparison of the fruit nutritional and chemical composition traits between these two clusters was found to be insignificant (Table S6).

Results of a correlation analysis between fruit chemical and mineral composition traits versus fruit morphological traits are presented in Table 5. Apart from the key fruit traits responsible for the cluster formation, seed and skin proportions and pericarp weight were also included in the correlation analysis as they are of interest to consumers. The ascorbic acid, TA, phenolic compounds, fructose, and glucose contents, as well as the mineral concentrations of P, Zn, and Cu, showed no correlation with the fruit morphological characteristics; hence, they are not depicted in Table 5.

Size- and weight-based fruit morphological traits generally correlated negatively with most fruit minerals. For instance, pulp weight negatively correlated with the TSS, protein, and all the mineral contents of the fruit. In contrast, pulp weight correlated positively with the fruit water content. Fruit seediness positively correlated with the TSS, protein, Ca, K, Mg, S, and B contents, but negatively with water content. The TSS content positively correlated with the skin proportion of the fruits.

Table 5. Spearman correlation coefficients between fruit morphological characteristics and fruit chemical and mineral composition from 105 guava trees summarized for all the regions.

Fruit morphological characteristics	**Fruit chemical and mineral composition**									
	TSS	protein	K	Ca	Mg	Na	S	B	Fe	Water
Fruit length	ns	ns	ns	-0.35***	ns	ns	ns	ns	ns	ns
Fruit width	ns	ns	-0.30**	-0.48***	ns	ns	-0.31**	ns	-0.37***	ns
Fruit weight	ns	ns	-0.34***	-0.52***	ns	ns	-0.31**	ns	-0.38***	ns
Pericarp	ns	ns	-0.37***	-0.48***	ns	ns	-0.32**	ns	-0.35***	ns
Exocarp	ns	ns	-0.33**	-0.44***	ns	ns	ns	ns	-0.44***	ns
Mesocarp	ns	ns	-0.33**	-0.44***	ns	ns	ns	ns	ns	ns
Pulp weight	-0.39***	-0.39***	-0.32**	-0.50***	-0.40***	-0.30**	-0.33**	-0.36***	-0.37***	0.36***
Pericarp (%)	0.32**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Skin (%)	0.33**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Seed (%)	0.41***	0.44***	0.56***	0.35***	0.46***	ns	0.40***	0.59***	ns	-0.43***

***Correlation is significant at $p \leq 0.001$ level. **Correlation is significant at $p \leq 0.01$ level. ns = correlation was not significant at $p \leq 0.01$ level. Only $r \geq 0.3$ at $p \leq 0.01$ values were considered as significant.

The PCA loading scatter plot was able to explain 46.5% of the total variation (Figure 3, Table S7); individual loading on the resultant three components are depicted in Table S7.

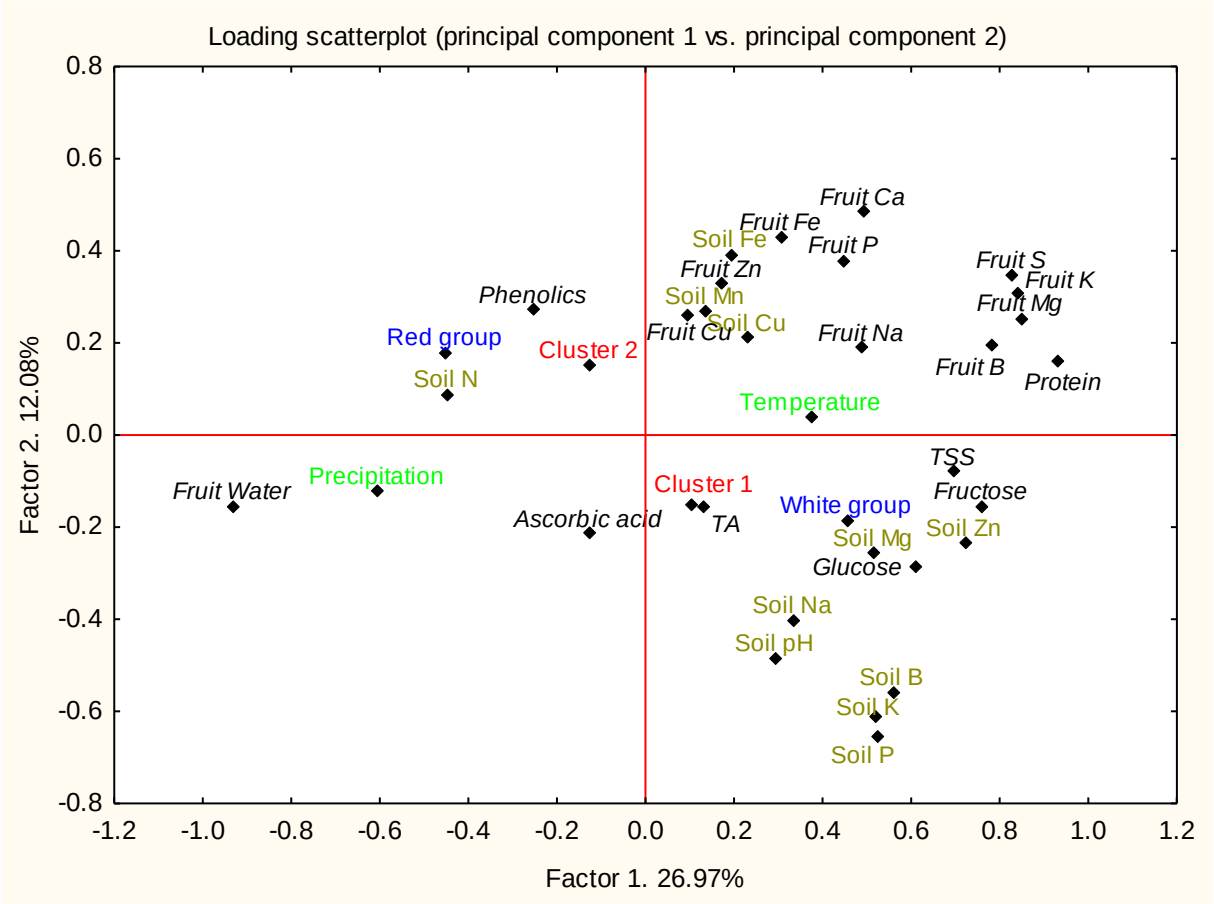

Figure 3. PCA performed on 50 samples for guava fruit chemical and mineral composition, soil nutrients, temperature, precipitation, and fruit morphological traits (see table 4 for cluster 1 and cluster 2 fruit traits). Variables placed close to each other influence the PCA model in similar ways.

Principal Component 1 had higher positive loadings of soil nutrients such as Zn, P, K, Mg, and B, together with fruit components such as TSS, fructose, protein, K, Mg, B and S. Also fruit water content and the environmental factor precipitation had higher but negative value loadings in this component. Principal Component 2 consisted of higher negative loadings of some soil nutrients—namely P, K, and B—but positive loadings of fruit minerals such as Ca and Fe. Principal Component 3 was positively loaded with soil trait N and fruit component TA, P, and Fe, but negatively with TSS. Principal Component 3 was also loaded negatively with environmental factor temperature and morphological traits of smaller fruits

(Cluster 1; see table S5), but positively with morphological traits of larger fruits (Cluster 2; see table S5). In addition, this component was loaded negatively with the white-fleshed fruits trait but positively with the red-fleshed fruits trait.

3.5. Discussion

3.5.1. The effect of temperature and precipitation on fruit chemical and mineral composition

The mean ascorbic acid content of fruits from the 128 guava trees from four regions of Kenya was 83.8 mg 100 g^{-1} FW. According to food composition tables, the ascorbic acid content of guava is estimated to be 228.3 mg 100 g^{-1} edible portion (Lukmanji et al. 2008), which is higher than the observed value in our sample. The observed differences could be due to variation in determination methods and state of the sample at the time of analysis. The samples in this study were partly transported over long distances to the laboratory and could only be analysed for ascorbic acid content the following day. The highest recorded mean value for ascorbic acid content was from the Eastern region (147.4 mg 100 g^{-1} FW), while the least was from the Rift Valley region (52.9 mg 100 g^{-1} FW); however, wide ranges were observed both within and among the regions. Based on mean annual temperature and total annual precipitation data from WorldClim—Global Climate Data: http://www.worldclim.org/bioclim; (Fick and Hijmans 2017) for individual accessions, annual precipitation correlated positively with the ascorbic acid content of the fruits in this study. However, the mean annual temperature correlated negatively but also insignificantly ($r < 0.3$, $P \leq 0.01$) with the ascorbic acid content. The effect of precipitation on ascorbic acid content of guava has so far not been reported. However, Gull et al. (2012) determined the ascorbic acid content in the pulp and peel of fully ripe guava fruits from three diverse regions of Pakistan as ranging from 129.5 mg 100 g^{-1} to 247.9 mg 100 g^{-1}. The ascorbic acid composition of the fully ripe fruits was found to vary with the regions, with higher values

recorded in the higher-temperature regions (mean max./min.) air temperature: 35/24°C) than in moderate and colder areas (mean max./min. air temperature: 33/21°C and 33/18°C, respectively). The variation was attributed mainly to climatic and soil factors. Contrarily, Thaipong and Boonprakob (2005) found higher contents of ascorbic acid in the slightly colder winter season (mean max./min. air temperature: 31.8/20.8°C) rather than in the hot summer season (mean max./min. air temperature: 33.6/24.5°C) in guava fruits grown in Thailand. The authors concluded that the effect of lower temperatures experienced in winter during fruit development could not only retard the excessive loss of respiratory substrates but also increase the translocation of photosynthates to other parts of the plant, including the fruits. It should be noted that the mean annual temperature for individual accessions in the present study (21.2°C), the mean minimum (16°C) and the mean maximum (23.7°C) are far below that reported by Gull et al. (2012) and Thaipong and Boonprakob (2005), which makes comparison of the results difficult. However, one may speculate that the cooling effect and slightly lower temperatures associated with precipitation are a possible reason for the positive correlation between ascorbic acid content and precipitation.

The observed TSS value in the present study (mean = 11.3% brix) is similar to that reported by El-Sisy (2013) in eight-year-old guava genotypes growing under uniform conditions for two seasons (ranging from 9.4% to 14.07%). El-Sisy (2013) recorded higher values in the first season as opposed to the second season, although both were under a uniform irrigation scheme, which points at the variation in temperature as among factors influencing TSS. The TSS in the present study positively correlated with mean annual temperature but negatively with annual precipitation. These results partly agree with those of Thaipong and Boonprakob (2005), in which lower TSS values during the summer season were attributed to the higher moisture content. However, the findings of Thaipong and Boonprakob (2005) contrast with the positive correlation observed between temperature and

TSS in the present study. A possible explanation for the negative correlation of TSS with precipitation could be the dilution effect, which is a result of higher soil moisture content leading to more water uptake and, hence, to water accumulation in the fruits as also shown by the positive correlation of fruit water content and precipitation (Table 2). In agreement with the observations of the present study, and using a ^{14}C tracer to compare the effects of elevated temperature on sugar and acid accumulation in mandarin fruit grown under tunnel house experiments, Marsh et al. (1999) reported a positive correlation between temperature and TSS. Fruit labelling with ^{14}C showed that rising canopy temperatures reduced the amount of incoming photosynthates partitioned to citrate and increased that allocated to sugars. A likely scenario could have also occurred in our study with guava.

The protein content of the guava fruit samples of the present study ranged from 0.24 g 100 g^{-1} to 1.16 g 100 g^{-1} FW with a mean of 0.53 g 100 g^{-1} FW, which is markedly lower than that reported in the food composition tables (2.6 g 100 g-1 edible portion; Lukmanji et al. 2008), possibly because freeze-dried samples were used in this study. Similar to TSS, the protein content also positively correlated with the mean annual temperature but negatively with the total annual precipitation. This trend was also observed with regard to most of the fruit minerals which were also lower (possibly due to use of freeze-dried samples) than that given in the food composition tables (except for Na and Fe) for Ca (18 mg 100 g^{-1}), K (417 mg 100 g^{-1}), Mg (22 mg 100 g^{-1}), Na (2.0 mg100 g^{-1}), P (40 mg 100 g^{-1}), Fe (0.3 mg 100 g^{-1}), Zn (0.2 mg 100 g^{-1}), and Cu (0.2 mg 100 g^{-1}) (Lukmanji et al. 2008). There currently appears to be no report on the relationship between climatic conditions and guava fruit protein and mineral composition for comparison; however, the dilution effect as a result of higher moisture content is also the likely reason for the negative correlation between precipitation and these fruit components. This can also be confirmed by the positive

correlation between the fruit water content and annual precipitation. The observed positive correlation with temperature requires further investigation.

3.5.2. Effect of soil nutrients on fruit chemical and mineral composition

Soil P positively and significantly correlated with the TSS contents of the fruits, as well as positively though insignificantly with fructose and glucose contents. Although there are no reports on the effect of P on guava sugar content, but higher sugar contents were reported in tomato with both higher and lower concentrations of P (Fandi et al. 2010). P is a key component in ATP synthesis. ATP is the principal energy-rich pyrophosphate required for starch synthesis. This energy can also be transferred to other co-enzymes such as uridine triphosphate and guanosine triphosphate which are required for sucrose and cellulose synthesis (Marschner 2012). The role this plays in glucose synthesis could contribute to the positive relationship between the P and TSS content of fruits such as guava. Consequently, Mg and its interaction with P have been observed to affect the activity of ATPases, mediating hydrolysis, and thus energy transfer (Marschner 2012). The positive correlation between soil Mg with fruit fructose and glucose contents could be a result of such interactions; however, more detailed studies are needed to confirm this.

Soil Zn content was also observed to increase the TSS and fructose contents of the fruits in this study. Zn plays various physiological functions and acts as a co-factor in many enzymatic reactions. It also has effects on photosynthesis, nucleic acid metabolism, and protein biosynthesis (Alloway 2004); hence, it could have contributed to the enhancement of the TSS and fructose levels in the present study.

3.5.3. Pulp colour influences chemical and mineral composition of guava fruits

The findings related to the fruit flesh colour depicted red-fleshed fruits as having higher phenolic content than the white-fleshed types. The results of this study were in agreement with those of Santos and Corrêa (2012), in which the pink- and red-fleshed guava

accessions recorded greater values for phenolic compound concentrations. In contrast, Hassimotto et al. (2005) found higher phenolic content in white guava pulp than in red guava pulp (160 vs. 124 mg 100 g^{-1} FW, respectively). Phenolic compounds are classified as phenolic acids, stilbenes, flavonoids, lignans, and tannins (Naczk and Shahidi 2004). Flavonoids account for the majority of the dietary phenols (Robbins 2003) and constitute most of the yellow, red, and blue colours in fruits (Lampila et al. 2009). A particular colour of guava flesh is therefore likely to reflect the accumulation of the respective flavonoid in the fruit and, hence, the phenolic content. Phenolic compounds have been reported to be affected by many other factors, among them variety, cultivation, species, area, and climatic conditions (Iqbal and Bhanger 2006; Wang and Lin 2000). These factors act in an interactive way; therefore, more focused studies on determining the contribution of flesh colour to the phenolic content of guava may be required.

The occurrence of flavonoids have been reported to also vary with the cultivars (Flores et al. 2015; Wang and Lin 2000) – thus could indicate genotypic variation among accessions of guava. Flavonoids have also been connected with various plant functions, including photosynthesis (Ampomah-Dwamena et al. 2015) and nutrient uptake. Accordingly, we found that the white-fleshed guavas accumulated more TSS and fructose than the red-fleshed types, which is in agreement with Choudhary et al. (2012) who studied the chemical composition of four guava cultivars under similar cultural conditions and found variations in TSS and non-reducing sugars, that were attributed partly to the variety of the fruit. Moreover, we also observed that the white-fleshed guava fruits accumulated more protein and some minerals (K, Mg, Na, S, and B), than the red-fleshed fruits. The variation in the accumulation of minerals in the fruit, such as K, Mg, S, and B, support the observation by Natale et al. (2002, 2007) that different guava cultivars vary in their nutrient uptake.

3.5.4. Larger and heavier fruits negatively correlate with their chemical and mineral composition

The size- and weight-based fruit traits correlated negatively with TSS, protein, and most fruit mineral contents. Fruits with higher pulp weight negatively correlated with TSS. In this regard, the results of the present study were similar to those of Thaipong and Boonprakob (2005)—larger fruits resulted in lower TSS and total sugar content in guava. Mehmood et al. (2014) and Singh et al. (2015) also reported poor accumulation of chemical compounds in large guava fruits, including TSS. Similarly, negative observations of fruit size and weight with TSS were observed during guava selection and breeding (Dinesh and Yadav 1998), in which the genotypic correlation was lower than the phenotypic correlation for TSS—thus, indicating greater effect of fruit size- and weight-based traits plus external factors, as soil and environment, affecting TSS. Accordingly, pulp weight positively correlated with the fruit water content—an indication that much of the juicy pulp core in large fruits mainly consisted of water. This could be confirmed by the positive correlation between the pericarp and skin proportions with TSS, implying a higher dilution effect of TSS in the pulp core but not or less in the peel portion.

In addition, the pulp weight of the fruits was negatively correlated with the fruit protein content and most of the minerals (K, Ca, Mg, Na, S, B, and Fe). Similarly, negative correlations were also observed between fruit weight-based traits and some fruit minerals mainly K, Ca, S, and Fe. Ca was also negatively correlated with fruit length and width, while K, S, and Fe showed negative correlations with fruit width. These negative correlations could still be attributed to the dilution effect of increasing fruit size on fruit mineral accumulation, as was also observed by Singh et al. (2015), Mehmood et al. (2014), and Dinesh and Yadav (1998) in guava. It was notable that seed proportion correlated positively with most of the fruit mineral and chemical constituents and negatively with fruit water content. One may assume that increased seed proportion is likely to take up the position of water in the fruit

pulp, thus reducing water accumulation in the pulp core of guava fruit, as evidenced by the negative correlation observed between seed proportion and fruit water content. Reduced water in the pulp core implies a reduced dilution effect for the fruit minerals and chemicals in the pulp. The lack of a significant correlation based on pericarp and skin proportions with most fruit minerals affirms that, unlike TSS, some minerals such as K, Ca, and Fe are likely to be evenly distributed within guava fruit. This is also confirmed by the observed negative correlations of the various fruit parts with some minerals—unlike the case of TSS which only negatively correlated with pulp weight. However, further research is necessary to ascertain this finding.

In conclusion, the ascorbic acid content positively correlated with annual precipitation. TSS positively correlated with temperature and soil P and Zn, and was also found to be higher in white-fleshed fruits and fruits having lower pulp weight and more seeds. The red-fleshed fruits contained more phenolic content than the white-fleshed types, while the white-fleshed fruits had more of TSS, protein, and some minerals. Larger fruits were generally observed to have a dilution effect on the fruit mineral content. Generally, most of the correlations were not strong, implying that more than just the studied factors influence the nutritional and chemical content of guava.

The relationship between climatic data on fruit traits such as ascorbic acid and TSS could help in the choice of guava production regions that maximize on these chemical components. The flesh colour of fruits provides the information necessary for the selection of fruits for various purposes—for example, sweeter white-fleshed fruits with more mineral content could be preferred for fresh consumption; larger, less sweet fruits with lower mineral content could be preferred for industrial processing. The positive role of soil elements such as P and Zn in enhancing fruit traits such as TSS and sugars could help in the establishment of a fertilizer regime that maximizes the respective fruit quality traits.

3.6. Supplementary information

Table S1. Geographical coordinates, altitude, mean annual temperature and annual precipitation of the 128 guava accessions collected from four regions of Kenya.

Accession number	Accession code	Region	Latitude [N°/S°]	Longitude [E°]	Altitude (m)	Mean annual temperature (°C)	Annual precipitation (mm)
1	KIL001	Coast	03.69568 °S	039.72340 °E	208	23.7	425
2	KIL002*♦	Coast	03.69580 °S	039.72343 °E	199	23.7	425
3	KIL003*♦	Coast	03.69679 °S	039.72604 °E	202	23.7	425
4	KIL004*♦	Coast	03.69518 °S	039.72219 °E	200	23.7	425
5	KIL009*♦	Coast	03.92239 °S	039.74352 °E	23	23.7	410
6	KIL010*	Coast	03.92240 °S	039.74314 °E	25	23.7	410
7	KIL011*	Coast	03.92226 °S	039.74282 °E	22	23.7	410
8	KIL012*	Coast	03.92228 °S	039.74283 °E	22	23.7	410
9	KIL013*♦	Coast	03.91339 °S	039.74015 °E	18	23.7	410
10	KIL014	Coast	03.91348 °S	039.74015 °E	17	23.7	410
11	KIL015*♦	Coast	03.91338 °S	039.73997 °E	18	23.7	410
12	KIL016*♦	Coast	03.91332 °S	039.73999 °E	21	23.7	410
13	KIL017*♦	Coast	03.91347 °S	039.73988 °E	20	23.7	410
14	KWA001	Coast	04.16923 °S	039.59783 °E	23	22.7	458
15	KWA002	Coast	04.16853 °S	039.59749 °E	19	22.7	458
16	KWA003	Coast	04.16856 °S	039.59748 °E	19	22.7	458
17	KWA005*♦	Coast	04.16494 °S	039.57737 °E	104	22.5	475
18	KWA006*♦	Coast	04.16495 °S	039.57743 °E	97	22.5	475
19	KWA007*♦	Coast	04.16496 °S	039.57764 °E	119	22.5	475
20	KWA008*♦	Coast	04.16782 °S	039.56780 °E	108	22.7	458
21	KWA009	Coast	04.16837 °S	039.56796 °E	92	22.7	458
22	KWA010*♦	Coast	04.16860 °S	039.56822 °E	94	22.7	458
23	KWA011*♦	Coast	04.34928 °S	039.53458 °E	22	22.3	469
24	KWA014	Coast	04.34318 °S	039.51459 °E	35	22.3	469
25	KWA015*♦	Coast	04.33752 °S	039.44971 °E	117	22.1	491
26	KWA016*	Coast	04.33753 °S	039.44975 °E	118	22.1	491
27	KWA017*♦	Coast	04.49746 °S	039.25124 °E	39	21.6	518
28	KWA018	Coast	04.49765 °S	039.25125 °E	45	21.6	518
29	KWA019*♦	Coast	04.49763 °S	039.25131 °E	41	21.6	518
30	KWA020*♦	Coast	04.49715 °S	039.25139 °E	45	21.6	518
31	KWA021	Coast	04.60348 °S	039.18504 °E	25	21.7	505
32	KWA023	Coast	04.60352 °S	039.18509 °E	20	21.7	505
33	KWA024	Coast	04.60323 °S	039.18452 °E	21	21.7	505
34	MOM006	Coast	03.96482 °S	039.73122 °E	15	23.7	410
35	MOM007*♦	Coast	03.96493 °S	039.73089 °E	14	23.7	410
36	MOM008	Coast	03.96229 °S	039.73233 °E	16	23.7	410
37	MER001*	Eastern	00.17234 °S	037.64283 °E	1564	20.5	1149
38	MER002*	Eastern	00.17239 °S	037.64275 °E	1545	20.5	1149
39	MER005*	Eastern	00.17249 °S	037.65120 °E	1479	20.5	1149

40	MER009*♦	Eastern	00.08721 °S	037.66675 °E	1455	20.5	1382
41	MER010*♦	Eastern	00.08726 °S	037.66695 °E	1452	20.5	1382
42	MER012*♦	Eastern	00.08564 °S	037.66451 °E	1478	16.8	1582
43	MER013*♦	Eastern	00.08536 °S	037.66438 °E	1481	16.8	1582
44	MER014*♦	Eastern	00.11461 °S	037.69637 °E	1384	20.5	1382
45	MER016*	Eastern	00.18701 °S	037.69572 °E	1290	20.8	1335
46	MER017*	Eastern	00.18693 °S	037.69600 °E	1288	20.8	1335
47	MER018*♦	Eastern	00.12048 °S	037.72087 °E	1393	20.5	1382
48	MER019*♦	Eastern	00.12024 °S	037.72074 °E	1385	20.5	1382
49	ELG001*♦	Rift Valley	00.64776 °N	035.51977 °E	2089	21.2	950
50	ELG002*♦	Rift Valley	00.64203 °N	035.52221 °E	2064	21.2	950
51	ELG003*♦	Rift Valley	00.64265 °N	035.52145 °E	2077	21.2	950
52	ELG004*♦	Rift Valley	00.64264 °N	035.52150 °E	2071	21.2	950
53	ELG005*♦	Rift Valley	00.67029 °N	035.51809 °E	2214	20.3	954
54	ELG007*♦	Rift Valley	00.64350 °N	035.51839 °E	2104	21.2	950
55	ELG008*♦	Rift Valley	00.64349 °N	035.51843 °E	2104	21.2	950
56	ELG009*♦	Rift Valley	00.64338 °N	035.51852 °E	2102	21.2	950
57	ELG010*	Rift Valley	00.64505 °N	035.51627 °E	2132	21.2	950
58	ELG011*♦	Rift Valley	00.63185 °N	035.52095 °E	2024	21.2	950
59	ELG012	Rift Valley	00.63469 °N	035.52243 °E	2031	21.2	950
60	ELG013*♦	Rift Valley	00.63766 °N	035.51977 °E	2079	21.2	950
61	ELG014*♦	Rift Valley	00.57152 °N	035.30377 °E	2142	17.1	1055
62	ELG015*♦	Rift Valley	00.57151 °N	035.30377 °E	2150	17.1	1055
63	ELG016*	Rift Valley	00.58574 °N	035.46054 °E	2317	16	1104
64	ELG019*	Rift Valley	00.58788 °N	035.46055 °E	2322	16	1104
65	ELG020	Rift Valley	00.66651 °N	035.53149 °E	1972	21.2	950
66	ELG021*♦	Rift Valley	00.66682 °N	035.53004 °E	1985	20.3	954
67	UAG018*	Rift Valley	00.64256 °N	035.52145 °E	2067	21.2	950
68	HOM001*	Western	00.59582 °N	034.57717 °E	1308	20.8	1659
69	HOM003*	Western	00.59585 °N	034.57596 °E	1307	20.8	1659
70	HOM004*	Western	00.59594 °N	034.57690 °E	1306	20.8	1659
71	HOM006*	Western	00.59596 °N	034.57690 °E	1306	20.8	1659
72	HOM007*	Western	00.59593 °N	034.57692 °E	1307	20.8	1659
73	HOM009*	Western	00.59600 °N	034.57698 °E	1305	20.8	1659
74	HOM010*	Western	00.59596 °N	034.57703 °E	1307	20.8	1659
75	HOM011*	Western	00.59603 °N	034.57717 °E	1302	20.8	1659
76	HOM012*	Western	00.60963 °N	034.58897 °E	1329	20.8	1659
77	HOM013*	Western	00.60974 °N	034.58366 °E	1335	20.8	1659
78	HOM014*	Western	00.60961 °N	034.58369 °E	1339	20.8	1659
79	HOM016*	Western	00.60961 °N	034.58374 °E	1337	20.8	1659
80	HOM017*	Western	00.60984 °N	034.58377 °E	1336	20.8	1659
81	HOM018*	Western	00.60610 °N	034.63214 °E	1463	20.8	1659
82	HOM019*	Western	00.60611 °N	034.63223 °E	1456	20.8	1659
83	HOM020*	Western	00.61762 °N	034.64497 °E	1498	20.8	1659
84	HOM021*	Western	00.61760 °N	034.64495 °E	1502	20.8	1659
85	HOM022*	Western	00.53904 °N	034.50943 °E	1242	20.8	1659

86	HOM023*	Western	00.53907 °N	034.50946 °E	1238	20.8	1659
87	HOM024*	Western	00.53907 °N	034.50945 °E	1240	20.8	1659
88	HOM025*	Western	00.53907 °N	034.50941 °E	1237	20.8	1659
89	HOM026*	Western	00.53908 °N	034.50942 °E	1238	20.8	1659
90	HOM027	Western	00.53906 °N	034.50946 °E	1242	20.8	1659
91	HOM028*	Western	00.53905 °N	034.50951 °E	1239	20.8	1659
92	HOM029*	Western	00.53893 °N	034.50956 °E	1240	20.8	1659
93	HOM030*	Western	00.53880 °N	034.50989 °E	1239	20.8	1659
94	HOM032*	Western	00.53987 °N	034.50855 °E	1246	20.8	1659
95	HOM035*	Western	00.72481 °N	034.45610 °E	1289	21.1	1526
96	HOM036*	Western	00.72479 °N	034.45597 °E	1290	21.1	1526
97	HOM039*	Western	00.72471 °N	034.45581 °E	1292	21.1	1526
98	HOM042*	Western	00.72455 °N	034.45533 °E	1283	21.1	1526
99	HOM043*	Western	00.72442 °N	034.45531 °E	1283	21.1	1526
100	HOM045*	Western	00.72436 °N	034.45530 °E	1285	21.1	1526
101	HOM046*	Western	00.72439 °N	034.45518 °E	1283	21.1	1526
102	HOM047*	Western	00.72412 °N	034.45534 °E	1265	21.1	1526
103	HOM048*	Western	00.72412 °N	034.45539 °E	1275	21.1	1526
104	KAK001*♦	Western	00.27951 °N	034.67358 °E	1419	20.6	1917
105	KAK002*♦	Western	00.27863 °N	034.67363 °E	1409	20.6	1917
106	KAK003	Western	00.27861 °N	034.67367 °E	1420	20.6	1917
107	KAK004*♦	Western	00.27791 °N	034.69564 °E	1447	20.6	1917
108	KAK005*	Western	00.27700 °N	034.69589 °E	1441	20.6	1917
109	KAK006*	Western	00.27777 °N	034.69579 °E	1443	20.6	1917
110	KAK007*	Western	00.24446 °N	034.82470 °E	1571	20.6	1917
111	KAK008*	Western	00.24442 °N	034.82479 °E	1572	20.6	1917
112	SIA001	Western	00.19481 °N	034.34081 °E	1297	21.8	1774
113	SIA002*♦	Western	00.19376 °N	034.33390 °E	1286	21.8	1774
114	SIA003*♦	Western	00.19423 °N	034.33385 °E	1280	21.8	1774
115	SIA004*	Western	00.13007 °N	034.42597 °E	1358	21.6	1740
116	SIA005	Western	00.13003 °N	034.42687 °E	1357	21.6	1740
117	SIA006*♦	Western	00.12687 °N	034.42089 °E	1340	21.6	1740
118	SIA007	Western	00.12680 °N	034.42102 °E	1342	21.6	1740
119	SIA008*♦	Western	00.12804 °N	034.42337 °E	1347	21.6	1740
120	SIA009*♦	Western	00.12810 °N	034.42309 °E	1347	21.6	1740
121	SIA010*	Western	00.13046 °N	034.42354 °E	1348	21.6	1740
122	SIA011*♦	Western	00.13008 °N	034.42255 °E	1349	21.6	1740
123	UNK001	Western	00.84360 °N	034.79930 °E	1684	16.8	1455
124	UNK002	Western	00.08413 °N	034.79875 °E	1688	20.3	1864
125	VIH001*♦	Western	00.08540 °N	034.79936 °E	1680	20.3	1864
126	VIH002	Western	00.08539 °N	034.79936 °E	1679	20.3	1864
127	VIH003*	Western	00.08532 °N	034.79938 °E	1682	20.3	1864
128	VIH004*♦	Western	00.84470 °N	034.79931 °E	1683	16.8	1455

*Accessions used for morphological characterization.
♦Accessions used for collection of soil samples.

Table S2: Sixty four selected morphological descriptors (23 quantitative and 41 qualitative ones) and their scale of measurement used in Kenyan guava characterization.

Morphological descriptor	**Scale of measurement**
tree	
Height	Scale: m
Trunk diameter	Scale: cm
Crown diameter	Scale: m
Number of main branches	Scale: [counts]
Crown shape	Nominal: irregular = 1, Broad-pyramidal = 2, spherical = 3
Tree growth habit	Nominal: irregular spreading = 1, upright = 2, drooping = 3
Trunk shape	Nominal: cylindrical = 1, funnel-shaped = 2, concave = 3, crooked/irregular = 4
Stem colour of young trees	Nominal: light brown = 1, green = 2
Stem colour of old trees	Nominal: light brown = 1, green = 2
Bark texture of young trees	Nominal: smooth = 1, rough = 2
Bark texture of old trees	Nominal: smooth = 1, rough = 2
Bark patchings	Nominal: absent = 0, slightly patchy = 1, patchy = 2, very patchy = 3
Fruit	
Stalk attachment	Ordinal: weak = 1, intermediate = 2, strong = 3
Length	Scale: cm
Width	Scale: cm
Thickness	Scale: cm
Weight	Scale: g
Pedicel length	Scale: mm
Shape	Nominal: obovate = 1, ovate = 2, roundish = 3, oblong = 4, deltoid = 5, Rhomboid = 6
Apex shape	Nominal: acute = 1, obtuse = 2, round = 3, Angular = 4
Stalk insertion	Nominal: oblique = 1, slightly oblique = 2, vertical = 3
Depth of fruit stalk cavity	Ordinal: absent = 0, shallow = 1, medium = 2, deep = 3, very deep = 4
Neck prominence	Ordinal: absent = 0, slightly prominent = 1, prominent = 2, very prominent = 3
Skin colour ripe fruit (ground colour)	Nominal: green = 1, Yellowish green = 2, Greenish yellow = 3
Skin colour ripe fruit (flush)	Nominal: none = 0, green with reddish blush = 1, yellow with reddish blush = 2
Skin surface texture	Nominal: smooth = 1, rough = 2, Ridged = 3
Longitudinal rib	Nominal: absent = 0, present = 1
Longitudinal grooves	Nominal: absent = 0, present = 1
Pulp	
Weight	Scale: g
Juiciness	Ordinal: slightly juicy = 1, juicy = 2, very juicy = 3
Texture of ripe fruit	Ordinal: soft = 1, intermediate = 2, firm = 3
Aroma	Ordinal: mild = 1, intermediate = 2, strong = 3
Colour	Nominal: pink =1, white = 2, red = 3, creamy = 4, creamy white = 5, yellowish pink = 6
Flavour	Ordinal: very acidic = 1, acidic = 2, moderately sweet = 3, sweet = 4, very sweet = 5
Exocarp	
Thickness	Scale: mm

Weight	Scale: g
Mesocarp	
Thickness	Scale: mm
Weight	Scale: g
Pericarp	
Thickness	Scale: mm
Weight	Scale: g
Skin proportion (ratio of skin to total fruit)	Scale: % (w/w) of total fruit
Seed	
Length	Scale: mm
Width	Scale: mm
Thickness	Scale: mm
50 seed weight	Scale: g
shape	Nominal: pear-shaped = 1, gourd-shaped = 2, reniform = 3, heart-shaped = 4, oblong = 5
Seed colour	Nominal: based on colour codes of the Royal Horticultural Society
Seed hardiness	Ordinal: soft = 1, intermediate = 2, hard = 3, very hard = 4
Seed taste	Ordinal: sweet = 1, sour = 2, bitter = 3, tasteless = 4
Seed proportion (ratio of seed to pulp)	Scale: % (w/w) of total fruit
Leaf	
Leaf attitude in relation to branch	Nominal: semi-erect = 1, horizontal = 2
Leaf growth habit	Nominal: spiral = 1, opposite = 2, alternate = 3
Leaf blade shape	Nominal: elliptic = 1, oblong = 2, ovate = 3, oblong lanceolate = 4
Leaf apex shape	Nominal: obtuse = 1, acute = 2, acuminate = 3, rounded = 4
Leaf base shape	Nominal: acute = 1, obtuse = 2, round = 3
Young leaf fragrance	Nominal: absent = 0, mild = 2, strong = 3
Old leaf fragrance	Nominal: absent = 0, mild = 2, strong = 3
Colour of young leaf	Nominal: light green = 1, light green with brownish tint = 2, light brick red = 3, reddish brown = 4, deep coppery tan = 5
Mature leaf colour	Nominal: green = 1, pale green = 2, dark green = 3
Young leaf hairiness	Nominal: absent = 0, slightly hairy = 1, hairy = 2, very hairy = 3
Central venation curvature	Nominal: absent = 0, present = 1
Margin undulations	Nominal: absent = 0, weak = 1, intermediate = 2, strong = 3, wavy = 4, entire = 5
Leaf length	Scale: cm
Leaf width	Scale: cm

Table S3. Fruit chemical and mineral composition of 50 randomly selected trees from four regions of Kenya for correlation with their soil nutrients.

	Region of collection					
Fruit chemical and mineral composition	**Rift Valley n=13**	**Western n=11**	**Coast n=19**	**Eastern n=7**	**Mean n=50**	***p*-value**
Ascorbic acid (mg 100 g^{-1} FW)	53.5 ^{b} (11.1-94.5)	116 ^{ab} (36.8-205.9)	78.0 ^{b} (11.5-279.1)	195.8 ^{a} (109.3-377.1)	96.5 (11.1-377.1)	0.001
Phenolics (mg 100 g^{-1} FW)	144.7 ^{a} (12.,3-167.5)	170.2 ^{a} (123.8-285.8)	153.9 ^{a} (113.7-248.7)	166.4 ^{a} (121.0-201.2)	156.8 (113.7-285.8)	0.110
TSS (% brix)	9.2 ^{b} (5.9-11.2)	10.3 ^{ab} (6.9-11.8)	12.9 ^{a} (8.1-20.0)	9.4 ^{b} (8.1-10.7)	10.9 (5.9-20.0)	0.000
TA (mg 100 g^{-1} FW)	0.79 ^{b} (0.62-0.96)	1.01 ^{ab} (0.63-2.25)	1.03 ^{ab} (0.60-1.54)	1.47 ^{a} (0.91-2.73)	1.02 (0.60-2.73)	0.004
Fructose (g 100 g^{-1} FW)	2.42 ^{b} (1.28-3.61)	2.24 ^{b} (1.00-3.24)	3.54 ^{a} (1.55-5.55)	2.20 ^{b} (0.88-2.80)	2.78 (0.88-5.55)	0.001
Glucose (g 100 g^{-1} FW)	1.07 ^{a} (0.52-1.57))	0.76 ^{a} (0.11-1.46)	1.34 ^{a} (0.14-2.41)	0.80 ^{a} (0.36-0.99)	1.07 (0.11-2.41)	0.067
Protein (g 100 g^{-1} FW)	0.42 ^{b} (0.26-0.55)	0.44 ^{b} (0.26-0.71)	0.66 ^{a} (0.38-1.16)	0.42 ^{b} (0.33-0.57)	0.51 (0.26-1.16)	0.000
Ca (mg 100 g^{-1} FW)	15.3 ^{a} (7.64-27.8)	13.7 ^{a} (7.38-28.3)	16.1 ^{a} (7.21-29.8)	11.6 ^{a} (6.45-14.1)	14.8 (6.45-29.8)	0.214
K (mg 100 g^{-1} FW)	269.6 ^{b} (106.3-391.5)	254.7 ^{b} (133.4-599.0)	388.2 ^{a} (208.3-704.8)	263.1 ^{ab} (201.3-376.4)	310.5 (106.3-704.8)	0.001
Mg (mg 100 g^{-1} FW)	8.03 ^{b} (3.43-16.9)	8.48 ^{b} (4.96-14.0)	12.4 ^{a} (7.91-17.4)	9.64 ^{ab} (8.07-12.9)	10.0 (3.43-17.4)	0.002
Na (mg 100 g^{-1} FW)	4.00 ^{a} (0.61-6.02)	0.61 ^{b} (0.23-1.07)	5.66 ^{a} (2.85-16.0)	0.75 ^{b} (0.37-1.54)	3.43 (0.23-16.0)	0.000
P (mg 100 g^{-1} FW)	18.3 ^{a} (11.4-32.0)	11.1 ^{b} (4.2-22.6)	15.3 ^{ab} (9.98-20.6)	12.7 ^{ab} (9.21-16.1)	14.8 (4.22-32.0)	0.015
S (mg 100 g^{-1} FW)	10.1 ^{b} (3.98-14.7)	10.0 ^{b} (6.34-21.2)	15.8 ^{a} (7.95-27.0)	10.6 ^{ab} (8.01-15.5)	12.3 (3.98-27.0)	0.001
Fe (mg 100 g^{-1} FW)	0.38 ^{a} (0.18-0.92)	0.27 ^{a} (0.11-0.67)	0.36 ^{a} (0.19-0.59)	0.41 ^{a} (0.20-0.82)	0.35 (0.11-0.92)	0.182
B (mg 100 g^{-1} FW)	0.15 ^{b} (0.08-0.21)	0.21 ^{ab} (0.08-0.35)	0.27 ^{a} (0.11-0.43)	0.20 ^{ab} (0.16-0.25)	0.21 (0.08-0.43)	0.001
Zn (mg 100 g^{-1} FW)	0.07 ^{a} (0.00-0.17)	0.07 ^{a} (0.00-0.40)	0.10 ^{a} (0.00-0.27)	0.10 ^{a} (0.00-0.18)	0.08 (0.00-0.40)	0.445
Cu (mg 100 g^{-1} FW)	0.14 ^{a} (0.06-0.52)	0.09 ^{b} (0.02-0.17)	0.09 ^{b} (0.05-0.17)	0.07 ^{b} (0.05-0.11)	0.10 (0.02-0.52)	0.064
Water content (%)	86.7 ^{a} (82.1-91.6)	87.1 ^{a} (80.9-92.3)	79.5 ^{b} (65.6-88.2)	87.6 ^{a} (83.7-90.2)	84.2 (65.6-92.3)	0.000

Values within the same row followed by the same letter are not significantly different at $p < 0.05$ according to Kruskall-Wallis test followed by pairwise comparisons. Values within parenthesis depict the range.

Table S4. Soil pH and nutrients of 50 soil samples collected under guava trees from four regions of Kenya.

	Region					
soil content	**Rift Valley n=13**	**Western n=11**	**Coast n=19**	**Eastern n=7**	**Mean n=50**	***p*-value**
pH	5.96 ^{a} (4.83-7.01)	5.66 ^{a} (4.33-7.20)	6.10 ^{a} (4.63-7.98)	6.10 ^{a} (5.36-7.27)	5.97 (4.33-7.98)	0.625
N (%)	0.23 ^{a} (0.14-0.43)	0.15 ^{a} (0.08-0.26)	0.07 ^{b} (0.03-0.24)	0.20 ^{a} (0.11-0.32)	0.15 (0.03-0.43)	0.000
C (%)	2.97 ^{a} (1.57-5.70)	1.62 ^{ab} (0.80-2.81)	0.90 ^{b} (0.44-2.37)	2.12 ^{a} (1.15-3.82)	1.77 (0.44-5.70	0.000
C/N ratio	13.0 ^{a} (9.5-17.4)	10.6 ^{b} (8.8-12.5)	12.4 ^{ab} (9.7-14.8)	10.5 ^{b} (9.5-11.8)	11.9 (8.8-17.4)	0.002
P_2O_5 (mg kg^{-1})	21.5 ^{b} (0.83-97.9)	19.9 ^{b} (2.00-69.0)	101.1 ^{a} (1.38-841.6)	48.5 ^{ab} (11.1-88.2)	55.2 (0.83-841.6)	0.030
K_2O (mg kg^{-1})	138.3 ^{b} (10.4-374.7)	283.0 ^{ab} (11.0-956.1)	656.8 ^{a} (4.17-4590)	487.1 ^{a} (285.2-816.4)	416.0 (4.17-4590)	0.040
Mg (mg kg^{-1})	134.2 ^{c} (46.9-252.6)	168.4 ^{bc} (49.4-462.1)	325.9 ^{a} (76.5-764.6)	270.2 ^{ab} (95.5-425.9)	233.6 (46.9-764.6)	0.006
B (mg kg^{-1})	1.59 ^{b} (0.21-3.62)	1.95 ^{b} (0.20-6.12)	4.06 ^{a} (0.49-15.76)	2.18 ^{ab} (0.63-3.51)	2.69 (0.20-15.76)	0.044
Cu (mg kg^{-1})	16.9 ^{a} (2.76-69.1)	24.4 ^{a} (7.12-57.6)	21.8 ^{a} (4.03-83.5)	10.9 ^{a} (4.52-26.3)	19.6 (2.76-83.5)	0.283
Mn (mg kg^{-1})	4764 ^{a} (1979-9358)	3614 ^{a} (815-6975)	4742 ^{a} (388-7693)	3865 ^{a} (1603-7731)	4377(388-9358)	0.474
Fe (mg kg^{-1})	757.1 ^{a} (250.3-1574)	785.1 ^{a} (335.3-1534)	803.3 ^{a} (236.7-1848)	738.5 ^{a} (369.5-1025)	778.2 (236.7-1848)	0.911
Zn (mg kg^{-1})	81.2 ^{a} (6.67-270.2)	73.9 ^{a} (10.2-205.0)	183.4 ^{a} (4.78-843.5)	67.2 ^{a} (20.4-217.5)	116.5(4.78-843.5)	0.462
Na (mg kg^{-1})	39.7 ^{a} (18.0-115.4)	43.3 ^{a} (18.2-140.8)	53.8 ^{a} (16.0-163.4)	64.0 ^{a} (25.0-226.9)	49.3 (16.0-226.9)	0.990

Values within the same row followed by the same letter are not significantly different at $p < 0.05$ according to Kruskall-Wallis test followed by pairwise comparisons. Values within parenthesis depict the range.

Table S5. Variation of the key morphological descriptors between the two identified morphological clusters of 105 guava samples.

	Morphological cluster			
Key descriptor	**Cluster 1 (n = 90) (Small fruits)**	**Cluster 2 (n = 15) (Big fruits)**	**Mean (n = 105)**	***p*-value**
Fruit length (cm)	4.28 [b] (2.97-5.39)	5.28 [a] (3.24-5.92)	4.42 (2.97-5.92)	0.000
Fruit width (cm)	4.14 [b] (2.60-5.34)	4.89 [a] (3.07-5.37)	4.24 (2.60-5.37)	0.000
Fruit weight (g)	45.9 [b] (21.5-95.0)	76.4 [a] (18.4-96.8)	50.2 (18.4-96.8)	0.000
Pericarp weight (g)	26.2 [b] (12.3-56.3)	47.3 [a] (10.8-68.5)	29.2 (10.8-68.5)	0.000
Exocarp weight (g)	13.7 [b] (5.5-24.0)	21.7 [a] (4.9-27.3)	14.9 (4.9-27.3)	0.000
Mesocarp weight (g)	12.0 [b] (2.3-33.1)	24.9 [a] (5.6-40.2	13.8 (2.3-40.2)	0.000
Pulp weight (g)	13.5 [b] (4.3-34.8)	20.2 [a] (4.5-27.9)	14.5 (4.3-34.8)	0.001

Values within the same row followed by the same letter are not significantly different at $p < 0.05$ according to the independent sample t-test. Values within parenthesis depict the range.

Table S6. Fruit chemical and mineral composition of 105 guava accessions from 4 regions of Kenya based on two morphological clusters.

Fruit chemical and mineral composition	Morphological cluster			
	Cluster 1 (n= 90)	**Cluster 2 (n= 15)**	**Mean (n= 105)**	***p*-value**
Ascorbic acid (mg 100 g^{-1} FW)	92.4 (9.8-377.1)	70.4 (23.9-140.0)	89.3 (9.8-377.1)	0.237
Phenolics (mg 100 g^{-1} FW)	152.8 (108.6-285.8)	143.8 (112.3-201.2)	151.5 (108.6-285.8)	0.272
TSS (% brix)	11.0 (5.9-20.0)	11.0 (6.0-14.1)	11.0 (5.9-20.0)	0.694
TA (mg 100 g^{-1} FW)	0.95 (0.60-2.30)	1.11 (0.70-2.70)	0.97 (0.60-2.70)	0.647
Fructose (g 100 g^{-1} FW)	2.78 (0.88-6.05)	3.24 (1.81-5.71)	2.84 (0.88-6.05)	0.172
Glucose (g 100 g^{-1} FW)	1.08 (0.10-2.80)	1.31 (0.72-2.41)	1.12 (0.10-2.80)	0.167
Protein (mg 100 g^{-1} FW)	0.50 (0.26-1.16)	0.56 (0.35-0.91)	0.51 (0.26-1.16)	0.092
Ca (mg 100 g^{-1} FW)	14.1 (4.98-29.8)	13.5 (7.09-26.2)	14.0 (4.98-29.8)	0.742
K (mg 100 g^{-1} FW)	303.6 (106.3-704.8)	306.2 (201.3-428.9)	303.9 (106.3-704.8)	0.540
Mg (mg 100 g^{-1} FW)	9.85 (3.43-18.8)	10.4 (6.62-16.2)	9.93 (3.43-18.8)	0.292
Na (mg 100 g^{-1} FW)	2.67 (0.23-16.0)	2.38 (0.56-6.02)	2.63 (0.23-16.0)	0.627
P (mg 100 g^{-1} FW)	13.8 (4.18-32.4)	15.9 (8.38-27.1)	14.1 (4.18-32.4)	0.260
S (mg 100 g^{-1} FW)	11.8 (3.21-27.0)	12.5 (9.59-18.2)	11.9 (3.21-27.0)	0.346
Fe (mg 100 g^{-1} FW)	0.33 (0.10-1.17)	0.32 (0.14-0.65)	0.33 (0.10-1.17)	0.993
B (mg 100 g^{-1} FW)	0.21 (0.08-0.43)	0.22 (0.13-0.36)	0.21 (0.08-0.43)	0.527
Zn (mg 100 g^{-1} FW)	0.07 (0.00-0.40)	0.07 (0.00-0.23)	0.07 (0.00-0.40)	1.00
Cu (mg 100 g^{-1} FW)	0.11 (0.02-0.52)	0.11 (0.06-0.22)	0.11 (0.02-0.52)	0.431
Water content (%)	84.6 (65.6-92.3)	83.2 (77.1-89.0)	84.4 (65.6-92.3)	0.156

There was no significant ($p < 0.05$) variation in the fruit chemical and mineral composition between the two clusters according to Mann-Whitney U-test. Values within parenthesis depict the range.

Table S7. Principal component analysis for the first three principal components from the PCA performed on 50 samples for guava fruit mineral and chemical composition, soil properties, temperature, precipitation, and fruit morphological traits.

Principal component analysis				
Number of components is 3				
Component	**Eigenvalues**	**% total variance**	**Cumulative eigenvalue**	**Cumulative %**
1	9.847704	28.13630	9.84770	28.13630
2	3.431655	9.80473	13.27936	37.94103
3	2.980329	8.51522	16.25969	46.45625

Variable	**Component 1**	**Component 2**	**Component 3**
Soil P	0.522502	-0.651341	0.257942
Soil K	0.516120	-0.607415	0.270057
Soil Mg	0.513624	-0.253515	0.277745
Soil pH	0.291867	-0.481520	0.285494
Soil B	0.555872	-0.555056	0.201729
Soil Cu	0.229013	0.213393	-0.097604
Soil Mn	0.134302	0.272558	-0.118987
Soil Fe	0.191074	0.394672	-0.057308
Soil Zn	0.719490	-0.231159	0.104476
Soil Na	0.332036	-0.399329	0.101003
Soil N	-0.449738	0.090707	0.428092
Ascorbic acid	-0.129239	-0.211722	0.203997
TA	0.100106	-0.149779	0.496962
TSS	0.692719	-0.077156	-0.369423
Phenolics	-0.254368	0.274513	0.092151
Protein	0.930105	0.162681	0.081566
Fruit Ca	0.490921	0.486956	0.159710
Fruit K	0.836569	0.310948	0.085002
Fruit Mg	0.845793	0.255386	0.041955
Fruit Na	0.486959	0.191402	-0.173206
Fruit P	0.446514	0.381473	0.348002
Fruit S	0.825804	0.348739	0.027387
Fruit Zn	0.171574	0.330380	-0.054288
Fructose	0.757403	-0.152597	-0.034986
Glucose	0.605603	-0.282825	0.056625
Fruit B	0.778867	0.195575	0.025969
Fruit Cu	0.094892	0.261821	0.146610
Fruit Fe	0.305063	0.432597	0.470091
Water (%)	-0.934266	-0.152044	-0.080925
Temperature	0.372731	0.041795	-0.281728
Precipitation	-0.606632	-0.120337	0.199947
Cluster 1	0.127922	-0.153963	-0.695523
Cluster 2	-0.127922	0.153963	0.695523
White group	0.453339	-0.182421	-0.468873
Red group	-0.453339	0.182421	0.468873

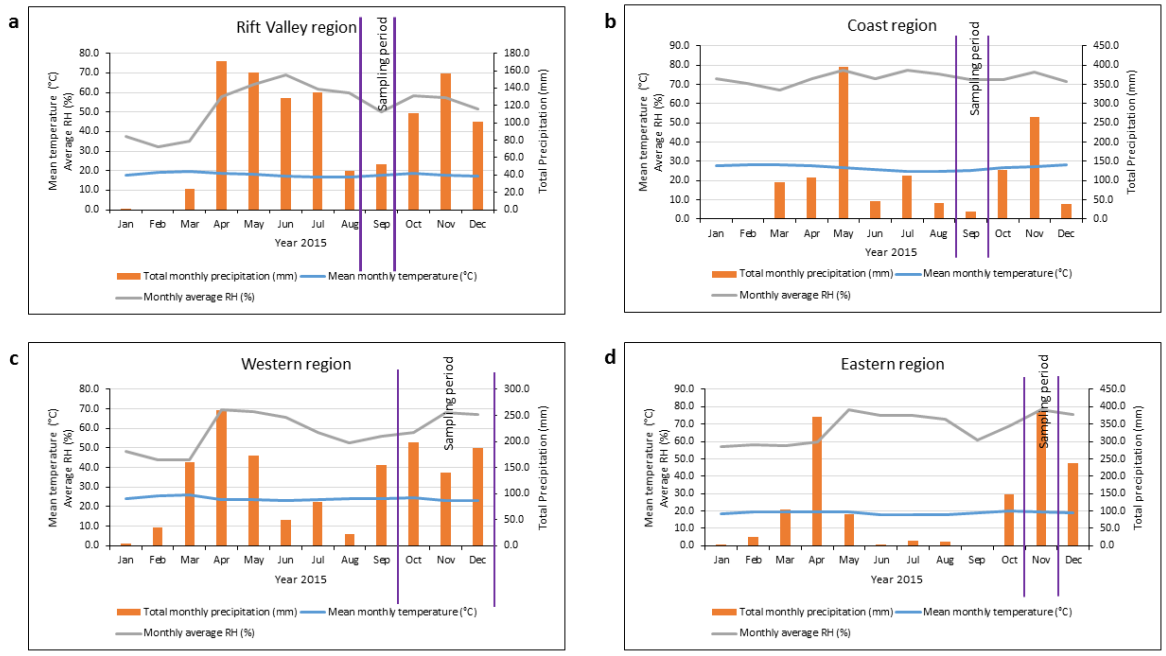

Figure S1. Mean monthly temperature, precipitation and Relative humidity (RH) of the four regions (a) Rift valley, (b) Coast, (C) Western and (d) Eastern, of guava fruit collection based on data from the nearest meteorological station for the year 2015. The periods when sampling was carried out is indicated.

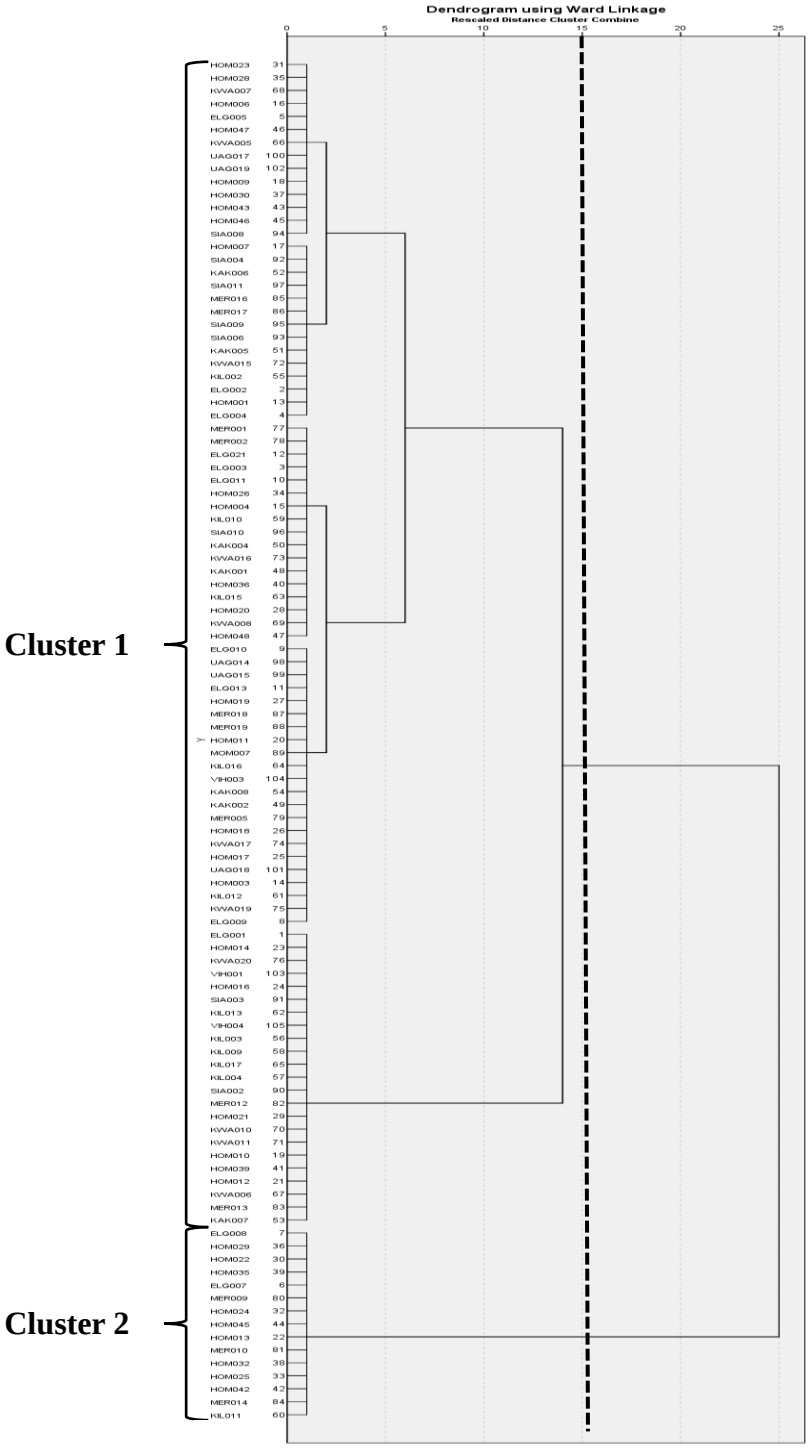

Figure S2. Dendrogram resulting from cluster analysis (Ward method, squared Euclidean distances, z-score standardization of variables) using seven key descriptors on 105 guava accessions. The dotted line indicate the cutting distance for cluster formation. Cluster 2 comprised of larger and heavier fruits compared to cluster 1.

Chapter four

4. Partitioning of dry matter and minerals in guava (*Psidium guajava* L.*)* accessions under salt stress: Implications for selection of adapted rootstocks for saline soils

Abstract

Common guava *(Psidium guajava* L.) is highly valued for the deliciousness of the fruit, which is a source of vitamins, minerals, and natural antioxidants. However, guava production faces salinity challenges in many guava-producing countries. The effect of sodium chloride (NaCl) salinity—0 mM (control), 10/20 mM (low), 20/40 mM (medium), and 40/80 mM (high)—supplied through a standard Hoagland nutrient solution to 10 genetically diverse guava accessions was investigated to test their level of tolerance in a six-week greenhouse experiment. Leaf number and leaf dry matter (DM) were significantly reduced at the medium and high salinity levels while root DM remained similar in all the treatments, however; differential accumulation of DM was observed in individual accessions. Root water content increased with rising salinity levels, whereas leaf water content was significantly reduced only at the high NaCl level. There was a decrease in the leaf potassium/sodium (K/Na) and calcium/sodium (Ca/Na) ratios with increasing salinity level, which could be attributed to the high accumulation of Na rather than to the replacement of K and Ca. The levels of leaf phosphorus (P) and sulphur (S) decreased with increasing salinity; however, leaf magnesium (Mg) did not show a clear trend. Leaf boron (B) and iron (Fe) were significantly reduced only at the high salinity level, whereas the other micronutrients remained unaffected. Differences among the accessions relative to the accumulation of Na were observed and positively correlated with the DM. Thus, the ability to maintain more DM under salt stress could serve as an indicator for salinity tolerance in guava and should be considered when selecting genotypes for adaptation to saline environments.

4.1. Introduction

Crop production worldwide is constrained by the effects of salinity resulting in ion toxicity, water deficiency for plant uptake and nutrient imbalance (Marschner 2012). Natural boundaries imposed by soil salinity also limit the nutritional potential of plants by lowering the quality of their products. Plant responses to water and salinity stresses are complex: They involve signal reception and transduction, followed by genetic and physiological responses (Munns and Tester 2008). The responses common to these stresses include osmolyte production, altering water transport, and scavenging reactive oxygen species (Keutgen and Pawelzik 2008; Marschner 2012). In particular, fruit production is very constrained in saline soils as most of the cultivated fruit tree species are not salt-tolerant (Saied et al. 2010). However, there are differences in salt tolerance among species and genotypes (Munns and Tester 2008). The selection of different genotypes under conditions of environmental stress is therefore one of the main tasks for exploiting the genetic variation in order to select and improve the stress-tolerant cultivars (Munns and Tester 2008).

The guava (*Psidium guajava* L.) plant is highly valued for its delicious tropical fruit, which is rich in vitamins, minerals, and natural antioxidants (Natale et al. 2002; Araújo et al. 2015; Flores et al. 2015). The guava tree is among the hardiest tropical fruit trees with regard to adaptation to diverse environmental conditions, and it outperforms most other fruit crops in productivity (Sharma et al. 2010). However, guava production faces salinity challenges in many guava-producing countries, such as Brazil (Cavalcante et al. 2007), Australia (Noble and West 1988), India (Singh et al. 2016), Kenya (Mugai 2004), and Sudan (Ali-Dinar et al. 1999). Guava has been ranked as salt-sensitive (da Silva et al. 2008) or reasonably salt-sensitive (Cavalcante et al. 2007) to moderately tolerant to salinity (Maas 1993; Ali-Dinar et al. 1999). The tolerance threshold for most cultivated guava varieties is generally reported to vary between 30 mM sodium chloride (NaCl) and 50 mM NaCl in the

rhizosphere (Ali-Dinar et al. 1999; Desai and Singh 1983). Ali-Dinar et al. (1999) found that the growth of guava seedlings in quartz sand was hardly affected by the 30 mM NaCl level but was reduced significantly at the 60 mM NaCl level.

Intraspecific variation has been reported to be high among the current guava cultivars (Sánchez-Urdaneta and Peña-Valdivia 2011; Mehmood et al. 2014). Consequently, variations in guava cultivars in response to salinity stress have been reported (Cavalcante et al. 2007; Francisco et al. 2016). Ali-Dinar et al. (1999), for example, observed more tolerance to salinity in guava cultivars with red fruit pulp than in those with white fruit pulp. Most of these studies, however, also focused on mechanisms to alleviate the effect of salt stress; therefore, their salinity experiments comprised treatments that could help plants tolerate salinity—for instance, they involved the application of nitrate fertilizers (Ali-Dinar et al. 1998), calcium nitrate (Ali-Dinar et al. 1999; Ebert et al. 2002), and organic manure (da Silva et al. 2008).

The objective of this study was to investigate the effects of salinity on the growth and mineral content of 10 genetically diverse guava accessions. To ensure a fair comparison of salinity tolerance, the treatments were uniformly supplied with a standard Hoagland nutrient solution through irrigation water with varying salt levels. Therefore, any observed variation in the individual accessions would mainly be due to the salt treatments. Results of this study can be used to select more salinity-tolerant guava accessions that can be used as rootstocks for cultivation of guava in saline soils.

4.2. Materials and Methods

4.2.1. Plant material sampling

Guava stem cuttings of about 1 cm in diameter and 15 cm in length were collected from four regions of Kenya **(Figure S1)**. The choice of the accessions was based on initial genetic clustering in which the individuals were highly differentiated from each other. The

selected accessions also differed in some morphological attributes **(Table S1).** From each of the accessions, 40 cuttings were taken and rooted for vegetative reproduction. In total, 400 plants were raised from the 10 accessions for the salinity experiment.

4.2.2. Experimental design and data collection

After successful rooting and development of the first leaves, the new plants were pruned to a height of 15 cm and the axillary buds were removed to inhibit lateral branching for homogenous plants. After four months, by which time the plants were well acclimatized, they were transferred into 3 l plastic pots containing washed quartz sand (0.6–1.2 mm Ø) and moved to the greenhouse for the salinity experiment at the University of Eldoret, Kenya.

The 400 guava plants were first arranged in a randomized complete block design with two blocks of 200 plants each. Prior to the introduction of the salinity treatment, each guava plant received 200 ml of standard Hoagland solution (Hoagland and Arnon 1950) per pot every other day to cover the nutrient requirements. This lasted one week (adaptation phase) to enable the plants to adapt to the greenhouse conditions. The greenhouse conditions consisted of natural tropical light conditions and mean minimum, average, and mean maximum temperatures of 19.4°C, 28.6°C, and 34.6°C, respectively. At the end of the week of adaptation (week 0), the initial data on plant height and leaf number were documented prior to the introduction of the NaCl treatments. These measurements were later recorded on a weekly basis after the commencement of the salt treatments—that is, from week 1 onwards. Each treatment involved the 10 accessions, with each accession replicated 10 times, resulting in 100 plants per treatment. The salt treatments were applied via an irrigation solution that was prepared by dissolving specified amounts of NaCl in standard Hoagland solution to correspond to 10 mM (low), 20 mM (medium), and 40 mM (high) NaCl, respectively. These also corresponded to electrical conductivities (ECs) of 1.40 dS m^{-1}, 2.34 dS m^{-1}, and 4.26 dS m^{-1}, respectively. Furthermore, the treatments included a control (0 mM NaCl) that

corresponded to EC of 0.46 dS m^{-1}. Throughout the experiment, each plant received 200 ml of irrigation solution between 9 a.m. and 10 a.m. every other day. The containers were uniformly perforated at the bottom to allow excess irrigation solution to pass through. The NaCl concentrations were doubled in the third week after the commencement of the salt treatments as follows: 0 mM (control), 20 mM (low), 40 mM (medium), and 80 mM (high) NaCl, corresponding to ECs of 0.46 dS m^{-1}, 2.80 dS m^{-1}, 4.74 dS m^{-1}, and 8.47 dS m^{-1}. The experiment was stopped after six weeks, when plants in the 40/80 mM NaCl treatment showed signs of severe salt stress with intensive leaf drops and chlorosis.

4.2.3. Sample preparation and measurements

At the end of the experiment, the leaves and stems were harvested, and fresh weights were determined. The roots were washed with clean tap water, allowed to dry for one hour in the greenhouse, and fresh weights were determined. Subsequently, all plant parts were dried to a constant weight at 65°C for 48 h and the dry matter (DM) was determined. In total, 1,200 samples comprising 400 leaf, 400 stem, and 400 root samples were sealed and stored in labelled plastic bags at room temperature until their grinding into fine powder.

4.2.4. Plant mineral analysis

The minerals were extracted from 100 mg each of the oven-dried and milled samples. Thereafter, 4 ml of concentrated nitric acid (HNO_3) and 2 ml of 30% hydrogen peroxide (H_2O_2) were added to each sample in a Teflon vessel. The samples were then wet-incinerated in a microwave at 200°C and 15 bar pressure for 75 minutes. Following this, the samples were transferred to 25 ml volumetric flasks and filled up to the total volume of 25 ml with pure water.

The mineral content in leaves—that is, macro (calcium [Ca], magnesium [Mg], phosphorus [P], potassium [K], sodium [Na], and sulphur [S]) and micro (boron [B], iron [Fe], manganese [Mn], and zinc [Zn]) elements—were determined using inductively coupled

plasma atomic emission spectroscopy (ICP-AES) (Vista-RL ICP-OES, Varian Inc., USA) (Wheal et al. 2011). Stem and root samples were analysed for Na concentration with a flame photometer (model BWB XP, BWB Technologies UK Ltd., UK). Prior measurements of Na content in the root and stem samples had not shown variations within similar treatments; hence, to save on time and cost, measurements for stems and roots considered only one block of the treatments (i.e. half of the total samples). The results were expressed as mg 100 g^{-1} dry weight (DW) of the sample. The Na content was also calculated at the plant level, taking into consideration the dry biomass at the end of the experiment for the leaves, stems, roots, and the entire plant. The results were accordingly expressed as mg per DW of the plant part or the entire plant.

4.3. Statistical analysis

All statistical analyses were performed using the statistical software package SPSS, version 20 (IBM Corp., Armonk, NY). For growth and DM parameters, samples from all 400 plants were used. For mineral analysis, however, samples from one block were used for stem and root (200 samples), while 240 samples were used for leaf mineral analysis, due to insufficient amount of leaf for some samples. However, for comparison of Na concentrations, same samples were used for the different plant organs. The data was statistically analysed using a two-way analysis of variance (ANOVA), considering the salt levels, the accession, and their interaction. Comparison of the means was assessed by a Tukey test at the 95% confidence interval. Furthermore, changes in growth parameters, DM, and Na content between the control and high salinity (40/80 mM NaCl) level within individual accessions was assessed for significance differences using a paired sample t-test.

4.4. Results

Plants in the control treatment did not show any signs of salt toxicity symptoms. At the low salinity level (10/20 mM NaCl), most plants showed only mild signs of salt injury:

The leaves were only less green compared to the control plants. At the medium (20/40 mM NaCl) salinity level, however, leaf chlorosis was moderately severe in most plants, with a few plants showing necrotic symptoms at the leaf margins. Mild leaf drops were observed in all plants at this salinity level, especially from Week 4, though with varying intensities among the accessions. Plants in the high salinity level (40/80 mM NaCl) showed symptoms of chlorosis and necrosis from Week 3 and the symptoms became more severe over time. All plants at this level exhibited severe leaf drops starting from the fourth week, with no observable differences among the accessions. **Figure 1** shows the salinity symptoms observed under different salt levels.

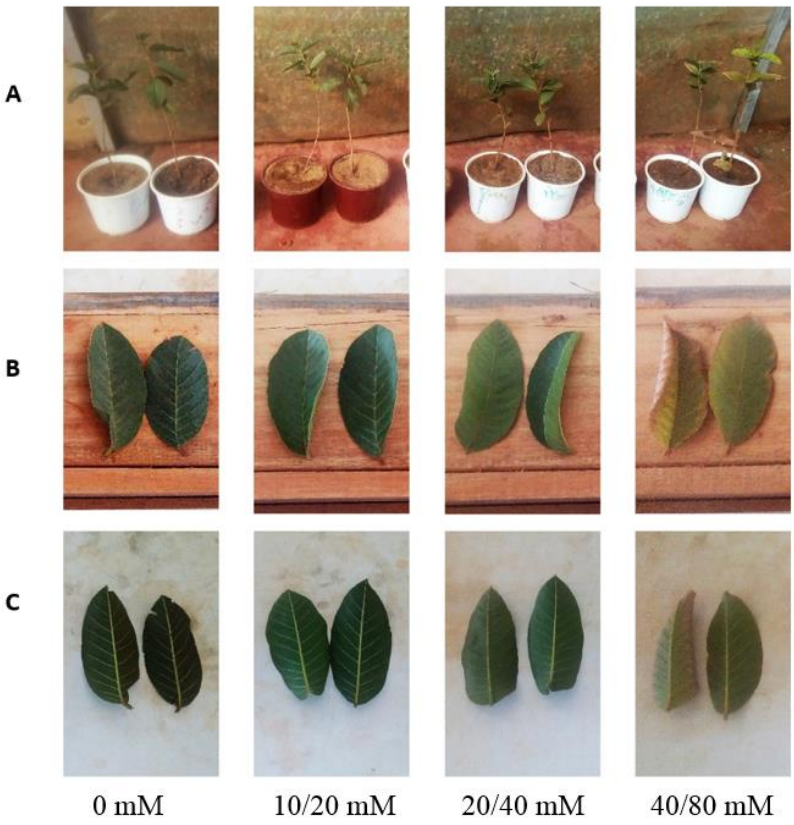

Figure 1. Observed salt injury symptoms at the end of the experiment in (A) guava plants, (B) upper side of the leaves, and (C) underside of the leaves. Symptoms became more pronounced from the medium (20/40 mM) to the high (40/80 mM) salt stress levels.

4.4.1. Growth parameters

Plant height increased from Week 0 with a mean of 42.8 cm to 47.7 cm in Week 6 (data not shown). Overall, plants in the control treatment had a mean height of 45.7 cm compared to 45.1 cm in the high salinity treatment (**Table 1**). Plant height was not significantly affected by NaCl treatments; however, a significant variation among the accessions was recorded based on the interaction between the accession and NaCl treatments (**Table 1)**. For instance, accession ELG009 gained more height (8%) at the 40/80 mM salt level compared to the control. On the other hand, accession HOM013 reduced most in height (9%) at the high salt level in comparison to the control. Nevertheless, these changes between the control and high salinity level in plant height within the accessions were found to be insignificant based on the paired sample t-test. The average number of leaves per plant increased steadily from Week 0 with a mean of 8.84 to 11.2 in Week 6 (data not shown). Leaf number, however, generally significantly reduced as a result of the NaCl treatments, with the control and low salinity treatments recording higher values compared to the medium and high salinity treatments (**Table 1, Figure 2**). The difference in leaf number between treatments was more observable from the fourth week and continued through to the end of the experiment (**Figure 2**). Compared to the control plants, the mean leaf number reduced by 8.33% in the 40/80 mM NaCl treatment. Differential variations in leaf number in individual accessions were also observed between the control and high salinity treatments. Accessions MER014, ELG009, and UAG014 recorded slight increases of 4.29%, 3.84%, and 0.53%, respectively, in the high NaCl treatments compared to the control. In contrast, all the remaining accessions had a reduction in leaf number with higher losses recorded in accessions KIL013 (21.4%), HOM016 (19.4%), and MER011 (15.3%). However, the reductions were significant only in accessions HOM016 and MER011 (**Table 1**).

Table 1. Effects of NaCl six weeks after starting the experiment on leaf number and plant height of 10 different accessions of guava collected from four regions of Kenya (n=10 per accession and treatment)

	Accessions										
NaCl (mM)	**ELG009**	**HOM013**	**HOM016**	**KIL013**	**KIL014**	**MER014**	**MER009**	**MER011**	**UAG014**	**VIH004**	**Mean**
					Leaf number						
0	$10.0^{bc} \pm 2.39$	$11.5^{a} \pm 1.91$	$9.81^{c} \pm 2.63$	$11.7^{a} \pm 2.90$	$10.8^{abc} \pm 2.72$	$11.0^{abc} \pm 2.48$	$10.6^{abc} \pm 2.89$	$11.3^{ab} \pm 2.62$	$10.7^{abc} \pm 2.97$	$10.7^{abc} \pm 2.50$	10.8 ± 2.66
10/20	$9.93^{bc} \pm 2.23$	$11.4^{ab} \pm 2.56$	$11.0^{ab} \pm 2.62$	$11.3^{ab} \pm 2.79$	$11.2^{ab} \pm 3.31$	$11.5^{a} \pm 2.79$	$10.0^{abc} \pm 2.78$	$10.6^{abc} \pm 3.32$	$11.1^{ab} \pm 2.63$	$9.34^{c} \pm 2.96$	10.7 ± 2.88
20/40	$10.8^{a} \pm 2.25$	$11.2^{a} \pm 2.20$	$9.21^{c} \pm 2.79$	$10.6^{ab} \pm 2.98$	$10.4^{abc} \pm 2.56$	$10.3^{abc} \pm 2.81$	$10.1^{abc} \pm 2.33$	$11.0^{a} \pm 2.78$	$10.7^{ab} \pm 2.83$	$9.34^{bc} \pm 2.43$	10.4 ± 2.67
40/80	$10.4^{abc} \pm 1.49$	$10.6^{abc} \pm 2.90$	$7.91^{d} \pm 2.55$	$9.20^{cd} \pm 3.22$	$9.60^{bc} \pm 3.06$	$11.5^{a} \pm 2.66$	$9.94^{bc} \pm 2.73$	$9.56^{bc} \pm 2.80$	$10.8^{ab} \pm 2.65$	$9.50^{bc} \pm 3.09$	9.90 ± 2.89
% change*	3.84	-8.05	-19.4	-21.4	-10.9	4.29	-6.58	-15.3	0.53	-11.2	-8.33
p*-value*	0.198	0.426	0.044	0.061	0.164	0.713	0.420	0.044	0.943	0.434	
					Plant height (cm)						
0	$46.0^{bc} \pm 6.05$	$49.6^{ab} \pm 9.34$	$40.4^{ef} \pm 6.68$	$49.4^{abc} \pm 4.56$	$53.0^{a} \pm 5.96$	$45.5^{bcd} \pm 15.6$	$41.0^{def} \pm 9.73$	$39.2^{f} \pm 6.26$	$47.9^{bc} \pm 10.1$	$44.6^{cde} \pm 10.2$	45.7 ± 9.90
20	$48.4^{ab} \pm 5.86$	$50.8^{a} \pm 6.93$	$41.9^{d} \pm 7.32$	$49.4^{ab} \pm 5.46$	$51.5^{a} \pm 5.40$	$43.1^{cd} \pm 6.94$	$41.7^{d} \pm 6.40$	$41.1^{d} \pm 7.09$	$46.2^{bc} \pm 7.28$	$43.6^{cd} \pm 10.7$	45.8 ± 8.00
20/40	$49.9^{ab} \pm 7.46$	$46.1^{bc} \pm 5.49$	$42.8^{cde} \pm 5.41$	$47.9^{ab} \pm 4.73$	$51.2^{a} \pm 6.49$	$43.2^{cd} \pm 14.0$	$43.0^{cd} \pm 8.64$	$38.5^{e} \pm 6.58$	$51.7^{a} \pm 10.2$	$41.3^{de} \pm 9.22$	45.6 ± 9.26
40/80	$49.7^{b} \pm 5.54$	$45.1^{cd} \pm 6.32$	$41.1^{ef} \pm 6.54$	$47.9^{bc} \pm 5.35$	$54.4^{a} \pm 6.43$	$46.0^{bc} \pm 10.6$	$40.0^{f} \pm 10.3$	$40.7^{ef} \pm 5.56$	$44.5^{cde} \pm 7.06$	$41.5^{def} \pm 6.17$	45.1 ± 8.40
% change*	8.12	-9.05	1.64	-3.14	2.75	1.12	-2.39	3.80	-7.21	-6.93	-1.31
p*-value*	0.137	0.159	0.714	0.570	0.458	0.935	0.604	0.503	0.185	0.362	

Data are expressed as mean values ± standard deviation. Different letters in each row indicate significant differences at $p \leq 0.05$. *Mean percent change between 0 mM NaCl and 40/80 mM NaCl. ***p*-value between 0 mM and 40 mM treatments according to a paired sample t-test. NaCl treatments were doubled after the first three weeks of the experiment.

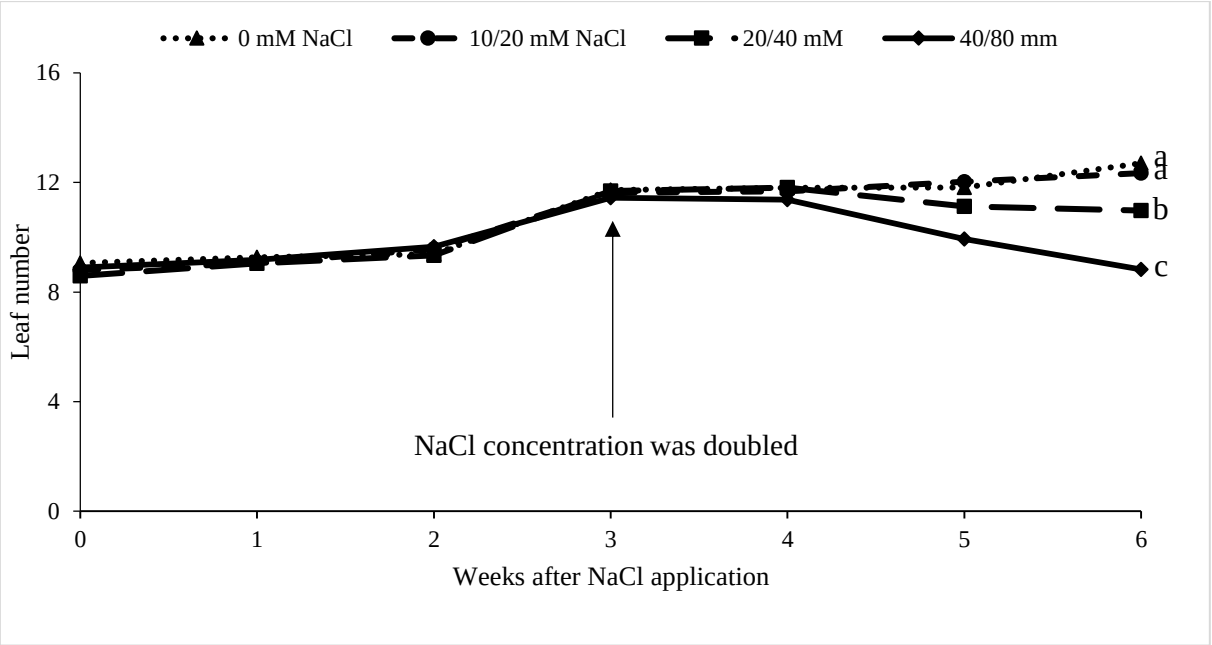

Figure 2. Leaf number of guava plants of 10 different accessions from Kenya (n=100 per treatment) as affected by different NaCl concentrations in the irrigation solution. Letters indicate significant differences at $p \leq 0.05$.

4.4.2. Plant dry matter

The mean total dry matter (DM) per plant was significantly reduced at the end of the experiment by 12% and 19% in the medium and high salinity levels, respectively, in comparison to the control **(Figure 3)**. The total DM in the low salinity treatments did not differ significantly from the control. With regard to the different plant parts, the highest values for DM were observed in the roots, followed by the leaves and stems. The reduction of DM in the leaves was the most distinct: It was reduced by 39% in the high 40/80 mM NaCl treatment and by 23% in the medium 20/40 mM NaCl treatments relative to the control. The reduction of DM in the stems was not as pronounced as observed for the leaves and was significant only in the high NaCl treatments, where DM was reduced by 18% in comparison to the control. Root DM was not significantly affected by the NaCl treatments. The shoot (i.e. leaves + stems)/root ratios ranged from 1.65 in the control to 1.19 in the high NaCl treatment; however, only the high NaCl treatment resulted in significant differences to the control.

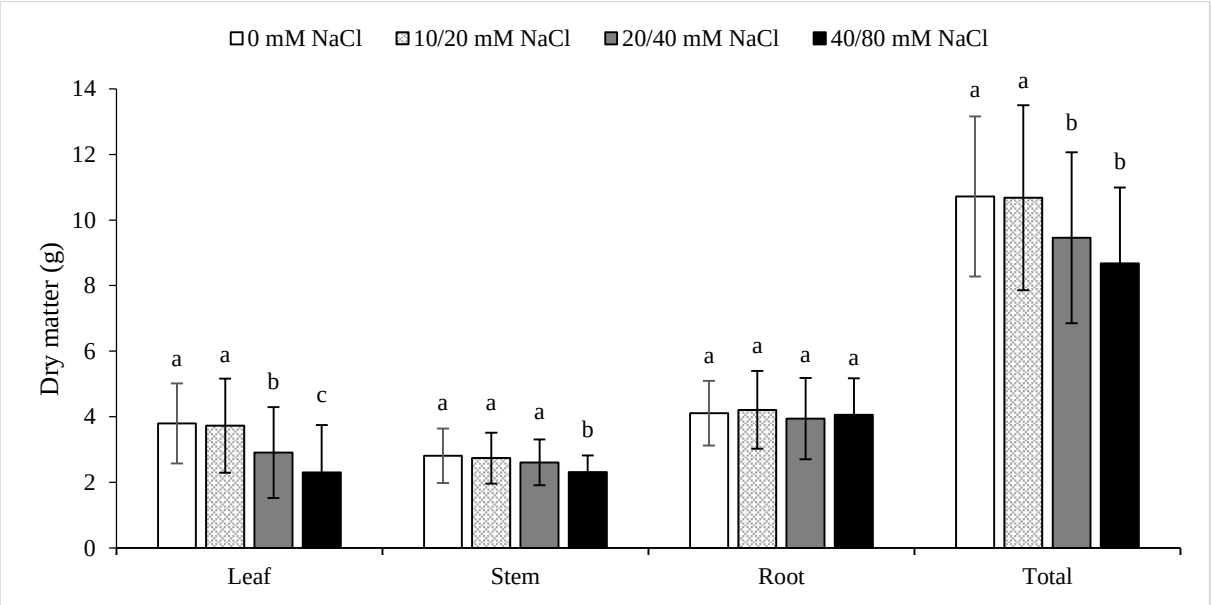

Figure 3. Effects of different NaCl treatments in irrigation solution on DM of different plant parts of guava plants at the end of the experiment. Bars show standard deviation of the mean. Letters indicate significant differences at $p \leq 0.05$.

With regard to the DM content of individual accessions, a reduction in leaf DM was recorded in all the accessions, ranging from 17.6% in ELG009 to 54.5% in HOM016 at the high salinity level compared to the control (**Table S2**). Accessions HOM016, UAG014, and KIL013 had high and significant reductions in their leaf DM of 54.5%, 52%, and 30.1%, respectively. Similar to the leaf DM, all the accessions also reduced in the stem DM content at the high salinity level in comparison to the control. Much of the stem DM loss was observed in accession UAG014 (32.4%), whereas the least was observed in accession ELG009 (4.46%), though both were not significant. However, three accessions—KIL013, KIL014, and VIH004—had 23.3%, 19.8%, and 18.5% reductions in their stem DM contents that were statistically significant. Only one accession, MER011 had a significant and the highest reduction in root DM content of 17.6% at the high salt level compared to the control. In contrast, half of the accessions were observed to increase root DM at the high salt level compared to the control, with the highest gain being observed in accession ELG009 (16.5%), though this was not statistically significant.

At the level of the whole plant, there was a reduction in total DM in all the accessions at the high salinity level, with differences ranging from 0.5% in ELG009 to 32% in HOM013. Only DM reductions in five accessions—HOM016 (26.6%), UAG014 (25.0%), MER011 (21.2%), KIL014 (19.8%), and KIL013 (17.5%)—were observed to be significant (**Table S2**).

Compared to the control, the relative water content in the leaves was significantly reduced by 21% in the high salinity treatments but remained unaffected in the rest of the treatments **(Figure 4)**. In the stems, no significant effect of salinity on the relative water content was observed. The relative water content in the roots increased with rising salinity levels by 16% in the medium NaCl treatments and by 22% in treatments with high NaCl concentration in comparison to the control. The total relative water content for the entire plant reduced significantly only in the high salinity level; it was statistically similar in the other treatments.

The leaf relative water content was similar for all the accessions in the control and medium salt treatments (**Table S3**). However, there was a decrease in the relative water content in all the individual accessions at the high salinity level in comparison to the control, with significant differences noted in accessions KIL013 (50.2%), KIL014 (46.7%), MER011 (39.6%), MER009 (14.4%), HOM016 (13.5%), and ELG009 (7.2%) (**Table S3**). There were no significant differences in the stem relative water content among the accessions and between the control and high salinity treatments. In the majority of the accessions, the relative root water content increased in the 40/80 mM treatments relative to the control with significant increases observed in accessions MER011 (81.6%), MER009 (67.5%), MER014 (39.4%), HOM016 (22.8%), HOM013 (20.8%), and UAG014 (4.6%). Compared to the control, a significant decrease in relative water content was found in accession KIL013

(20.1%), and a significant increase was found in accession HOM013 (4.2%) in the high NaCl level (**Table S3**).

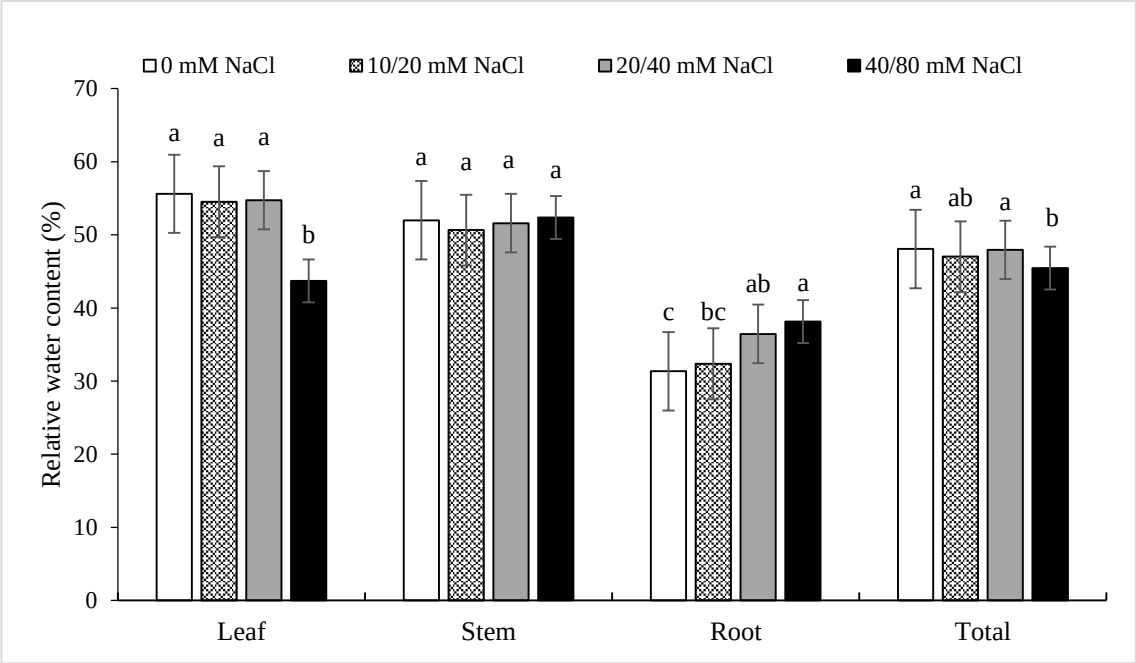

Figure 4. Effects of different NaCl treatments in irrigation solution on relative water content (%) of guava plants at the end of the experiment (n=10 per accessions and treatment). Bars show standard deviation of the mean. Letters indicate significant differences at $p \leq 0.05$.

4.4.3. Mineral analysis

4.4.3.1. Concentration of Na in leaves, stems, and roots

The Na concentration in the leaves, stems, and roots based on 100 mg of the sample are shown in **Table S4.** For all the plant organs, there was a progressive rise in the average Na concentration from the control treatment to the highest salinity level. The Na in the shoot (leaves + stems) was observed to sharply increase relative to the roots, as depicted by the shoot/root ratios. The shoot/root ratio increased from 0.6 in the control to 2.4 and 4.0 in the medium and high salinity levels, respectively. Accordingly, at the plant level, considering the DM—for leaves, roots, and the whole plant—plants in the low and medium salinity levels recorded similar values that were significantly higher than the control but lower than those in the high salinity treatment. The stem Na content differed only at the high salinity level at which the highest value was recorded (**Table S5)**.

In the case of individual accessions, accumulation of Na based on 100 mg of the sample was observed to vary based on plant parts and NaCl treatments (**Table 2**). Interestingly, leaf Na concentration among the accessions varied even in the control prior to the introduction of the treatments. At the low NaCl level, the accessions did not differ in their leaf Na concentration. With a further increase to moderate and high salt stress, significant variations among the accessions were observed. The accessions differentially accumulated Na in their leaves: Accession ELG009 had the highest values at the high salinity level; this was 45 times more than the control. The least accumulation of leaf Na at the high salinity level in comparison to the control was in accession HOM013; here, the accumulation of leaf Na was 16 times higher than the control. These differences between the control and the higher salinity level were significant for all accessions, except MER011. Significant differences among the accessions in stem Na concentration were observed only at the high salinity level. The differences in Na concentration between the control and high salinity level increased from nine-fold in accessions HOM013 and HOM016 to 68-fold in accession KIL014. All stem Na changes between the control and 40/80 mM treatments were significant except for accession KIL013 and MER011. The root Na concentration among the accessions was observed to vary in low and medium salinities but not in the control and high salinity treatments. The changes between the control and high salt level were lower than those observed for leaves and stems—increasing from three-fold to five-fold, and were all significant for all the accessions (**Table 2).**

At the whole plant level, considering plant DM, accession ELG009 still accumulated the highest leaf Na content, and this was 37 times higher in the high salinity treatment than in the control (**Table S6**). The least change was observed in accession HOM016 (seven times higher than the control). The changes between the Na content in the control plants and those under high salt treatments in accessions KIL013, KIL014, MER014, and UAG014 were not

significant. Regarding the stem, two accessions, KIL013 and MER011, did not differ in their Na content between the control and the high salinity level. Considering the root, only accession VIH004 did not have a significant accumulation of Na (**Table S6**). At the whole plant level, there was a significant difference in Na content between the control and 40/80 mM treatments in all the accessions. The Na content increased by four-fold in HOM016 to 11-fold in ELG009 and KIL013 in the 40/80 mM plants compared to the control (**Table S6).**

Table 2. Leaf, stem, and root Na concentration of 10 guava accessions six weeks after initiation of salt treatment (n=5 per accession and treatment)

Plant part	NaCl (mM)	Accession									
		ELG009	**HOM013**	**HOM016**	**KIL013**	**KIL014**	**MER009**	**MER011**	**MER014**	**UAG014**	**VIH004**
Leaf	0	$33.4^{bc} \pm 11.3$	$106.6^{a} \pm 30.0$	$36.1^{bc} \pm 10.7$	$62.7^{abc} \pm 43.9$	$89.9^{ab} \pm 52.6$	$32.8^{bc} \pm 22.5$	$23.5^{c} \pm 11.5$	$21.7^{c} \pm 20.0$	$42.4^{bc} \pm 46.6$	$27.3^{c} \pm 17.0$
	10/20	$384.1^{a} \pm 220.9$	$281.9^{a} \pm 253.6$	$246.8^{a} \pm 148.6$	$514.4^{a} \pm 273.2$	$496.1^{a} \pm 282.8$	$77.5^{a} \pm 76.8$	$441.8^{a} \pm 667.2$	$47.5^{a} \pm 19.7$	$260.9^{a} \pm 236.1$	$122.5^{a} \pm 89.9$
	20/40	$671.1^{ab} \pm 673.8$	$864.4^{ab} \pm 474.5$	$694.6^{ab} \pm 320.7$	$1410.4^{a} \pm 1010.8$	$1067.9^{ab} \pm 359.0$	$337.2^{b} \pm 321.9$	$1049.5^{ab} \pm 557.0$	$197.0^{b} \pm 209.2$	$477.4^{ab} \pm 282.2$	$648.4^{ab} \pm 839.3$
	40/80	$1487.2^{abc} \pm 460.2$	$1669.2^{abc} \pm 802.7$	$1366.2^{abc} \pm 477.3$	$2618.2^{a} \pm 854.9$	$2352.3^{ab} \pm 838.9$	$1118.5^{bc} \pm 486.8$	$1231.2^{bc} \pm 1177.0$	$590.5^{c} \pm 510.1$	$1346.0^{abc} \pm 731.7$	$998.9^{bc} \pm 419.5$
	Fold change*	45	16	38	42	26	34	38	25	32	37
	p-value**	0.001	0.006	0.001	0.004	0.001	0.003	0.054	0.043	0.006	0.002
Stem	0	$34.7^{a} \pm 16.1$	$20.3^{a} \pm 5.7$	$30.9^{a} \pm 22.9$	$25.8^{a} \pm 7.8$	$24.3^{a} \pm 2.7$	$21.1^{a} \pm 4.3$	$25.3^{a} \pm 6.2$	$15.6^{a} \pm 9.0$	$20.4^{a} \pm 7.9$	$18.1^{a} \pm 9.0$
	10/20	$121.3^{a} \pm 42.8$	$67.3^{a} \pm 36.4$	$84.3^{a} \pm 28.3$	$94.8^{a} \pm 28.4$	$124.4^{a} \pm 45.8$	$85.7^{a} \pm 46.6$	$108.4^{a} \pm 112.8$	$80.0^{a} \pm 46.8$	$107.7^{a} \pm 41.6$	$64.3^{a} \pm 33.2$
	20/40	$236.3^{a} \pm 217.6$	$120.7^{a} \pm 47.2$	$259.5^{a} \pm 199.2$	$272.7^{a} \pm 184.8$	$156.4^{a} \pm 134.1$	$207.0^{a} \pm 159.6$	$215.0^{a} \pm 136.7$	$87.5^{a} \pm 42.4$	$133.2^{a} \pm 53.9$	$120.7^{a} \pm 50.3$
	40/80	$499.5^{ab} \pm 300.5$	$173.0^{b} \pm 54.8$	$287.2^{b} \pm 102.8$	$1196.0^{ab} \pm 1252.9$	$1658.6^{a} \pm 1145.6$	$207.3^{b} \pm 105.8$	$720.3^{ab} \pm 560.7$	$237.4^{b} \pm 94.0$	$608.9^{ab} \pm 311.7$	$210.1^{b} \pm 58.0$
	Fold change*	14	9	9	46	68	10	28	15	30	12
	p-value**	0.026	0.003	0.007	0.105	0.033	0.018	0.050	0.006	0.014	0.002
Root	0	$162.8^{a} \pm 57.9$	$151.1^{a} \pm 39.8$	$150.1^{a} \pm 65.1$	$165.3^{a} \pm 56.4$	$159.0^{a} \pm 26.3$	$120.2^{a} \pm 20.4$	$121.7^{a} \pm 29.0$	$107.8^{a} \pm 26.2$	$120.2^{a} \pm 18.8$	$114.1^{a} \pm 18.7$
	10/20	$317.7^{ab} \pm 74.6$	$298.0^{abc} \pm 55.9$	$280.6^{abc} \pm 67.7$	$339.7^{a} \pm 45.2$	$287.1^{abc} \pm 52.9$	$213.1^{c} \pm 19.2$	$259.5^{abc} \pm 29.5$	$218.2^{bc} \pm 27.6$	$301.0^{abc} \pm 59.2$	$255.8^{abc} \pm 28.2$
	20/40	$444.0^{a} \pm 102.7$	$423.3^{ab} \pm 92.0$	$331.5^{ab} \pm 46.7$	$391.3^{ab} \pm 71.9$	$381.2^{ab} \pm 63.5$	$412.2^{ab} \pm 32.4$	$431.8^{ab} \pm 118.2$	$285.7^{b} \pm 65.0$	$444.7^{a} \pm 53.0$	$338.0^{ab} \pm 56.3$
	40/80	$688.3^{a} \pm 81.9$	$474.0^{a} \pm 113.5$	$419.9^{a} \pm 72.1$	$690.2^{a} \pm 258.0$	$678.7^{a} \pm 125.1$	$468.5^{a} \pm 133.1$	$541.1^{a} \pm 107.7$	$431.8^{a} \pm 144.4$	$606.2^{a} \pm 154.8$	$429.2^{a} \pm 106.0$
	Fold change*	4	3	3	4	4	4	4	4	5	4
	p-value**	0.001	0.004	0.001	0.012	0.000	0.001	0.004	0.006	0.002	0.004

Data are expressed as mean values in mg $100g^{-1}$ DW ± standard deviation. Different letters in each row indicate significant differences at $p \leq 0.05$. *Fold change between 0 mM NaCl and 40/80 mM NaCl. ***p*-value between 0 mM and 40 mM treatments according to a paired sample t-test. NaCl treatments were doubled after the first three weeks of the experiment.

4.4.3.2. Macronutrients in leaves (P, K, Ca, Mg, and S)

The results of leaf macronutrient accumulation are presented in **Table 3**. In comparison to the control, leaf K concentrations were significantly reduced by 13% and 14% in the low 10/20 mM NaCl and medium 20/40 mM NaCl treatments, respectively. In the high 40/80 mM NaCl treatment, K concentration did not differ from the control treatment. In relation to leaf Na concentration, leaf K/Na ratio was successively reduced with an increase in salinity. No changes in the Ca concentrations of the guava leaves were observed under conditions of salinity stress. However, similarly to the K/Na ratios, the Ca/Na ratios were reduced significantly in the higher salinity levels.

Leaf P concentration was similar in treatments with salt stress but statistically lower than in the control plants. Changes in leaf Mg concentrations in response to increasing NaCl salinity were significant only in the lower 10/20 mM salinity level, while the medium and high salinity levels were similar to the control plants. Leaf S concentration showed a declining trend with a rising salinity level, accounting for 25%, 33%, and 37% in the low, medium, and high NaCl treatments; hence, the reduction in moderate salt stress was not significantly different from the low and high salt stress.

Table 3. Concentration of K, Ca, P, Mg, and S, and K/Na and Ca/Na ratios in leaves of guava plants as affected by different concentrations of NaCl in irrigation solution (n=60).

NaCl (mM)	K	Ca	P	Mg	S	K/Na ratio	Ca/Na ratio
0	$1941.7^{a} \pm 414.1$	$1460.9^{a} \pm 304.6$	$232.0^{a} \pm 75.0$	$199.6^{a} \pm 38.0$	$277.7^{a} \pm 63.0$	$73.0^{a} \pm 53.7$	$49.2^{a} \pm 29.9$
10/20	$1689.1^{b} \pm 355.2$	$1375.7^{a} \pm 207.8$	$187.2^{b} \pm 38.6$	$180.9^{b} \pm 25.3$	$208.1^{b} \pm 59.6$	$25.1^{b} \pm 32.8$	$17.9^{b} \pm 21.7$
20/40	$1675.5^{b} \pm 396.4$	$1495.0^{a} \pm 229.5$	$176.5^{b} \pm 39.6$	$193.1^{ab} \pm 39.8$	$184.9^{bc} \pm 38.6$	$7.9^{c} \pm 18.3$	$5.9^{c} \pm 12.4$
40/80	$1809.0^{ab} \pm 361.0$	$1482.2^{a} \pm 281.1$	$175.9^{b} \pm 43.4$	$189.5^{ab} \pm 39.9$	$174.2^{c} \pm 30.8$	$1.9^{c} \pm 1.7$	$1.5^{c} \pm 1.2$
Mean (n=240)	1778.8 ± 395.0	1453.5 ± 261.2	192.9 ± 56.0	190.8 ± 36.7	211.2 ± 63.9	27.0 ± 42.9	18.6 ± 26.9

Data are expressed as mean values in mg 100 g^{-1} DW ± standard deviation. Different letters in each column indicate significant differences at $p \leq 0.05$. NaCl treatments were doubled after the first three weeks of the experiment.

4.4.3.3. Micronutrients in leaves (B, Fe, Mn, and Zn)

The results of the micronutrient analysis of the leaf samples are depicted in **Table S7**. B remained the same in the low and medium salinity levels, but was reduced significantly in the high salinity level. A similar trend was observed with respect to Fe concentration. There was no variation in the accumulation of Mn and Zn in leaves, which indicates that they remained unaffected by all the salinity treatments.

4.4.3.4. Correlation among growth traits, DM, and Na content

A positive correlation between the changes in leaf number and that of the whole plant DM was observed (**Table 4**.). A change in leaf DM positively correlated with the change in root DM and DM change of the entire plant, while stem and root DM changes positively correlated with the change in the DM of the whole plant. In addition, leaf DM change was positively correlated with the change in the Na content of the leaf and that of the entire plant but negatively with that of the stem Na content. The change in root DM positively correlated with the change in the Na content of the leaf, root, and that of the entire plant. The entire plant DM change positively correlated with the change in the leaf Na, root Na, and Na content of the entire plant (**Table 4**). The change in leaf Na content positively correlated with that of the root and whole plant, while the change in stem Na content only positively correlated with that of the whole plant.

Table 4. Correlation in the percent changes between the 0 mM and 40/80 mM Na content, DM, and growth parameters of 10 guava accessions from Kenya after six weeks of salt treatment

	Plant height	Leaf number	Leaf DM	Stem DM	Root DM	Plant DM	Leaf Na	Stem Na	Root Na
Leaf number	0.101								
Leaf DM	0.106	0.240							
Stem DM	0.218	-0.004	0.247						
Root DM	-0.118	0.255	0.341*	0.002					
Plant DM	0.055	0.286*	0.817***	0.380**	0.757***				
Leaf Na	-0.042	0.178	0.579***	0.272	0.342*	0.615***			
Stem Na	0.119	-0.018	-0.287*	0.248	0.039	-0.030	0.008		
Root Na	-0.109	0.183	0.167	0.015	0.773***	0.547***	0.281*	0.104	
Plant Na	0.005	0.187	0.442**	0.262	0.565***	0.675***	0.748***	0.410**	0.623***

***Correlation is significant at the $p \leq 0.001$ level. **Correlation is significant at the $p \leq 0.01$ level. *Correlation is significant at the $p \leq 0.05$ level.

4.5. Discussion

The salt injury was evident in all salinity treatments except the control and was more pronounced in the higher salinity level. The symptoms began with slight leaf cupping, followed by leaf chlorosis on the lower older leaves, which progressed to the upper younger leaves. Eventually, in the medium and high salinity treatments, the leaves became necrotic and started to fall off. Thus, the reduced leaf number in the medium and high salinity treatments was due to salt injury in the older leaves which could no longer expand and dilute the salt as was also observed by Munns and Tester (2008). However, the individual accessions were observed to have varying degrees to which the salt could be tolerated, for instance accessions ELG009, MER014 and UAG014 produced more leaves relative to the control, though not significantly. The remaining accessions reduced in leaf number at varying degrees in comparison to the control—indicative of existing genetic differences in salt tolerance as pointed out by Munns and Tester (2008). The salt-related toxicity symptoms observed are also consistent with observations in other experiments involving guava (Ebert et al. 2002; da Silva et al. 2008).

The plant height in the salinity treatments of the present study were not significantly different from those of the unstressed plants. However, variations among accessions were noted: Accessions ELG009, HOM016, KIL014, MER014, and MER011 increased in plant height at the high salt level in comparison to the control, while the other accessions reduced. Several earlier studies involving guava have reported varying effects of salinity on plant height (Ali-Dinar et al. 1999; Cavalcante et al. 2007; da Silva et al. 2008; Singh et al. 2016). These studies described varied observations with regard to the salinity levels. For instance, Cavalcante et al. (2007) reported a decrease in plant height during the initial development of the guava seedlings when irrigated with water with EC of more than 1.5 dS m^{-1}. Singh et al. (2016) observed an increase in the plant height of guava seedlings up to an EC of 1.4 dS m^{-}

^{1} relative to the control but with a decrease at an EC above 2.0 dS m^{-1} that was comparable to the control. The differences relative to these growth parameters in the different studies could be due to the duration of exposure to salinity, growing conditions, plant age, and genotype, as was also reported by Maas (1993).

Leaf DM was significantly reduced in the medium 20/40 and high 40/80 mM NaCl treatments, whereas DM in stems was significantly reduced only in the high salinity treatments. Root DM was not significantly affected by all salinity levels. However, the accessions were observed to have varied responses with regard to leaf, stem, and root DM at the various salinities. Ali-Dinar et al. (1998) reported a reduction in the DM production of leaves and stems with increasing salinity, even as root DM increased. However, da Silva et al. (2008) observed that salinity reduced DW in roots but increased DW in leaves, and therefore suggested that the roots were more affected by salinity than the shoot. These different findings may be due to the varying longevity of the experiments, as they were set up for 200 days (da Silva et al. 2008) and 12 weeks (Ali-Dinar et al. 1998), but they could also be due to genetic differences, as observed in the 10 accessions of the present study. Accession ELG009 was able to maintain and gain DM for leaf and root at the low, medium, and high salinities, and lost the least DM at the total plant level. Accession HOM013, on the other hand, lost the highest DM at the whole plant level. The decrease in leaf DM in the 20/40 mM and 40/80 mM NaCl treatments in this study could be attributed to the reduction of leaf area and the reduced number of leaves. Decreased DM production under saline conditions is attributed to a higher expenditure of metabolic energy and decreased carbon gain, along with the adaption to salinity, as observed by Netondo et al. (2004) and reflected in the reduction of leaf DM in the present study.

The relative water content of the leaves was significantly lower only at the high salinity level compared to the control. The stem relative water content remained similar in

all the treatments while that of the roots increased with increasing salinity. The decrease of leaf water content at the high salinity level and the observed cupping of leaves in this study may be considered a response to water stress (Koller 1990) due to the decreased osmotic potential caused by salinity in the root zone (Munns 2002). Owing to increased osmotic potentials in the root zone, water availability reduces for plants under salinity. Therefore, water uptake and turgor are reduced, whereby subsequently the stomata are closed. This, in turn, leads to decreased transpiration and photosynthesis (Mastrogiannidou et al. 2016). The reduced water uptake by the plant is likely to reduce the overall water content of plant leaves and stems. The stem is a conduit for water and food transport to the leaves in plants; therefore, the effect of reduced water content is expected to be less felt compared to the leaves. Thus, similar values are observed in the stem water content for all the salinities. On the other hand, an increase in the root water content was observed with a rise in the salinity level in most accessions under the current study. Such an increase in relative water content as a result of salinity in some plants has been attributed to compensate the possible morphological changes to salinity (Saied et al. 2003). Additionally, the increased succulence in some plants could be a morphological feature to avoid excessive ion concentration in their tissues as observed by Larcher (2003). Moreover, at the high 40/80 mM NaCl treatment, the water content in the roots increased significantly while the leaves experienced a significant reduction, which might indicate either that water transportation to the leaves was hindered due to reduced transpiration, and/or that the leaf injury was already severe, resulting in reduced leaf area, as was also reported by da Silva et al. (2008).

The leaf Na content varied in all treatments for all the 10 genetically diverse accessions, except in the low NaCl treatment. Variations among accessions in the control prior to the introduction of treatments supplemented with NaCl could be attributed to the fact that the Hoagland solution contained salts (e.g. sodium molybdate) which were

differentially accumulated, already indicating genotypic variation in salt uptake among the guava accessions. The similarity of Na uptake at the low salinity level is likely to be due to low concentrations of salt in the solution that did not significantly affect plant uptake by altering the osmotic potential (Munns and Tester 2008). However, with a further increase in salinity, differences in the accumulation of Na in leaves emerged among the accessions which could be attributed to their genotypic differences. There was no differential accumulation of Na in the stem until the high 40/80 mM NaCl treatment in all accessions. This coincided with the time in which there was severe leaf drop, and therefore, much of the Na remained in the stem and could not leave the transpiration stream. In contrast, the root Na concentration varied at the lower and medium salinities but was similar for all accessions at the highest salinity level. It has been proposed that Na in roots might accumulate up to a certain saturation level, and when this is exceeded, leaf Na content increases (Esechie and Rodriguez 1998). Guava was inefficient at excluding Na from the transpiration stream, as greater amounts of Na were accumulated in the leaves with increasing salinity (Munns 1993). At the high salinity level, the root Na increased possibly due to reduced transpiration as a result of reduced leaf number observed from the severe leaf drop.

Accession ELG009 (from the Rift Valley region) showed a tendency towards higher Na accumulation while accession HOM016 (from the Western region) accumulated the least Na based on the leaf, stem, root, and entire plant DM. Nevertheless, these accessions and all the others in this study accumulated significant amounts of Na at the high salinity level compared to the control based on whole plant DM. The positive correlations observed between leaf and root DM with the Na content of the plant seems to play a role in ameliorating the effects of Na toxicity within the plant—as salinity tolerance is usually determined by the percentage of biomass production in saline versus control conditions over a period of time (Martin et al. 1994). Accessions such as ELG009, KIL013, MER009 and

MER014 were able to maintain a higher whole plant DM despite also accumulating appreciable Na content at the high salinity level compared to the control—indicative of their higher degree of salinity tolerance compared to the other accessions.

Salinity is also associated with the plant's ability to maintain balanced K levels under saline conditions (Blumwald et al. 2000). K is an important osmoticum which is antagonistic to Na, and it is generally believed that K is replaced by Na in plant tissues with an increase in the NaCl levels (Kozlowski 1997; Marschner 2012). The leaf K/Na ratios in the present study sharply reduced with rising salinity levels. Notably, the leaf K content decreased slightly in relation to the control, though the decrease was not statistically significant. Therefore, the observed decrease in the K/Na ratio in this study is mainly attributed to the severe accumulation of Na rather than to the replacement of K, as was also suggested by Negrão et al. (2017). Similarly, a lower K/Na ratio was observed in guava by Ebert et al. (2002) in the control treatment in relation to treatments ameliorated by the application of calcium nitrate ($Ca[NO_3]_2$). Accordingly, Ebert et al. (2002) did not observe replacement of K by Na in guava seedlings treated with ($Ca[NO_3]_2$) under saline conditions. This may also indicate that K was available under salt stress. According to He and Cramer (1993), changes in K/Na ratios with rising salinity levels do not interact with K-Na selectivity; therefore, neither of them was found to be correlated with salt resistance and may not represent a reliable criterion for the selection of tolerant species. Similar observations were made for Ca/Na ratios during the experiment, with the Ca content remaining unaffected by salinity in the experiment, even though the Ca/Na ratio decreased with increasing salinity. When exposed to Na, plants were found to respond directly and specifically to the enhancement of cytosolic Ca (Munns and Tester 2008). Ca has been reported to ameliorate the performance of plants under saline conditions as it can enhance membrane selectivity for K (Ebert et al. 2002; Marschner 2012) and may prevent toxic ion accumulations in cells (Cramer et al.

1987). Therefore, there seems to be a threshold level for Ca to ameliorate salinity effects in guava plants. Ebert et al. (2002) observed enhanced K/Na and Ca/Na ratios with application of 10 mM ($Ca[NO_3]_2$).

Relatively little is known about the effect of NaCl salinity on Mg, P, and S accumulation in plants, including guava. In the present experiment, salinity-induced changes in leaf Mg content slightly decreased relative to the control; however, the medium and high salinity levels were statistically similar to the control. In contrast, Makhija et al. (1980) reported that Mg levels in the leaves of guava seedlings increased with rising salinity levels. Mastrogiannidou et al. (2016), however, found a negative correlation between salinity and leaf Mg content in pomegranate when provided with half-strength Hoagland solution. Further investigation to ascertain the effect of salinity on Mg uptake in guava is therefore required. The P content showed a tendency to decrease with increasing salinity in the present study. Similarly, Makhija et al. (1980) reported significant reductions of P concentrations in guava. According to Grattan and Grieve (1998), the effects of salinity on P uptake vary within plant species and according to experimental conditions, such as in the developmental stage of plants, type, and level of salinity, as well as the P regime. Reduced P availability under saline conditions may not only be a result of ionic-strength effects that lead to decreased availability of P, but can also be associated with desorption-dissolution reactions which control P release from the soil (Grattan and Grieve 1998). With regard to leaf S content, to the knowledge of the authors of this paper, there is no report on the effects of salinity on S accumulation in the leaves of guava plants. The study found that S content was significantly decreased with rising salinity levels. In other studies, reduced S content was also observed in the leaves of annuals, such as tomato plants (Balliu et al. 2015), maize (Ausma et al. 2017), and pea (Mor and Manchanda 1992), when exposed to NaCl salinity. Ausma et al. (2017) ascribed the measured decrease of S in maize to reduced sulphate

content. Mor and Manchanda (1992) suggested that reduced foliage S content may result from a hampered S translocation caused by chloride (Cl) at the root–shoot interface. Sulphate is assimilated in the roots by a proton gradient and transported to the chloroplasts in xylem vessels via a transpiration stream (Nazar et al. 2011). Therefore, the observed decrease in leaf S content with rising salinity levels in the present experiment may be attributed to both reduced translocation of S caused by Cl at the root–shoot interface and reduced transportation due to reduced evapotranspiration as a result of reduced leaf number and possibly stomatal closure.

Currently, there is no report on the effect of NaCl-induced salinity on the micronutrient composition of guava. In this study, relatively uniform B, Fe, Mn, and Zn contents were observed, with B and Fe being statistically lower only at the high salinity level. Grattan and Grieve (1998) observed that the solubility of Fe and Zn decreases further with increasing salinity in tomato. However, other observations have been made in different plants. For instance, Fe, Mn, and Zn concentrations increased in tomato and soybean under salinity (Balliu et al. 2015). Hu and Schmidhalter (2001) found that the effect of salinity on the foliage B, Fe, Mn, and Zn content of wheat were complex and depended on the levels of macronutrients and salinity. Therefore, further investigation on the effect of NaCl-induced salinity on the micronutrient content of guava is necessary.

4.6. Conclusion

All 10 accessions in the present study exhibited salt injury symptoms at varying levels, and this was severe in the high salinity level. The accessions had varied responses with regard to plant height and leaf number, showing varying degrees of tolerance. A reduction in leaf DM also varied among the accessions although significant reductions were observed in the medium and high salinity levels, while the leaf water content in the high salinity level reduced significantly. Root DM remained stable under all salinity treatments

with root water content rising with increasing salinity levels; hence, water transport to the leaves may have been hindered by reduced transpiration as a result of reduced leaf number. The accessions varied in their DM production at different salinity levels relative to the control, with accession ELG009 maintaining more DM. A reduction in leaf chlorophyll content was observed, suggesting that the chlorophyll formation process was inhibited by NaCl. A similarity in root Na concentration at the high salinity level in all the accessions indicates that the lethality level was reached regardless of the genetic differences of the accessions. Overall, a decrease in the K/Na and Ca/Na ratios was observed; however, similar amounts of K and Ca were still maintained in the leaves at all salinity levels, which was indicative of the accumulation of Na rather than the replacement of K and Ca by Na. The P and S levels reduced with rising salinity, while the B and Fe concentrations reduced only significantly at the high salinity level. Guava was not effective at excluding Na from the transpiration stream as high amounts of Na were accumulated in leaves with increasing salinity until 40 mM NaCl, which is when the plants began to die. However, accessions which were able to maintain more leaf number, plant height, and biomass such as ELG009, MER009, and MER014 could be targeted for adaptation to saline environments at salinity levels below 40/80 mM.

4.7. Supplementary information

Table S1. Geographical coordinates and characteristics of guava accessions used for the salinity experiment.

No.	Accession name	Region	Latitude [N°/S°]	Longitude [E°]	Elevation [m]	Criteria for selection	Flesh colour
1	KIL013	Coast	03.91339 °S	039.74015 °E	18	Highest exocarp thickness	Red
2	KIL014	Coast	03.91347 °S	039.73988 °E	20	Highest Ca levels (36.5 mg 100 g^{-1}	Red
3	MER009	Eastern	00.08721 °S	037.66675 °E	1,455	Very salty fruits	Red
4	MER014	Eastern	00.11461 °S	037.69637 °E	1,384	Highest pulp weight	White
5	MER011	Eastern	00.08583 °S	037.66500 °E	1,474	Highly differentiated based on genetic clustering	White
6	ELG009	Rift Valley	00.64338 °N	035.51852 °E	2,102	Unique roundish leaves; Hidden fruits; High insect infestation; Fleshy fruits with few but heavy seeds	Red
7	UAG014	Rift Valley	00.57152 °N	035.30377 °E	2,152	Highly differentiated based on genetic clustering	Red
8	VIH004	Western	0.084470 °N	034.79931 °E	1,683	Largest tree ever collected; DBH* 45.3 cm	Red
9	HOM013	Western	00.60974 °N	034.58366 °E	1,335	Highly differentiated based on genetic clustering	Red
10	HOM016	Western	00.60984 °N	034.58377 °E	1,336	Most seedy	Red

*DBH = Diameter at breast height

Table S2. Dry matter of the leaf, stem, root and whole plant of ten guava accessions from Kenya after six weeks of salt treatment (n=10 per accession and treatment).

		Accessions										
Plant part	**NaCl (mM)**	ELG009	HOM013	HOM016	KIL013	KIL014	MER009	MER011	MER014	UAG014	VIH004	Mean (n=100)
Leaves	0	$3.23^{bcd} \pm 0.60$	$4.63^{a} \pm 1.17$	$3.52^{abcd} \pm 0.96$	$4.88^{a} \pm 0.64$	$3.97^{abcd} \pm 1.32$	$3.01^{cd} \pm 1.23$	$2.80^{d} \pm 0.68$	$3.63^{abcd} \pm 1.21$	$3.94^{abc} \pm 1.46$	$4.37^{abc} \pm 1.10$	3.80 ± 1.22
	10/20	$3.27^{bc} \pm 1.82$	$5.15^{a} \pm 1.05$	$4.07^{abc} \pm 1.05$	$4.63^{ab} \pm 1.12$	$4.29^{abc} \pm 0.93$	$2.59^{c} \pm 1.31$	$2.63^{c} \pm 1.18$	$3.42^{abc} \pm 1.43$	$3.90^{abc} \pm 1.16$	$3.37^{abc} \pm 1.29$	3.73 ± 1.44
	20/40	$3.70^{a} \pm 1.18$	$3.41^{a} \pm 1.85$	$2.79^{a} \pm 1.40$	$2.93^{a} \pm 1.86$	$3.53^{a} \pm 1.46$	$2.59^{a} \pm 1.11$	$1.88^{a} \pm 0.54$	$2.76^{a} \pm 1.43$	$2.95^{a} \pm 1.27$	$2.57^{a} \pm 0.79$	2.91 ± 1.38
	40/80	$2.66^{a} \pm 0.83$	$2.23^{a} \pm 0.94$	$1.60^{a} \pm 1.27$	$3.41^{a} \pm 2.43$	$2.02^{a} \pm 2.04$	$2.00^{a} \pm 0.87$	$1.95^{a} \pm 1.34$	$2.59^{a} \pm 1.12$	$1.89^{a} \pm 1.12$	$2.83^{a} \pm 1.40$	2.32 ± 1.45
	% change*	-17.6	-51.8	-54.5	-30.1	-49.1	-33.6	-30.4	-28.7	-52.0	-35.2	-38.9
	p-value**	0.482	0.120	0.020	0.034	0.089	0.123	0.068	0.084	0.000	0.110	
Stems	0	$2.69^{ab} \pm 0.69$	$3.31^{a} \pm 0.64$	$2.47^{ab} \pm 0.73$	$3.35^{a} \pm 0.70$	$3.29^{a} \pm 1.01$	$2.44^{ab} \pm 0.35$	$2.09^{b} \pm 0.51$	$2.46^{ab} \pm 0.52$	$3.18^{a} \pm 1.10$	$2.87^{ab} \pm 0.88$	2.81 ± 0.83
	10/20	$2.70^{ab} \pm 0.72$	$3.40^{a} \pm 0.75$	$2.41^{ab} \pm 0.91$	$3.10^{ab} \pm 0.56$	$3.15^{ab} \pm 0.44$	$2.32^{b} \pm 0.73$	$2.44^{ab} \pm 0.68$	2.75^{a}b ± 0.95	$2.56^{ab} \pm 0.66$	$2.52^{ab} \pm 0.73$	2.73 ± 0.78
	20/40	$2.85^{a} \pm 0.77$	$2.62^{a} \pm 0.87$	$2.44^{a} \pm 0.49$	$2.74^{a} \pm 0.69$	$3.06^{a} \pm 0.80$	$2.58^{a} \pm 0.68$	$2.20^{a} \pm 0.41$	$2.33^{a} \pm 0.74$	$2.96^{a} \pm 0.66$	$2.31^{a} \pm 0.45$	2.61 ± 0.70
	40/80	$2.57^{a} \pm 0.36$	$2.34^{ab} \pm 0.61$	$2.05^{ab} \pm 0.43$	$2.57^{a} \pm 0.29$	$2.64^{a} \pm 0.62$	$2.30^{ab} \pm 0.56$	$1.76^{b} \pm 0.22$	$2.33^{ab} \pm 0.38$	$2.15^{ab} \pm 0.58$	$2.34^{ab} \pm 0.46$	2.31 ± 0.51
	% change*	-4.46	-29.3	-17.0	-23.3	-19.8	-5.74	-15.8	-5.3	-32.4	-18.5	-17.8
	p-value*	0.183	0.111	0.054	0.021	0.039	0.116	0.096	0.092	0.065	0.005	
Roots	0	$3.89^{a} \pm 1.05$	$4.29^{a} \pm 0.80$	$4.10^{a} \pm 0.84$	$4.35^{a} \pm 0.76$	$4.68^{a} \pm 0.64$	$3.97^{a} \pm 1.13$	$3.47^{a} \pm 0.98$	$4.35^{a} \pm 1.03$	$3.95^{a} \pm 1.14$	$4.09^{a} \pm 1.23$	4.11 ± 0.98
	10/20	$4.32^{a} \pm 1.00$	$4.70^{a} \pm 1.02$	$3.88^{a} \pm 1.07$	$4.94^{a} \pm 0.60$	$4.24^{a} \pm 0.60$	$3.91^{a} \pm 1.25$	$3.62^{a} \pm 1.51$	$4.25^{a} \pm 1.68$	$4.33^{a} \pm 1.36$	$3.89^{a} \pm 1.21$	4.21 ± 1.19
	20/40	$4.83^{a} \pm 1.22$	$4.22^{a} \pm 1.37$	$3.72^{a} \pm 1.26$	$4.08^{a} \pm 0.94$	$4.46^{a} \pm 1.29$	$3.60^{a} \pm 1.54$	$3.46^{a} \pm 0.97$	$3.82^{a} \pm 1.57$	$3.90^{a} \pm 0.66$	$3.27^{a} \pm 0.98$	3.94 ± 1.24
	40/80	$4.53^{a} \pm 1.17$	$3.72^{ab} \pm 1.02$	$3.75^{ab} \pm 0.94$	$4.40^{a} \pm 0.73$	$4.88^{a} \pm 0.76$	$3.76^{ab} \pm 0.83$	$2.86^{b} \pm 0.81$	$4.01^{ab} \pm 1.06$	$4.29^{ab} \pm 1.07$	$4.36^{a} \pm 1.57$	4.06 ± 1.11
	% change*	16.5	-13.3	-8.54	1.15	4.27	-5.29	-17.6	-7.82	8.61	6.60	-1.22
	p-value**	0.176	0.967	0.794	0.919	0.224	0.136	0.045	0.433	0.669	0.861	
Whole plant	0	$9.81^{abc} \pm 1.69$	$12.2^{ab} \pm 2.27$	$10.1^{abc} \pm 1.52$	$12.6^{a} \pm 1.35$	$11.9^{ab} \pm 2.54$	$9.41^{bc} \pm 2.43$	$8.35^{c} \pm 1.58$	$10.4^{abc} \pm 2.10$	$11.1^{abc} \pm 2.90$	$11.3^{abc} \pm 2.77$	10.7 ± 2.44
	10/20	$10.3^{ab} \pm 2.96$	$13.3^{a} \pm 1.65$	$10.4^{ab} \pm 2.55$	$12.7^{a} \pm 1.59$	$11.7^{ab} \pm 1.19$	$8.83^{b} \pm 2.80$	$8.70^{b} \pm 2.39$	$10.4^{ab} \pm 3.79$	$10.8^{ab} \pm 2.78$	$9.79^{ab} \pm 2.74$	10.7 ± 2.82
	20/40	$11.4^{a} \pm 1.87$	$10.2^{ab} \pm 3.67$	$8.95^{ab} \pm 2.12$	$9.75^{ab} \pm 2.51$	$11.0^{ab} \pm 2.86$	$8.77^{ab} \pm 2.66$	$7.54^{b} \pm 1.19$	$8.91^{ab} \pm 2.95$	$9.81^{ab} \pm 1.87$	$8.15^{ab} \pm 1.75$	9.46 ± 2.60
	40/80	$9.76^{a} \pm 1.83$	$8.30^{ab} \pm 1.85$	$7.41^{ab} \pm 1.38$	$10.4^{a} \pm 2.37$	$9.54^{ab} \pm 2.47$	$8.05^{ab} \pm 1.98$	$6.58^{b} \pm 1.99$	$8.92^{ab} \pm 2.22$	$8.33^{ab} \pm 2.02$	$9.53^{ab} \pm 2.81$	8.68 ± 2.31
	% change*	-0.51	-32.0	-26.6	-17.5	-19.8	-14.5	-21.2	-14.2	-25.0	-15.7	-18.9
	p-value**	0.834	0.093	0.023	0.030	0.023	0.098	0.028	0.120	0.042	0.228	

Data are expressed as mean values in grams ± standard deviation. Different letters in each row indicate significant differences at $p \leq 0.05$. *Mean percent change between 0 mM NaCl and 40/80 mM NaCl treatments. ***p*-value between 0 mM and 40 mM treatments according to a paired sample t-test. NaCl treatments were doubled after the first three weeks of the experiment.

Table S3. Relative water content of the leaf, stem, root and whole plant of ten guava accessions from Kenya after six weeks of salt treatment (n=10 accessions per treatment).

Plant part	NaCl (mM)	Accession									
		ELG009	HOM013	HOM016	KIL013	KIL014	MER009	MER011	MER014	UAG014	VIH004
Leaves	0	56.3^{a} ± 5.12	52.4^{a} ± 6.43	55.8^{a} ± 3.71	54.8^{a} ± 3.51	55.5^{a} ± 1.64	58.7^{a} ± 3.23	57.5^{a} ± 8.93	53.2^{a} ± 16.3	57.1^{a} ± 8.36	54.7^{a} ± 4.80
	10/20	57.0^{a} ± 7.77	53.8^{ab} ± 3.60	52.7^{ab} ± 3.80	55.8^{ab} ± 2.50	55.9^{ab} ± 5.03	54.1^{ab} ± 5.41	53.4^{ab} ± 2.97	50.1^{b} ± 5.05	55.5^{ab} ± 2.80	56.9^{a} ± 4.33
	20/40	55.9^{a} ± 2.50	53.4^{a} ± 14.2	54.7^{a} ± 3.90	51.6^{a} ± 17.0	54.6^{a} ± 12.2	54.8^{a} ± 4.56	55.2^{a} ± 4.04	55.0^{a} ± 3.59	55.0^{a} ± 3.21	57.0^{a} ± 3.72
	40/80	52.3^{a} ± 4.35	46.9^{abc} ± 11.0	48.3^{abc} ± 11.9	27.3^{c} ± 19.7	29.6^{b}c ± 19.1	50.3^{ab} ± 11.4	34.7^{abc} ± 22.3	50.2^{ab} ± 9.72	48.1^{abc} ± 13.3	49.6^{ab} ± 15.4
	% change*	-7.2	-10.4	-13.5	-50.2	-46.7	-14.4	-39.6	-5.6	-15.8	-9.4
	p-value**	0.019	0.521	0.008	0.049	0.002	0.040	0.019	0.627	0.150	0.087
Stems	0	53.3^{a} ± 4.58	47.8^{a} ± 9.06	54.6^{a} ± 14.8	54.2^{a} ± 8.64	57.6^{a} ± 9.54	46.4^{a} ± 11.6	49.2^{a} ± 10.8	54.8^{a} ± 10.2	50.0^{a} ± 12.5	51.9^{a} ± 6.49
	10/20	52.9^{a} ± 4.89	47.5^{a} ± 12.8	51.7^{a} ± 18.4	53.9^{a} ± 2.99	49.8^{a} ± 5.78	53.2^{a} ± 9.95	46.6^{a} ± 9.81	44.9^{a} ± 5.23	52.7^{a} ± 9.24	53.0^{a} ± 6.44
	20/40	50.9^{a} ± 2.70	51.9^{a} ± 8.04	48.8^{a} ± 6.38	54.5^{a} ± 4.38	55.8^{a} ± 7.14	48.3^{a} ± 9.23	46.5^{a} ± 13.3	53.6^{a} ± 4.93	53.7^{a} ± 7.90	52.0^{a} ± 8.80
	40/80	52.6^{a} ± 6.69	51.7^{a} ± 7.82	50.9^{a} ± 10.0	54.2^{a} ± 9.05	58.8^{a} ± 8.46	47.6^{a} ± 17.0	54.1^{a} ± 5.22	52.2^{a} ± 12.1	51.3^{a} ± 6.35	50.4^{a} ± 13.1
	% change*	-1.3	8.2	-6.7	-0.1	2.2	2.6	9.9	-4.9	2.7	-2.9
	p-value**	0.584	0.913	0.900	0.654	0.589	0.237	0.060	0.438	0.646	0.854
Roots	0	33.6^{ab} ± 13.9	34.8^{ab} ± 10.8	30.1^{ab} ± 9.23	40.4^{a} ± 12.5	34.4^{ab} ± 10.0	23.1^{b} ± 8.51	22.9^{b} ± 14.3	27.7^{ab} ± 12.4	32.9^{ab} ± 8.98	33.6^{ab} ± 13.8
	10/20	29.4^{a} ± 10.3	38.7^{a} ± 10.7	35.6^{a} ± 12.9	33.1^{a} ± 10.2	31.9^{a} ± 11.4	27.9^{a} ± 9.51	31.6^{a} ± 15.9	28.1^{a} ± 15.3	34.2^{a} ± 7.66	33.1^{a} ± 12.1
	20/40	35.7^{a} ± 13.2	35.2^{a} ± 12.5	35.7^{a} ± 16.3	38.2^{a} ± 13.4	40.7^{a} ± 13.8	33.7^{a} ± 25.8	30.7^{a} ± 17.2	33.7^{a} ± 26.0	42.0^{a} ± 18.8	38.8^{a} ± 10.8
	40/80	38.3^{a} ± 18.7	42.1^{a} ± 12.0	37.0^{a} ± 5.87	38.0^{a} ± 10.2	39.3^{a} ± 8.76	38.7^{a} ± 14.5	41.6^{a} ± 7.84	38.6^{a} ± 7.88	34.5^{a} ± 5.60	33.5^{a} ± 14.3
	% change*	14.1	20.8	22.8	-5.9	14.3	67.5	81.6	39.4	4.6	-0.2
	p-value**	0.274	0.034	0.041	0.603	0.120	0.002	0.006	0.006	0.003	0.361
Whole plant	0	47.7^{a} ± 4.83	45.0^{a} ± 3.47	46.8^{a} ± 5.72	49.8^{a} ± 3.37	49.2^{a} ± 5.48	42.7^{a} ± 4.80	43.2^{a} ± 7.23	45.2^{a} ± 5.84	46.7^{a} ± 5.97	46.7^{a} ± 4.21
	10/20	46.4^{a} ± 4.56	46.7^{a} ± 5.96	46.7^{a} ± 6.62	47.6^{a} ± 2.49	45.9^{a} ± 5.86	45.1^{a} ± 6.31	43.9^{a} ± 7.26	41.0^{a} ± 5.25	47.5^{a} ± 3.11	47.7^{a} ± 3.68
	20/40	47.5^{a} ± 4.82	46.8^{a} ± 6.75	46.4^{a} ± 6.76	48.1^{a} ± 6.08	50.4^{a} ± 4.63	45.6^{a} ± 9.49	44.1^{a} ± 6.73	47.4^{a} ± 8.62	50.2^{a} ± 6.53	49.3^{a} ± 5.00
	40/80	47.7^{a} ± 7.04	46.9^{a} ± 3.84	45.4^{a} ± 5.56	39.8^{a} ± 7.34	42.6^{a} ± 7.43	45.5^{a} ± 8.76	43.5^{a} ± 7.10	47.0^{a} ± 6.78	44.6^{a} ± 4.73	44.5^{a} ± 7.43
	% change*	0.0	4.2	-3.1	-20.1	-13.4	6.5	0.6	3.9	-4.4	-4.8
	p-value**	0.314	0.001	0.891	0.037	0.132	0.118	0.445	0.136	0.702	0.527

Data are expressed as percent ± standard deviation. Different letters in each row indicate significant differences at $p \leq 0.05$. *Mean percent change between 0 mM NaCl and 40/80 mM NaCl treatments. ***p*-value between 0 mM and 40 mM treatments according to a paired sample t-test. NaCl treatments were doubled after the first three weeks of the experiment.

Table S4. Concentration of Na in different plant parts and shoot/root ratio of guava seedlings as affected by different levels of NaCl in the irrigation solution six weeks after salt treatment.

NaCl (mM)	Leaves (n = 60)	Stems (n = 50)	Roots (n = 50)	Shoot/Root (n = 50)
0	$47.6^{d} \pm 39.7$	$23.7^{b} \pm 11.2$	$137.2^{d} \pm 41.7$	$0.56^{c} \pm 0.35$
10/20	$316.0^{c} \pm 377.0$	$108.5^{b} \pm 98.4$	$277.1^{c} \pm 59.4$	$1.56^{bc} \pm 1.44$
20/40	$713.1^{b} \pm 607.7$	$166.2^{b} \pm 121$	$388.4^{b} \pm 85$	$2.42^{b} \pm 2.03$
40/80	$1477.8^{a} \pm 879.1$	$579.8^{a} \pm 711$	$542.8^{a} \pm 166$	$3.97^{a} \pm 2.92$
Mean	638.6 ± 780.6	219.6 ± 420	336.4 ± 179.1	2.13 ± 2.29

Data are expressed as mean values in mg 100 g^{-1} DM ± standard deviation. Different letters in each column indicate significant differences at $p \leq 0.05$. NaCl treatments were doubled after the first three weeks of the experiment.

Table S5. Accumulation of Na in different plant parts and total Na per plant DM of guava seedlings as affected by different levels of NaCl after six weeks of salt treatment.

NaCl (mM)	Leaves (n = 50)	Stems (n = 50)	Roots (n = 50)	Whole plant (n = 50)
0	$2.09^{\mathbf{c}} \pm 1.87$	$0.72^{\mathbf{b}} \pm 0.41$	$5.74^{\mathbf{c}} \pm 2.11$	$8.55^{\mathbf{c}} \pm 8.55$
10/20	$13.1^{\mathbf{b}} \pm 16.4$	$3.32^{\mathbf{b}} \pm 3.17$	$12.1^{\mathbf{b}} \pm 4.75$	$28.5^{\mathbf{b}} \pm 28.5$
20/40	$19.3^{\mathbf{b}} \pm 15.3$	$4.30^{\mathbf{b}} \pm 3.25$	$15.5^{\mathbf{b}} \pm 6.07$	$39.1^{\mathbf{b}} \pm 39.1$
40/80	$30.7^{\mathbf{a}} \pm 28.1$	$13.5^{\mathbf{a}} \pm 17.9$	$22.5^{\mathbf{a}} \pm 10.7$	$66.7^{\mathbf{a}} \pm 66.7$

Data are expressed as mean values in mg per dry weight ± standard deviation. Different letters in each column indicate significant differences at $p \leq 0.05$. NaCl treatments were doubled after the first three weeks of the experiment.

Table S6. Total Na content per plant part (leaf, stem and root), and for the whole plant DM of ten guava accessions from Kenya after six weeks of salt treatment (n=5 per accession and treatment).

		Accession									
Plant part	NaCl (mM)	ELG009	HOM013	HOM016	KIL013	KIL014	MER014	MER009	MER011	UAG014	VIH004
Leaves	0	$1.18^{ab} \pm 0.36$	$4.27^{a} \pm 1.67$	$1.67^{ab} \pm 0.79$	$2.73^{ab} \pm 1.82$	$4.30^{a} \pm 1.08$	$1.09^{ab} \pm 0.99$	$1.38^{ab} \pm 1.27$	$0.58^{b} \pm 0.26$	$2.51^{ab} \pm 3.41$	$1.18^{ab} \pm 0.89$
	10/20	$17.3^{a} \pm 18.7$	$14.5^{a} \pm 14.7$	$7.88^{a} \pm 1.99$	$30.0^{a} \pm 15.9$	$23.0^{a} \pm 13.8$	$2.23^{a} \pm 1.25$	$3.55^{a} \pm 4.41$	$20.5^{a} \pm 34.4$	$9.11^{a} \pm 7.51$	$2.82^{a} \pm 2.74$
	20/40	$13.6^{bc} \pm 12.7$	$19.0^{bc} \pm 11.1$	$24.6^{abc} \pm 12.0$	$29.5^{ab} \pm 16.7$	$44.5^{a} \pm 15.3$	$4.49^{c} \pm 3.24$	$11.1^{bc} \pm 7.04$	$21.8a^{bc} \pm 6.73$	$11.3^{bc} \pm 7.90$	$12.8^{bc} \pm 15.7$
	40/80	$43.4^{a} \pm 26.0$	$44.6^{a} \pm 28.8$	$12.1^{a} \pm 8.37$	$58.0^{a} \pm 45.6$	$38.7^{a} \pm 34.8$	$19.6^{a} \pm 23.1$	$20.2^{a} \pm 10.3$	$11.9^{a} \pm 6.60$	$32.2^{a} \pm 35.6$	$26.8^{a} \pm 14.5$
	Fold change*	37	10	7	21	9	18	15	21	13	23
	p-value**	0.023	0.040	0.038	0.053	0.096	0.153	0.018	0.019	0.111	0.015
Stems	0	$1.15^{a} \pm 0.66$	$0.61^{a} \pm 0.26$	$0.83^{a} \pm 0.71$	$0.87^{a} \pm 0.23$	$0.86^{a} \pm 0.20$	$0.42^{a} \pm 0.28$	$0.56^{a} \pm 0.17$	$0.58^{a} \pm 0.25$	$0.76^{a} \pm 0.40$	$0.55^{a} \pm 0.27$
	10/20	$7.09^{a} \pm 6.29$	$2.38^{a} \pm 1.69$	$2.30^{a} \pm 0.79$	$3.17^{a} \pm 1.27$	$5.16^{a} \pm 4.73$	$2.87^{a} \pm 2.59$	$2.37^{a} \pm 1.02$	$3.34^{a} \pm 3.82$	$3.15^{a} \pm 1.67$	$1.38^{a} \pm 0.35$
	20/40	$3.41^{a} \pm 1.27$	$3.23^{a} \pm 2.02$	$5.79^{a} \pm 3.07$	$8.06^{a} \pm 6.73$	$4.04^{a} \pm 2.44$	$1.88^{a} \pm 0.62$	$5.57^{a} \pm 3.95$	$4.50^{a} \pm 2.77$	$3.75^{a} \pm 1.31$	$2.80^{a} \pm 1.36$
	40/80	$12.7^{ab} \pm 7.11$	$4.26^{b} \pm 2.33$	$5.43^{b} \pm 1.93$	$30.1^{ab} \pm 29.4$	$40.8^{a} \pm 31.2$	$4.92^{b} \pm 1.97$	$4.06^{b} \pm 1.55$	$13.5^{ab} \pm 10.9$	$15.0^{ab} \pm 10.4$	$4.00^{b} \pm 0.83$
	Fold change*	11	7	7	35	47	12	7	23	20	7
	p-value**	0.024	0.023	0.008	0.091	0.046	0.008	0.006	0.056	0.040	0.001
Roots	0	$5.71^{a} \pm 5.71$	$5.57^{a} \pm 5.57$	$5.62^{a} \pm 5.62$	$7.42^{a} \pm 7.42$	$8.07^{a} \pm 8.07$	$4.51^{a} \pm 4.51$	$5.66^{a} \pm 5.66$	$4.41^{a} \pm 4.41$	$5.27^{a} \pm 5.27$	$5.11^{a} \pm 5.11$
	10/20	$13.3^{ab} \pm 6.38$	$15.2^{ab} \pm 5.25$	$10.6^{ab} \pm 2.38$	$17.7^{a} \pm 3.96$	$11.4^{ab} \pm 2.40$	$10.5^{ab} \pm 5.12$	$9.14^{ab} \pm 2.73$	$11.1^{ab} \pm 4.80$	$13.9^{ab} \pm 4.41$	$7.88^{b} \pm 2.61$
	20/40	$22.4^{a} \pm 7.60$	$16.9^{abc} \pm 6.22$	$9.36^{c} \pm 3.33$	$15.4^{abc} \pm 1.83$	$20.3^{ab} \pm 4.24$	$12.4^{abc} \pm 4.12$	$14.5^{abc} \pm 7.90$	$15.7^{abc} \pm 5.63$	$17.6^{abc} \pm 4.48$	$10.6^{bc} \pm 2.00$
	40/80	$33.1^{a} \pm 1.06$	$18.3^{ab} \pm 8.02$	$15.7^{ab} \pm 4.60$	$32.4^{ab} \pm 15.8$	$30.7^{ab} \pm 8.85$	$15.8^{ab} \pm 7.72$	$17.3^{ab} \pm 8.60$	$14.2^{b} \pm 4.69$	$28.4^{ab} \pm 7.84$	$18.7^{ab} \pm 10.8$
	Fold change*	6	3	3	4	4	4	3	3	5	4
	p-value**	0.000	0.019	0.012	0.033	0.003	0.018	0.041	0.002	0.005	0.069
Whole plant	0	$8.04^{abc} \pm 2.55$	$10.5^{abc} \pm 1.94$	$8.13^{abc} \pm 2.57$	$11.0^{ab} \pm 3.77$	$13.2^{a} \pm 1.36$	$6.02^{bc} \pm 1.67$	$7.60^{bc} \pm 1.22$	$5.57^{c} \pm 1.71$	$8.55^{abc} \pm 3.77$	$6.84^{bc} \pm 2.53$
	10/20	$37.7^{ab} \pm 27.4$	$32.1^{ab} \pm 12.8$	$20.7^{ab} \pm 3.73$	$50.9^{a} \pm 16.2$	$39.6^{ab} \pm 10.9$	$15.6^{b} \pm 8.12$	$15.1^{b} \pm 6.98$	$34.9^{ab} \pm 33.6$	$26.2^{a} \pm 10.3$	$12.1^{b} \pm 4.36$
	20/40	$39.4^{bc} \pm 11.6$	$39.1^{bc} \pm 17.1$	$39.8^{bc} \pm 13.0$	$52.9^{ab} \pm 20.9$	$68.9^{a} \pm 11.0$	$18.8^{c} \pm 5.68$	$31.1^{bc} \pm 8.21$	$42.0^{bc} \pm 4.91$	$32.7^{bc} \pm 7.02$	$26.1^{c} \pm 15.3$
	40/80	$89.1^{abc} \pm 31.0$	$67.1^{bcd} \pm 28.6$	$33.2^{d} \pm 12.0$	$120.4^{a} \pm 31.5$	$110.2^{ab} \pm 26.3$	$40.3^{cd} \pm 27.3$	$41.6^{cd} \pm 16.1$	$39.7^{cd} \pm 13.4$	$75.6^{abcd} \pm 28.1$	$49.5^{cd} \pm 19.1$
	Fold change*	11	6	4	11	8	7	5	7	9	7
	p-value**	0.001	0.004	0.001	0.012	0.000	0.001	0.004	0.006	0.002	0.004

Data are expressed as mean values in mg per dry weight ± standard deviation. Different letters in each row indicate significant differences at $p \leq 0.05$. *Fold change between 0 mM NaCl and 40/80 mM NaCl treatments. ***p*-value between 0 mM and 40 mM treatments according to a paired sample t-test. NaCl treatments were doubled after the first three weeks of the experiment.

Table S7. Concentration of B, Fe, Mn, and Zn in leaves of guava seedlings as affected by different concentrations of NaCl in irrigation solution six weeks after salt treatment (n=60)

NaCl (mM)	B	Fe	Mn	Zn
0	$6.7^{a} \pm 1.8$	$26.3^{a} \pm 7.7$	$14.9^{a} \pm 12.3$	$3.8^{a} \pm 0.9$
10/20	$6.0^{ab} \pm 1.6$	$24.1^{a} \pm 5.8$	$15.7^{a} \pm 10.0$	$3.5^{a} \pm 0.8$
20/40	$6.5^{ab} \pm 1.3$	$24.7^{a} \pm 8.2$	$19.5^{a} \pm 13.4$	$3.4^{a} \pm 0.8$
40/80	$5.9^{b} \pm 1.6$	$17.7^{b} \pm 7.2$	$19.1^{a} \pm 9.2$	$3.7^{a} \pm 1.1$
Mean	6.3 ± 1.6	23.2 ± 8.0	17.3 ± 11.5	3.6 ± 1.0

Data are expressed as mean values in mg 100 g^{-1} DW ± standard deviation. Different letters in each column indicate significant differences at $p \leq 0.05$. NaCl treatments were doubled after the first three weeks of the experiment.

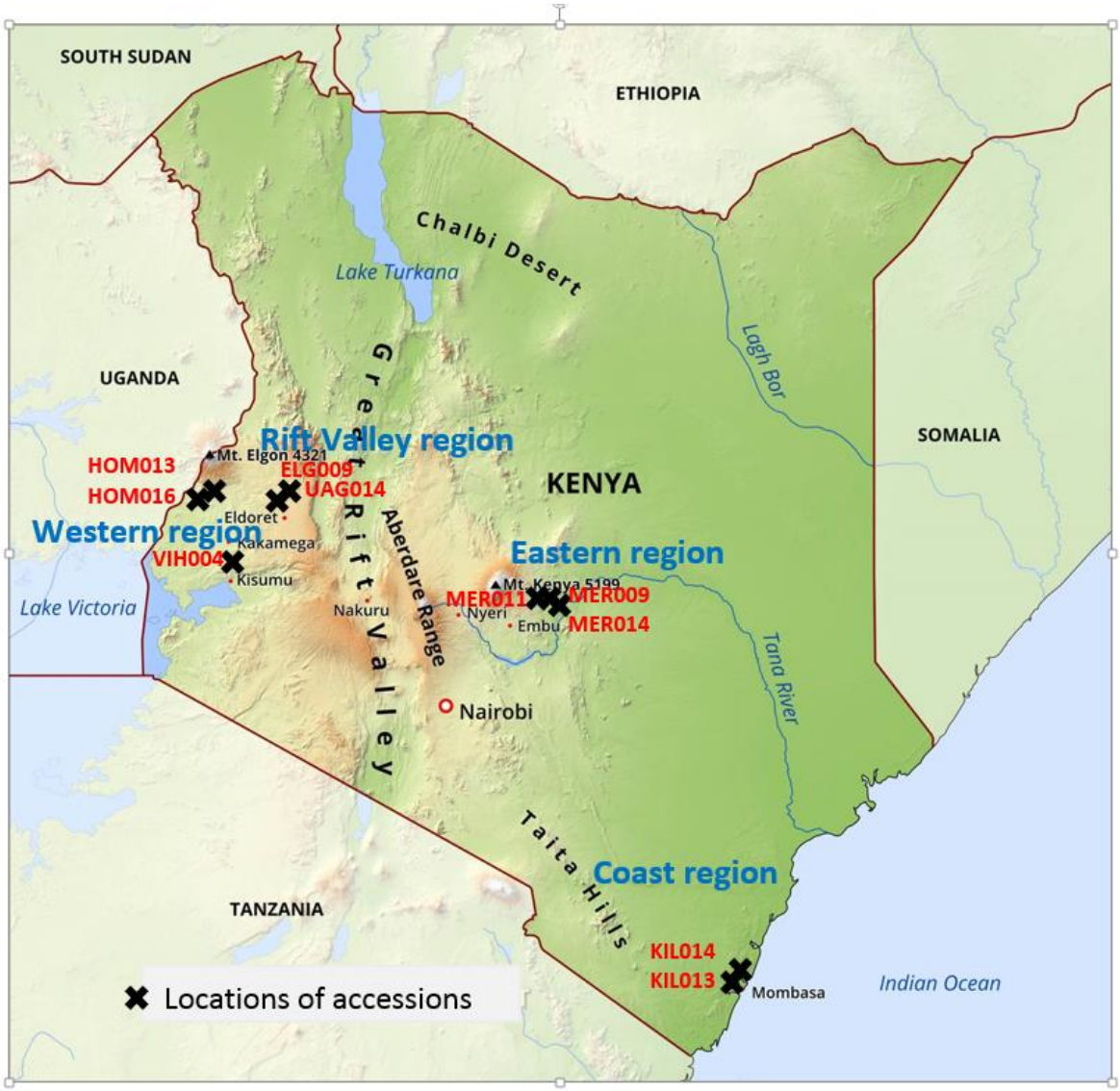

Figure S1. Sample collection sites for the 10 selected guava accessions (location icon followed by accession name (red colour) in four regions of Kenya (Coast, Eastern, Rift Valley, and Western).

Chapter five

5. General discussion

Suitability for production and improvement of quality traits in plants from genetically diverse parents is based on prior information regarding genetic variability and genetic divergence present (Jana et al. 2015; Pommer and Murakami 2009). The analysis of the diversity of Kenyan guava germplasm using SSR markers revealed non-existence of duplicate accessions; therefore, each accession was genetically distinct from the others. The low levels of observed heterozygosity (mean = 0.312) with respect to expected heterozygosity (mean = 0.630) indicated limited gene exchange among the accessions, including those that existed within the same geographical locations. This was also confirmed by the high average fixation index (F_{IS} = 0.511) and high intra-regional genetic heterogeneity as depicted by AMOVA. These results are in agreement with those reported in guava e.g. by Grattapaglia et al. (2012) and Sitther et al. (2014). Consequently, lack of robust well supported (bootstrap values >50%) genetic clusters based on NJ-phylogenetic tree was observed – and fruits with different flesh colours could be found clustering together. However, this observation both agrees and disagrees with some prior studies on guava. For instance, similar to our findings, Mehmood et al. (2013) observed a high range in genomic DNA diversity using iPBS markers in open pollinated guava that only separated into small multiple clusters. On the contrary, Chen et al. (2007), Kanupriya et al. (2011) and Coser et al. (2012) observed genetic differentiation in guava based on region of origin and fruit flesh colour. These differences could be attributed to the sample populations used in these studies and also on the genetic markers.

Conventional propagation in guava is made through seeds and this has led to clonal degradation of some improved (usually used for commercial fruit production) varieties as a result of variations brought by cross pollination (25-40%) (Mehmood et al. 2013). High

genetic differentiation of guava in some studies elsewhere could therefore be due to use of both improved and uncultivated varieties that facilitated crossing at some point in time. For instance, (Coser et al. 2012) observed a higher genetic similarity among improved cultivars than between uncultivated and improved cultivars. The sampled Kenyan guava accessions in this study exist in varying environments, ranging from homesteads and in the wild. Some of the accessions were planted hence selected by the farmers, while others derive from seed naturally. In addition, the plant material could be of different origin since the time guava was introduced in Kenya is not known – hence seed material is likely to have been moved by communities in the past, leading to a lack of strong genetic grouping in Kenyan guava accessions. This implies that most of the individual accessions in this study are genetically diverse single genotypes presenting a wide genetic variation that can be tapped to improve both quality and production potential of guava fruit.

The diversity in the fruit morphological and, chemical and mineral composition of the accessions studied herein also attest to the extent of genetic variation present. For instance, analysis and hierarchical clustering of the morphological traits identified two clusters based on fruit tree, leaf and fruit traits. The clusters were distinct from each other mainly based on seven key descriptors that were only fruit based. Similar to our results, Mehmood et al. (2014) also found more variation in guava mainly based on fruit traits. Accordingly, Coser et al. (2012) reported that guava trees with red- and white-fleshed fruits were traditionally distinguished by fruit traits such as shape. Coser et al. (2012) further grouped the red-fleshed guavas in two sub-groups based on their geographical locations, altitude and climatic factors. It is likely that the farmers selected their fruit trees based on their suitability to adapt and produce more yield according to their environmental conditions. The occurrence of varied morphological traits, especially fruit-based as key discriminants in our guava accessions regardless of the geographical regions and existing climatic factors in

these regions, could therefore imply that not much selection has been done – and therefore concurs with the results of our SSR characterization. It also implies that, not much has been done at a farmer level to improve the quality and productivity of guava fruit.

The results of the effect of climatic factors (temperature and precipitation), soil minerals and fruit morphological traits (both flesh colour-, size- and weight-based traits) revealed that each of these factors affected the fruit chemical and mineral composition. In agreement with our results, Thaipong and Boonprakob (2005) found in guava fruits grown in Thailand more accumulation of ascorbic acid in the winter season (mean min./max. air temperature: 31.8/20.8°C) rather than in the summer season (mean min./max. air temperature: 33.6/24.5°C). We also observed variations in chemical and mineral composition of the fruits in respect to the soil minerals and fruit morphological traits. Similarly, variation in guava fruit chemical and mineral composition have been reported to vary with soil minerals as well as plant cultivars (Natale et al. 2007). Likewise, variation based on morphological traits has also been reported (Mehmood et al. 2014; Singh et al. 2015) – in which large fruits are observed to have a dilution effect of their mineral composition. It should however, be noted that the correlations in our study were not strong (i.e. $r < 0.7$) indicating that the fruit chemical and mineral composition is a complex of traits and influenced by an interaction of the studied factors and more others e.g. plant genotype as was also observed by (Natale et al. 2007) in only two guava cultivars. Our accessions were highly differentiated based on both SSR data and morphological traits. For instance, based on fruit flesh colour, the white-fleshed fruits were depicted as having higher contents of protein, K, Mg, Na, S and B compared to the red-fleshed fruits. Additionally, the wide ranges in chemical and mineral composition of the fruits irrespective of their geographical origins and environmental conditions points to their high genetic variation.

Strategies that can enhance guava fruit quality and performance include identification of tolerant accessions to salinity stress. Plants' tolerance to saline stress has been found to depend on the species and is genetically instigated (Munns and James 2003). Owing to prevalence of saline conditions in guava growing regions e.g. Kenya (Mugai 2004), Brazil (Cavalcante et al. 2007) and India (Singh et al. 2016), we selected ten genetically diverse guava accessions from the genetic grouping of the NJ-phylogenetic tree constructed from SSR data for assessment of their tolerance to salinity. The plants were raised by rooting cuttings – which is an effective propagation method in guava (Kareem et al. 2013) for production of true to type and quality plants. Generally, all the ten accessions exhibited salt injuries as was also reported in other experiments with guava (Ali-Dinar et al. 1999; Ebert et al. 2002). The leaf dry matter was found to vary the most while root dry matter remained similar in all the accessions. Total plant dry matter production at high salinity relative to dry matter in non-saline conditions (Martin et al. 1994) revealed genetic differences in salt tolerance among the accessions, with accession ELG009 being outstanding with the least reduced dry matter (only 0.6%). Genetic differences in Na exclusion is also highly correlated with differences in salinity tolerance (Munns and James 2003).Variations were observed with respect to accumulation of Na both in the leaves and roots of individual accessions at salinity levels below 40/80 mM NaCl. For instance, accession MER014 was able to accumulate less Na while accession KIL013 accumulated the highest Na content. This therefore implies that, at a NaCl concentration of less than 40 mM, some accessions are able to reduce its accumulation while others can accumulate high concentrations but continue to survive. This could therefore help in identification of rootstocks tolerant to salinity from the existing genetic variation.

5.1. Conclusion

Composition of genotypes can be greatly affected by environmental conditions – the lower the environmental effects, the lower the effect on plant genotype composition (Perfectti and Camacho 1999). Our accessions were genetically diverse and could have contributed significantly to both the observed morphological variation and to fruit chemical and mineral composition. Environmental factors can be minimized by studying the interaction between genotype and environment in targeted accessions so as to select those with preferred traits e.g. high mineral composition, high productivity and tolerance to adverse environmental conditions like drought and salinity.

One way to carry out guava improvement in Kenya is through breeding using the available guava germplasm. Such breeding approach has been effectively implemented in some tropical fruits such as avocado, orange and mango (Samson 2003). Alternatively, a decentralized participatory strategy previously developed by the World Agroforestry Centre (ICRAF) and its partners can be employed. The strategy brings together the local people who are the farmers and the scientific community. The existing knowledge of the local community regarding fruit tree use and cultivation is then enhanced by the scientific knowledge in terms of collection, selection and propagation (Asaah et al. 2011; Jamnadass et al. 2011). This approach ensures ownership and management of the resources by the communities themselves, hence greater and more rapid adoption (Lombard and Leakey 2010; Tchoundjeu et al. 2010). As considerable genetic variation exists on a regional level in the Kenyan guava germplasm, participatory selection involving communities may present a substantial success.

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Summary

Common guava (*Psidium guajava* L.) fruit has a significant nutritional and medicinal potential besides its economic importance, yet is still underutilized in terms of production and consumption. The current global guava fruit production is based only on a few cultivars selected mainly based on their morphological traits, hence much of the genetic variation remains untapped. There is also limited knowledge about the impact of climatic conditions, soil properties and fruit morphological traits on the chemical and mineral composition of the guava fruit. Additionally, guava production currently faces challenges of salinity in many guava-producing countries. The objectives of this study were therefore to determine the genetic diversity, nutritional variability and salinity tolerance among accessions of guava collected from four different regions of Kenya (Coast, Eastern, Rift Valley and Western). The genetic diversity was assessed on 177 guava accessions using 13 simple sequence repeat (SSR) markers. Out of the 177 trees used for SSR analysis, a relationship between climatic factors and, chemical and mineral composition of fruits from128 trees was determined. Correlations were also performed for fruit chemical and mineral composition with their morphological traits and soil mineral contents, respectively. The effect of sodium chloride (NaCl) salinity—0 mM (control), 10/20 mM (low), 20/40 mM (medium), and 40/80 mM (high) was also investigated on ten genetically diverse accessions in a six week greenhouse experiment. Results of the genetic analysis revealed that most accessions generally clustered into multiple weakly supported groups with only 46 out of 177 accessions being supported by bootstrap values above 50%. There was a lack of clear-cut genetic groups along geographical origins and fruit flesh colour. Results of fruit chemical and mineral composition revealed a positive correlation between ascorbic acid composition and total annual precipitation while the total soluble solids (TSS) was positively linked with mean annual temperature, and was high in white-fleshed fruits. The mineral content of the fruits

mainly reduced with an increase in most of the fruit weight- and size-based morphological traits and also with the total annual precipitation. Results of the salinity experiment revealed genetic differences in dry matter accumulation by some accessions. Moreover, variations were observed among the accessions with respect to accumulation of Na, and was positively correlated to dry matter (DM). Generally, guava was not so effective in excluding Na from the transpiration stream. For guava conservation, selection and improvement, we recommend sampling many individual accessions covering the geographical range of the species based on our genetic analysis results. The information on fruit chemical and mineral composition could act as a guide in the selection of specific regions for guava fruit production, selection of accessions for improvement programmes, and the design of appropriate fertilizer regimes that enhance guava fruit composition. Differences among the accessions relative to DM production and accumulation Na at varying salinities should be considered for selecting genotypes for adaptation to saline environments. The interaction between genotype, environmental conditions and fruit morphological traits should be used to select accessions with preferred traits – for instance, those with high chemical and mineral composition, high productivity and tolerance to adverse conditions such as salinity.

Acknowledgement

The field work for this research was funded by the International Fund for Agricultural Development (IFAD) and the European Commission under the World Agroforestry Centre (ICRAF) project "Tree crops development in Africa to benefit the poor". Thanks to Deutscher Akademischer Austauschdienst (DAAD) and the Kenyan government through National Commission for Science, Technology and Innovation (NACOSTI) for providing me a PhD scholarship; and to all farmers as well as field and lab staff at the ICRAF. The SSR fragment analysis was done at the Department of Forest Genetics and Forest Tree Breeding, University of Goettingen, Germany. I appreciate Mrs. Alexandra Dolynska for the technical assistance. The technical staff at the Division of Quality of Plant Products, University of Goettingen are also appreciated for their assistance with laboratory protocols and aspects to do with the analysis of nutritional properties of guava.

I acknowledge Prof. Dr. Anthony Whitbread (formerly of the Crop Sciences Department, University of Goettingen), for having read my proposal and also accepted me as a PhD student at the University. I also thank Prof. Dr. Reiner Finkeldey (formerly at the Department of Forest Genetics and Forest Tree Breeding, University of Goettingen) who also agreed to co-supervise my work and allowed the use of their laboratory. Consequently, I appecriate the advise and help of Prof. Dr. Konstantin Krutovsky and Dr. Markus Mueller (both of the Department of Forest Genetics and Forest Tree Breeding, University of Goettingen) regarding SSR fragment analysis and revising the genetic manuscript. Prof. Dr. Katja Kehlenbeck formerly of the World Agroforestry Centre, (currently at Rhine-Waal University of Applied Sciences, Faculty of Life Sciences) assisted with the formulation of the hypotheses and subsequently the proposal. She was also instrumental in field work and morphological characterization of guava in Kenya as well as reading of the manuscripts. Thank you so much. I also would not forget Dr. Naumann Marcel (Division of Quality of Plant Products, University of Goettingen) for his tireless effort at advising and facilitating the various phases of this study, and reading of the manuscripts. Much appreciation goes to Prof. Dr. Elke Pawelzik for finally accepting me as a PhD student in her Division (Division of Quality of Plant Products, University of Goettingen) when the former supervisors had to leave for other positions elsewhere. I acknowledge the much support she offered regarding the various aspects of the analysis, evaluation of my proposal, progress reports and reading of the manuscripts.

Lastly, I appreciate my parents, brothers and sisters and, my loving wife, Joan and son, Joshua for they had to bear with my long absence from home during this study. I love you and may the Almighty God bless you abundantly.

Declaration

Appendix 4:

DECLARATIONS

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form.

   Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

   Göttingen, 17.05.2018

   .....
   (Signature)

   CHIVEU CHEMULANGA JOSIAH
   (Name in block capitals)

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorised aid.

   Göttingen, 17.05.2018

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Publications

Chiveu, CJ, Dangasuk, OG, Omunyin, ME. and Wachira, FN. (2009). Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd. based on ISSR Markers. *Silvae Genetica* 58(1/2): 20-28.

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